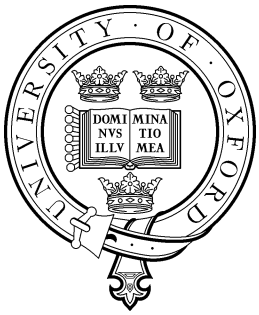
The Influence of Farm-Level Factors on Midge Abundance and Transmission of Arboviruses



Lara Ellen Harrup

Christ Church College

University of Oxford

A thesis submitted for the degree of

Doctor of Philosophy

Hilary 2010

This research was conducted in collaboration with
The Institute for Animal Health

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Abstract

Since 1998 bluetongue virus (BTV) has posed a significant risk to the health of European livestock. The incidence and prevalence of BTV infection is highly dependent on the distribution and abundance of its *Culicoides* vectors. An understanding of the drivers of vector abundance and distribution are therefore required for accurate estimation of transmission risk and for the development of cost-effective targeted vector control strategies. In northern Europe BTV transmission occurs via the Palaearctic vector groups, *Culicoides obsoletus* s.l. and *C. pulicaris* s.l. There is, however, a paucity of detail regarding the ecology of the constituent species within these groups. The issue of habitat use by larval and adult Palaearctic BTV vectors was addressed within this thesis using a multidisciplinary approach, combining statistical modelling and GIS techniques with detailed ecological surveys of adult and larval Palaearctic BTV vector populations.

Within this thesis the drivers of BTV outbreak occurrence and vector abundance were first investigated at a regional level in mainland Europe. The results revealed that although both the climatic and non-climatic environmental predictors can accurately describe the risk of BTV outbreak occurrence they fail to explain a significant amount of the variation in Palaearctic BTV vector group abundance. This was potentially the result of additional, as yet undefined, local-scale drivers of spatial variation and/or variation in the environmental preferences of the constitute species of the Obsoletus and Pulicaris groups. In light of this, spatial variation in adult and immature Palaearctic BTV vector abundance was investigated to species level at the within-farm scale in southern England. This work first examined the environmental correlates of *Culicoides* larval development sites within and between farms using both direct larval sampling and emergence trapping. Larval habitats were successfully identified and a habitat modification control method was tested. A semiochemical-baited trapping technique was then devised and tested and then utilised to make habitat-specific population estimates of livestock-associated *Culicoides* for investigation of the drivers of the spatial variation in adult abundance at the within-farm scale. Using multivariate statistics, both BTV vector larval occurrence and adult abundance were related to climatic and non-climatic environmental predictors. As a result of this work, drivers of Palaearctic BTV vector occurrence and abundance were identified that will contribute significantly to improving the understanding of BTV transmission patterns.

Acknowledgements

I would like to thank my supervisors both at the Institute for Animal Health, Dr. Simon Carpenter and Prof. Philip Mellor and in the Department of Zoology at the University of Oxford, Prof. David Rogers and Dr. Bethan Purse now at the Centre for Ecology and Hydrology, for their invaluable guidance throughout my project, without which the completion of this thesis would not have been possible.

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"If you blow on a conch, guiding your breath deep into the twists of its pearly coil, it produces the sound of Om. Some say that this is the beginning of all things"

Contents

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List of Abbreviations

Common abbreviations are defined below, specialist abbreviations are defined below and on their first use within the text.

A1	Annual Amplitude
A2	Biannual Amplitude
A3	Triannual Amplitude
AHS	African Horse Sickness
AIC	Akaike's Information Criterion
ANOVA	Analysis of Variance
AOI	Area of Interest
AR	Auto Regressive
AUC	Area Under the Curve
AVHRR	Advanced Very High Resolution Radiometer
BBSRC	Biotechnology and Biological Research Council
BT	Bluetongue
Bti	*Bacillus thuringiensis* serovar *israelensis*
BTV	Bluetongue virus
°C	Degrees Centigrade
C.	*Culicoides*
CDC	Center for Disease Control
cm	Centimetres
CO_2	Carbon Dioxide
CL2000	CORINE (see below) Land Cover 2000
CORINE	Coordination of Information on the Environment
CMS	Carter Miller Stress Index
D^2	Deviance Explained
D_{xy}	Bias-corrected Somers D_{xy} Rank Correlation
d.f.	Degrees of Freedom
DCA	Detrended Correspondense Analysis
DEM	Digital Elevation Model
DEFRA	Department for Environment, Farming and Rural Affairs
DIC	Deviance Information Criterion
dLST	Day-time Land Surface Temperature
DWSI	Disease Water Stress Index
EAG	Electroantennogram
EIP	Extrinsic Incubation Period
EO-1	Earth Observing Mission One
ETM	Enhanced Thematic Mapper
EU	European Union
EVI	Enhanced Vegetation Index
FN	False Negative
FP	False Positive

Continued overleaf...

GCP	Ground Control Point
GIS	Geographic Information System
GAM	General Additive Model
GLM	Generalized Linear Model
GPS	Global Positioning System
h	Hour
HDI	Human Development Index
i.d.	Internal Diameter
IAH	Institute for Animal Health
IFOV	Instantaneous Field of View
IHS	Intensity Hue Saturation
JNCC	Joint Nature COnservation Committee
K	Kelvin
kg	Kilogram
L	Location
LAI	Leaf Area Index
LST	Land Surface Temperature
m	Metre
m^2	Metres squared
MAX	Maximum
ms^{-1}	Metres per second
MCMC	Markov Chain Monte Carlo
$mg.l^{-1}$	Milligrams per litre
$mg.h^{-1}$	Milligrams per hour
ml	Millilitres
$ml.min^{-1}$	Millilitres per minute
$ml.h^{-1}$	Millilitres per hour
MIN	Minimum
MIR	Mid-InfraRed Reflectance
mm	Millimetres
MLV	Modified Live Vaccine
MODIS	Moderate Resolution Imaging Spectroradiometer
n	Sample size
nm	Nanometre
NASA	National Aeronautics and Space Administration
NERC	Natural Environment Research Council
NDVI	Normalized Difference Vegetation Index
nLST	Night-time Land Surface Temperature
NOAA	National Oceanic and Atmospheric Administration
NTF	National Transfer Format (UK Geographic Data Standard - BS7567)
OIE	Office International des *Épizooties* (World Organization for Animal Health)
OR	Odds Ratio
OS	Ordnance Survey
OSGB	Ordnance Survey Great Britain
OVI	Onderstepoort Veterinary Institute

Continued overleaf...

P1	Annual phase
P2	Biannual phase
P3	Triannual phase
PCNM	Principal Coordinates of Neighbour Matrices
PCR	Polymerase Chain Reaction
ppm	Parts per Million
PVCu	Unplasticised Polyvinyl Chloride
QCAL	Digital Number
R_0	Basic Reproduction Number
R_a^2	Adjusted Redundancy Statistic
RDA	Redundancy Analysis
RMSE	Root Mean Squared Error
RNA	Ribonucleic Acid
ROC	Reciever Operator Characteristic
S.D.	Standard Deviation
S.E.	Standard Error
s.l.	*Sensu Latu*
SLC	Scan Line Corrector
sp.	Species
s.s.	*Sensu Stricto*
SPOT	Systeme Pour l'Observation de la Terre
SRTM	Shuttle Radar Topography Mission
TAIR	Air Temperature
TC	Tassel Cap
TM	Thematic Mapper
TN	True Negative
TP	True Positive
UK	United Kingdom
US	United States of America
USGS	United States Geological Society
UTM	Universal Transverse Mecator
UV	Ultra Violet
V	Variance
VIF	Variance Inflation Factor
VPD	Vapour Pressure Deficit
Wm^{-2}	Watts per square metre
WNV	West Nile Virus
WWF	World Wildlife Fund
$\bar{x}$	Arithmetic Mean
Z.p	Zero-inflated Poisson distribution
Z.nb	Zero-Inflated negative binomial distribution
Z.geo	Zero-nflated geometric distribution
$\pm$	Plus or minus

Chapter 1

General Introduction

1.1 *Culicoides* as Arbovirus Vectors

To date, approximately 30 *Culicoides* species have been implicated in the transmission of pathogens and parasites to man and other animals (Meiswinkel et al., 2004b,a). Over 50 arboviruses have been isolated from *Culicoides*, mostly within the families Bunyaviridae (20 viruses), Reoviridae (19 viruses) and Rhabdoviridae (11 viruses) (Coetzer et al., 1994; Mellor et al., 2000). A large proportion of these viruses (45 %), have not been isolated from other arthropod groups [for review see Meiswinkel et al. (1994)]. Of these, bluetongue virus (BTV) [Orbivirus: Reoviridae] is currently of greatest economic importance in Europe and is notifiable to the Office International des *É*pizooties (OIE) (OIE, 2006). Diseases are classified as notifiable if they (*i*) have the potential for very serious and rapid spread irrespective of national borders, (*ii*) are of serious socio-economic or public health consequence, and (*iii*) are of major importance in the international trade of animals and animal products (OIE, 2009b).

1.1.1 Bluetongue Virus

Bluetongue virus is an infectious, non-contagious virus, which replicates in all species of wild and domestic ruminants and is the causative agent of the disease bluetongue (BT). The virus has a double-stranded RNA genome consisting of 10 segments contained within three concentric shells of structural proteins that form the subcore, outer core and outer capsid (Grimes et al., 1998). Globally BTV exists as 24 confirmed serotypes [BTV 1 to 12 (Howell, 1960); BTV 13 to 16 (Howell, 1970); BTV 17 to 24 (B.J. Erasmus, unpublished data)], which are distinguished on the basis of the antigenic profile of its major outer capsid protein VP2, and to a lesser extent VP5 [see Figure 1.1 on page 2].

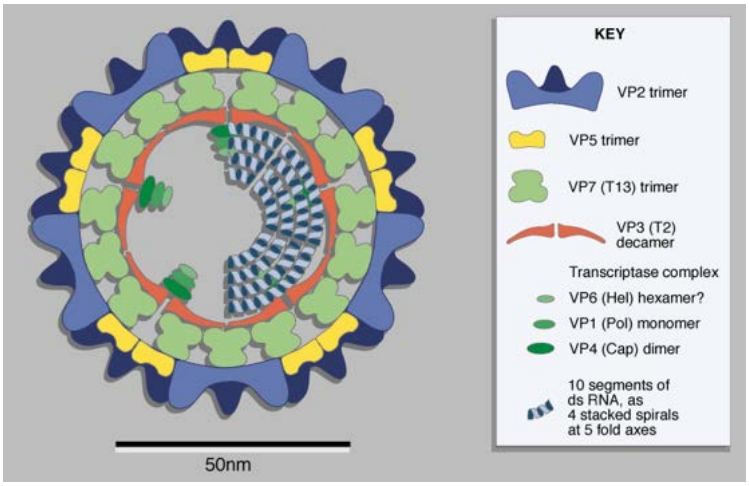


Figure 1.1. Schematic diagram of the structure of the bluetongue virus particle, based on structural data from several sources [reproduced from Mertens et al. (2005)]

Bluetongue virus is maintained in the field through continual cycles of replication between *Culicoides* vectors and susceptible ruminant hosts (Du Toit, 1944; Foster et al., 1963) [see Figure 1.6 on page 10]. Bluetongue itself is primarily regarded as a disease of sheep, with clinical signs most commonly observed in certain fine wool and mutton breeds (Hutcheon, 1880, 1902; Spreull, 1905; Verwoerd and Erasmus, 2004) and some deer species (Hoff and Hoff, 1976). The majority of indigenous breeds of sheep (Parsonson, 1990a; Verwoerd and Erasmus, 2004) and wild ruminants (Frolich et al., 2005; Prasad et al., 1998; Ruiz-Fons et al., 2008) in most 'endemic' areas tend to be sub-clinically affected. These areas of high-level endemic immunity, combined with the use of vaccination mean that the vast majority of BTV infections remain 'silent' worldwide, until the virus comes into contact with naïve and BT-susceptible ruminant populations. Sub-clinically infected cattle play an important role in BTV's amplification and transmission by allowing undetected spread to occur prior to the outbreak being discovered (Verwoerd and Erasmus, 2004). For example, BTV-3 apparently circulated without being detected for at least a year (2002/2003) on the French island of La Réunion in the Indian Ocean. Circulation was only detected during a retrospective survey using previously collected sera (Breard et al., 2005).

In immunologically naïve BT-susceptible sheep, clinical signs of BTV infection include pyrexia, depression, nasal discharge, excessive salivation, facial oedema, con-

junctivitis, hyperaemia and ulceration of the oral mucosa, coronitis, muscle weakness and death [see Figure 1.2 below]. The frequency and severity of clinical signs varies substantially with breed, individual and the strain of the virus involved [for review see MacLachlan and Gard (2009)]. Infection can lead to significant direct costs to the farmer, including a loss of condition, reduced milk yield, infertility and abortion (Osburn, 1994). Indirect costs as a result of livestock trade restrictions and from the cost of surveillance to monitor the spread of the virus are also substantial and are commonly underestimated by economic analyses (Hoar et al., 2004; Hoogendarn, 2007; MacLachlan, 2006; Mulhern, 1985).

(a). **Depression** (b). **Facial Oedema and Excessive Salivation** (c). **Conjunctivitis**

(d). **Coronitis and Lameness** (e). **Inter-Dermal Haemorrhaging** (f). **Death**

Figure 1.2. Progressive clinical signs of BT in naïve sheep [photographs provided by K.E. Darpel, ©Institute for Animal Health]

Although uncommon, severe clinical signs and mortality have been observed sporadically in naïve cattle populations infected with BTV (Bekker et al., 1934); examples include outbreaks in Israel, Spain and Portugal (Campano Lopez and Sanchez Botija,

1958; Lopez and Sanchez, 1958; Manso Ribeiro and Noronha, 1958; Parsonson, 1990b). Morbidity in cattle has also been one of the features of the BTV-8 outbreak in northern Europe, (Dal Pozzo et al., 2009; Darpel et al., 2007; EFSA, 2007; Elbers et al., 2008a,b). The probable causes of this phenomenon, which may include lack of previous exposure of the host populations and virulence of the virus strain circulating, have yet to be investigated in detail.

1.1.1.1 The Current State of Bluetongue Virus in Europe

Cyprus is thought to have suffered regular incursions of BTV since at least 1924 (Gambles, 1949). The only recorded incursions of BTV into the rest of Europe prior to 1998, however, were in the Iberian Peninsula, between 1956 to 1960 (BTV-10) (Manso-Ribeiro et al., 1957; Sellers et al., 1979) and between 1979 to 1980 on several Greek Islands in the Aegean Sea (BTV-4) (Dragonas, 1981; Vassalos, 1980).

In 1998 outbreaks of BTV-9 were identified on several Greek islands in the Aegean Sea close to the Turkish coast (ANON, 1998a,b, 2000), starting what was to become the most prolonged and devastating series of BTV outbreaks in recorded history (Mellor and Wittmann, 2002). Between 1998 and 2005 twelve European countries in the Mediterranean Basin were affected (Purse et al., 2005; Mellor and Wittmann, 2002) [see Figure 1.4 on page 6], with 1.8 million sheep known to have been killed, through contracting BT or through being destroyed as part of control measures (Mellor and Wittmann, 2002; Purse et al., 2006, 2005). During this period the BTV outbreaks were restricted to countries in the Mediterranean Basin, but they involved multiple serotypes (Mellor, 2002; Purse et al., 2005; Mertens and Mellor, 2003) and at least three independent entry points, from sub-Saharan Africa via Morocco or Tunisia/Algeria, and from the Middle East via Turkey [for review see Maan et al. (2008a) and Figure 1.3 on page 5]. Sero-surveys in these countries of apparent origin had previously indicated that BTV had been circulating at intervals for several decades prior to these incursions [for review see Gibbs and Greiner (1994), Purse et al. (2005) and Taylor et al. (1985)].

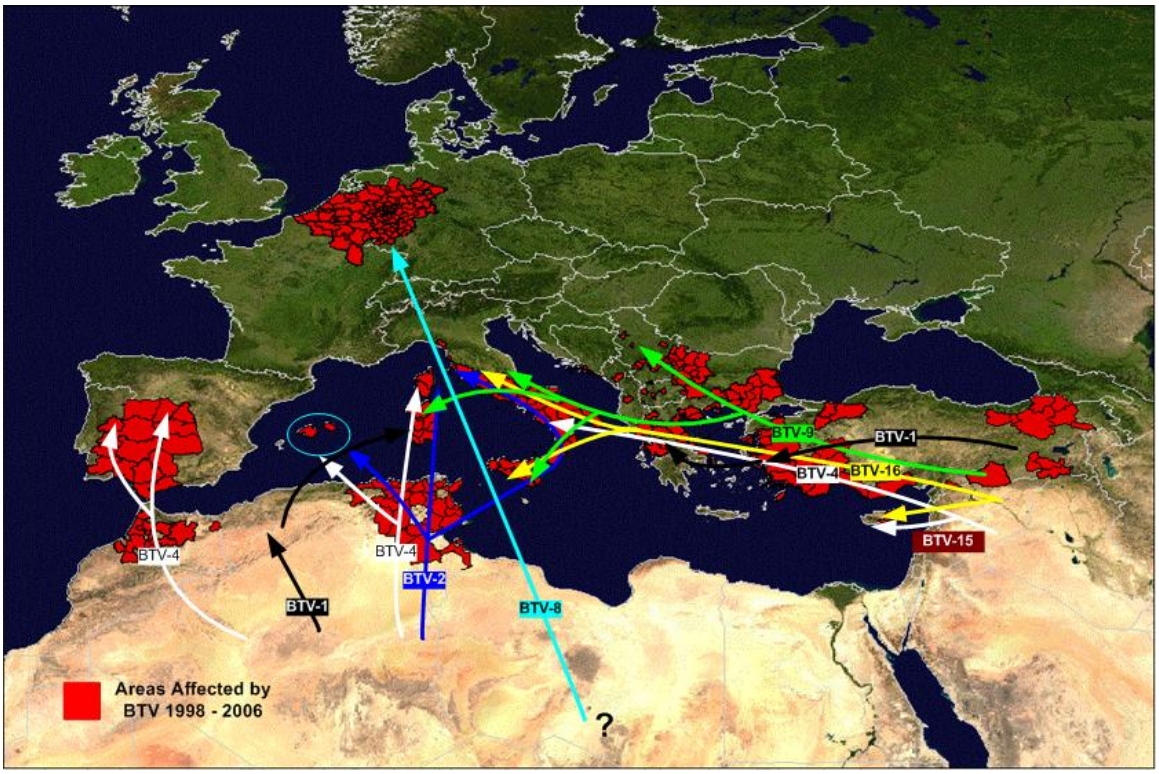
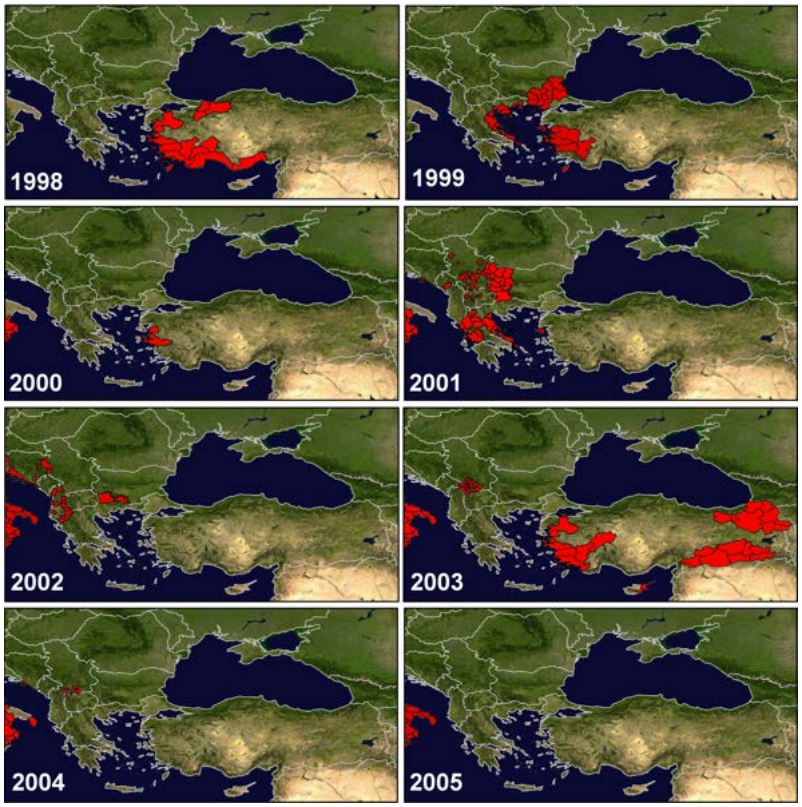
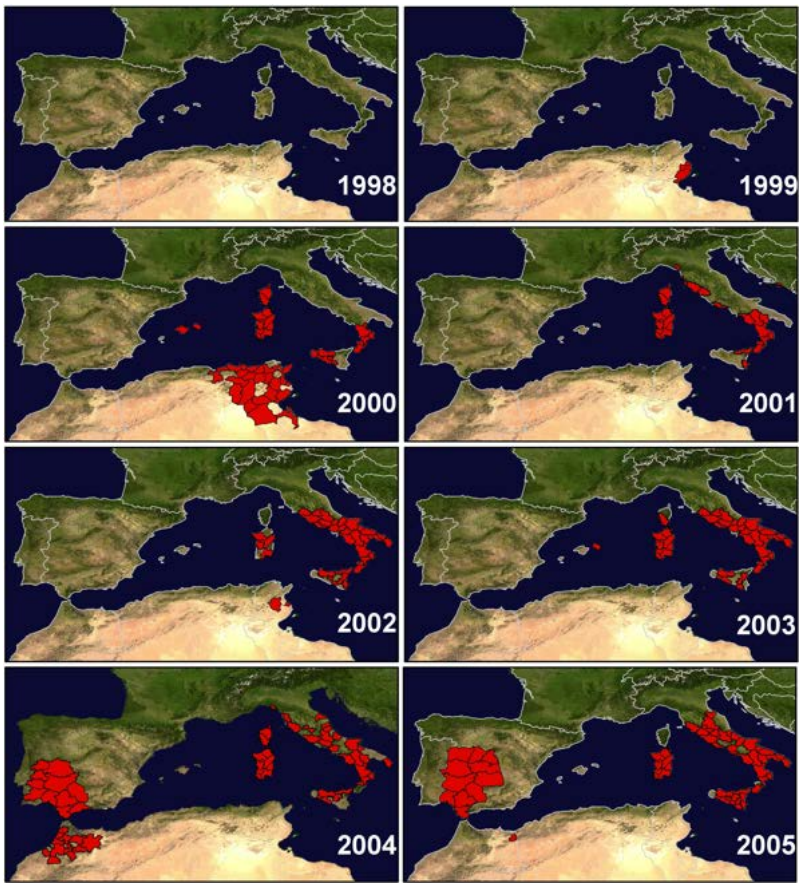


Figure 1.3. Bluetongue virus serotype spread in the Mediterranean Basin 1998-2006 [modified from Maan et al. (2008a)]

Since 1998, with the exception of 2002, new introductions of BTV strains into Europe have occurred every year. In August 2006, however, a second step change in the epidemiology of the virus occurred with the first recorded case of BTV reported in northern Europe, in the Maastricht region of the Netherlands (ANON, 2006i,h). This outbreak occurred some 5° of latitude further north than had ever previously recorded in Europe and was later discovered to be BTV-8, a serotype that was not circulating in the Mediterranean countries (EFSA, 2007; International Society for Infectious Diseases, 2006; OIE, 2007). The BTV-8 strain was also not known to be active in any of the regions bordering the Mediterranean Basin, although surveillance was limited. Phylogenetic analysis has since identified the strain as genetically distinct from American, Asian or vaccine strains of BTV-8 and most closely resembling an isolate collected from sub-Saharan Africa (Maan et al., 2008b). Its mode of entry into northern Europe remains unknown (EFSA, 2007; Mintiens et al., 2008).



(a). **Eastern Mediterranean Basin**



(b). **Western Mediterranean Basin**

Figure 1.4. Bluetongue virus activity in the Mediterranean Basin by year, from 1998 to 2005 [based on data from various sources, see Mellor et al. (2008) Appendix G on page 303]

Since its introduction in 2006 BTV-8 has continued to expand its geographic range with transmission recorded in 17 countries [see Table 1.1 below]. Although vaccination and other control methods are ongoing, the OIE now consider BTV-8 endemic in Austria, Belgium, France, Germany, Luxembourg, the Netherlands, Spain and Switzerland (OIE, 2009a).

Country	Year First Recorded
Netherlands	2006 (ANON, 2006i,h)
Belgium	2006 (ANON, 2006b)
Germany	2006 (ANON, 2006d)
France (including Corsica)	2006 (ANON, 2006c)
Luxembourg	2006 (ANON, 2006f)
Czech Republic	2007 (ANON, 2007f)
Switzerland	2007 (ANON, 2007e)
Denmark	2007 (ANON, 2007a)
United Kingdom*	2007 (ANON, 2007g)
Austria	2008 (ANON, 2008a)
Hungary	2008 (ANON, 2008b)
Italy (including Sardinia)	2008 (ANON, 2008c)
Sweden	2008 (ANON, 2008f)
Norway	2008 (ANON, 2009c)
Slovakia	2008 (ANON, 2008d)
Spain	2008 (ANON, 2008e)
Greece (restricted to the Island of Lesbos)	2009 (ANON, 2009b)

*BTV-8 transmission not recorded in the year following incursion

Table 1.1. European countries with recorded BTV-8 transmission, August 2006 to January 2010

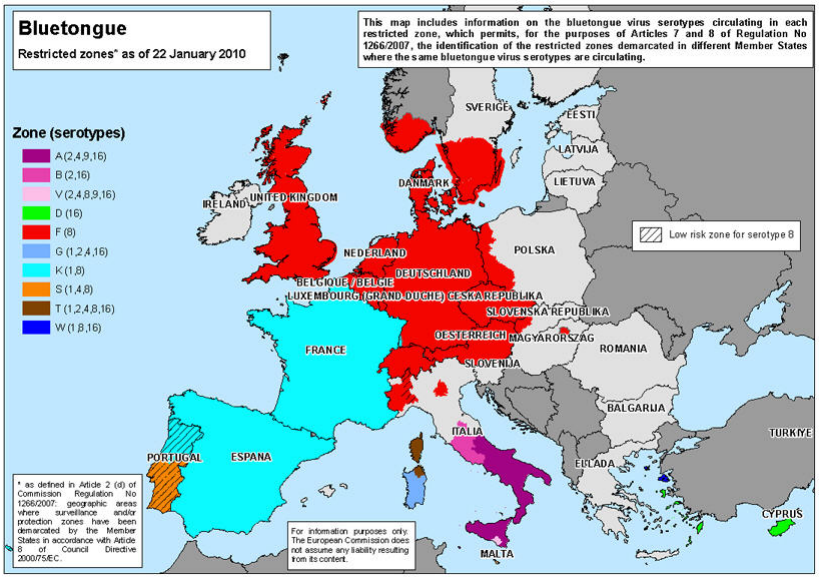


Figure 1.5. Bluetongue restriction zones in Europe active from the 22^{nd} January 2010, current as of 12^{th} February 2010 [reproduced from EFSA (2010)]

1.1.1.1.1 BTV Transmission in the UK

The first case of BTV-8 in the UK was detected on the 15^{th} September near Ipswich, Suffolk (ANON, 2007g; DEFRA, 2007). The most likely entry route was identified as wind-borne movement of infected vectors from the continent (Gloster et al., 2007a,b, 2008), with overnight winds from the Ostend area of Belgium on the 4^{th} and 5^{th} of August, providing suitable conditions for entry of the vectors responsible for the first outbreaks (Gloster et al., 2008). The UK outbreak was restricted primarily to the east and south east of England (DEFRA, 2008c). During 2007 to 2008, 138 holdings and approximately 1,000 ruminants were identified as having BTV through clinical and serological surveys (DEFRA, 2009a; OIE, 2009c). Of the countries where transmission has been recorded only the UK has not reported further cases of transmission in the year following incursion. Pending the results of further surveillance and proof of disease-free status the whole of the UK excluding Northern Ireland remains within a BTV-8 protection zone [see Figure 1.5 on page 7].

Other BTV serotypes currently active in Europe, that could threaten the UK, include a BTV-1 strain first identified in July 2007 in Andalusía, Spain (ANON, 2007d). It is likely this outbreak spread from the major epizootic of BTV-1 that was ongoing in Morocco (ANON, 2006g), Algeria (ANON, 2006a) and Tunisia (ANON, 2007h) in 2006 and 2007. The BTV-1 strain has subsequently expanded into Portugal (ANON, 2007c), Sardinia (ANON, 2006e) and France (ANON, 2007b), and into areas outside the range of *C. imicola*, with cases recorded as far north as Brittany (DEFRA, 2008a). Although vaccination and other control methods are ongoing the OIE now considers BTV-1 endemic in Spain and Portugal [see Figure 1.5 on page 7 for current European restrictions zones].

Infections, but not transmission, of two additional serotypes were recorded in northern Europe in 2008, BTV-6 in the Netherlands (ANON, 2008g; International Society for Infectious Diseases, 2008b), Germany (ANON, 2009a; International Society for Infectious Diseases, 2008d) and Belgium, and BTV-11 in Belgium (De Clercq et al., 2009; FAVV, 2009). Phylogenetic analysis has shown that both these strains are

genetically almost identical to the seed viruses used for the development of the Modified Live Vaccines (MLVs) produced by Onderstepoort Biological Products, South Africa (International Society for Infectious Diseases, 2008a,c, 2009). BTV-6 and BTV-11 have not previously been reported in Europe and MLVs against them have not been used in Europe. No further reports of these serotypes have been made in Europe in 2009.

1.1.1.2 Bluetongue Virus Transmission and Vectors

Bluetongue virus is not contagious between mammalian hosts, although transmission via infected germplasms has been reported (Sellers and Taylor, 1980) and transplacental transmission has been demonstrated (Gibbs et al., 1979; Worwa et al., 2009). This latter phenomenon was initially thought to be a laboratory artefact of the use of tissue culture passaged viruses (Kirkland and Hawkes, 2004) and was considered to be a rare event in the natural cycle of BTV globally (McLachlan et al., 1992; Melville et al., 1995). Following reports of apparent transplacental transmission of BTV-8 in northern Europe (Menzies et al., 2008), however, this assumption is being reevaluated (Wilson et al., 2008a). Transovarial transmission in *Culicoides* has not been demonstrated (Jones and Foster, 1971; Nunamaker et al., 1990), but BTV RNA fragments have been detected in *Culicoides* larvae and pupae (White et al., 2005). The majority of BTV transmission between ruminant hosts therefore occurs via *Culicoides* vectors [see Figure 1.6 on page 10].

Bluetongue virus infections of ruminants occur throughout temperate and tropical regions of the world, where the distributions of those species of *Culicoides* that are biological vectors of the virus (Du Toit, 1944; Foster et al., 1963) coincident with temperatures that are suitable for virus replication in and transmission by the vectors. Prior to 1998, it was assumed that the only epidemiologically relevant vector in Europe was *C. imicola*. This assumption arose from the fact that the limited epizootics caused by BTV in Europe had been restricted to the areas of Iberia and certain Greek Islands below 40°N, which were known to support populations of *C. imicola* (Baylis, 2002; Purse et al., 2005; Mellor, 2002).

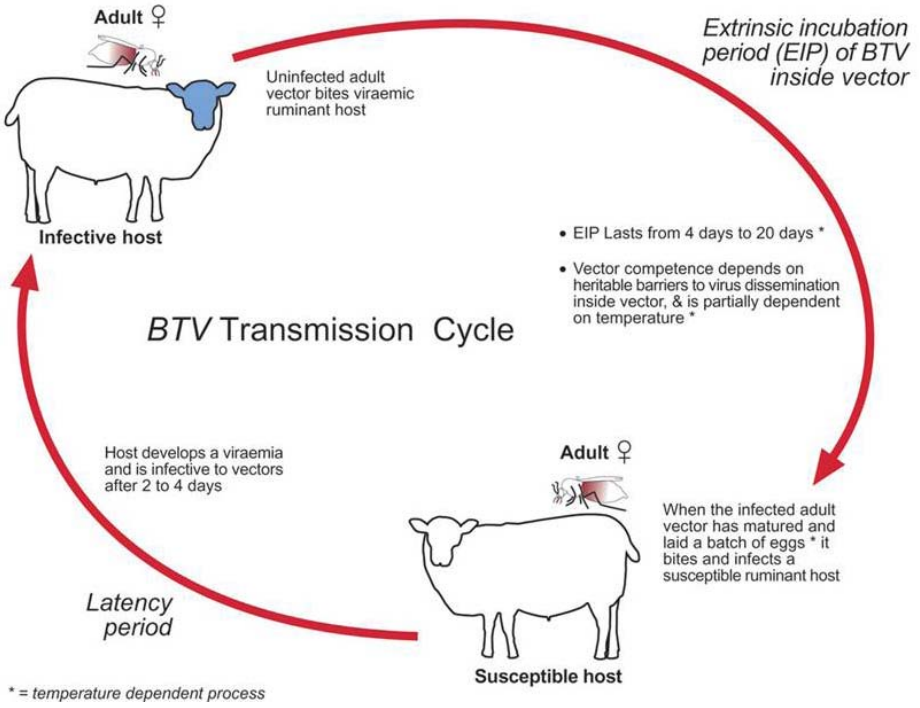


Figure 1.6. A generalised transmission cycle for BTV [reproduced from Purse et al. (2005)]

The dramatic westward and northward expansion from the Mediterranean Basin into mainland Europe, in part due an apparent northwards, climate-mediated expansion of the range of the traditional Afro-Asiatic vector *C. imicola* into southern Europe [for review see Purse et al. (2005)]. Some BTV outbreaks, however, have occurred in areas in the Mediterranean Basin where *C. imicola* has been shown to be absent, either spatially or temporally, including northern Greece, European Turkey, the Balkans (Djuricic et al., 2004; Mellor et al., 1984; Nomikou et al., 2003; Purse et al., 2006) and most recently in northern Europe, where *C. imicola* has never been found (De Deken et al., 2008; EFSA, 2007; Meiswinkel et al., 2007, 2008a,c) [see Figure 1.7 below]. The extension of the 2007 *C. imicola* range displayed in Figure 1.7 (Purse et al., 2008) into Switzerland (46°N) is a result of the collection of a single *C. imicola* (Cagienard et al., 2006). The area in which the *C. imicola* individual was collected has since been resampled and no further specimens of *C. imicola* have been collected suggesting that a locally breeding population of *C. imicola* is not present in Switzerland (Cagienard et al., 2006).

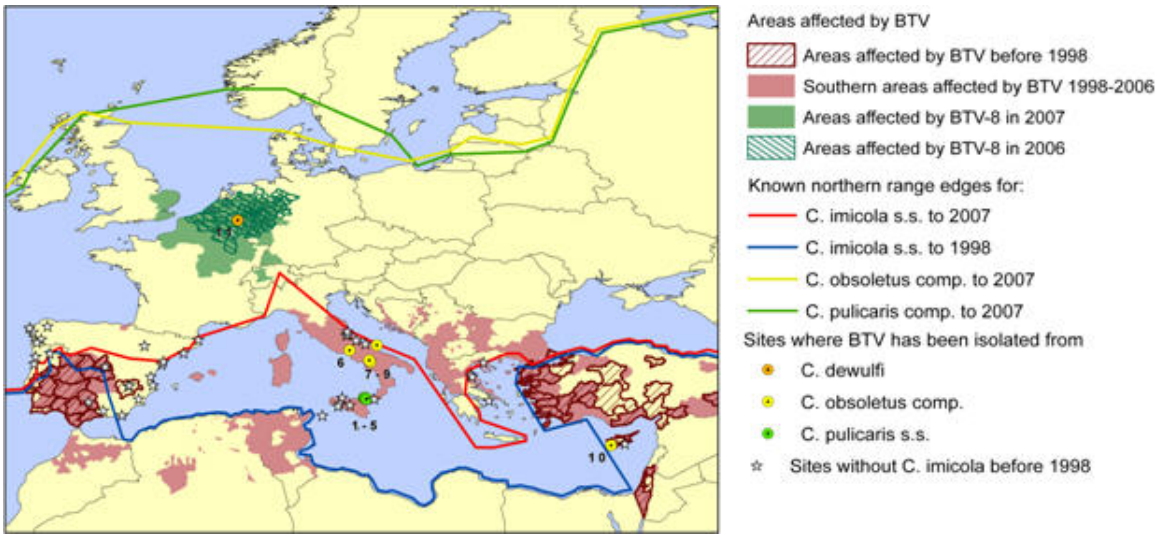


Figure 1.7. The changed distribution of bluetongue virus and its vectors in Europe [reproduced from Purse et al. (2008)]

There is increasing evidence that Palaearctic vectors, which were previously thought to be of minor epidemiological significance are involved in the transmission of BTV in outbreaks occurring in Europe outside the range of *C. imicola* [for review see Carpenter et al. (2006, 2009)]. The vectors that have been suggested to be involved are two species groups *Culicoides obsoletus* and *Culicoides pulicaris* [hereafter referred to as *C. obsoletus sensu lato* (s.l.) and *C. pulicaris* s.l], the females of which are morphologically cryptic. *Culicoides obsoletus* s.l. consists of four species in the UK (*C. obsoletus* Meigen, *C. dewulfi* Goetghebuer, *C. chiopterus* Meigen and *C. scoticus* Downes and Kettle), and falls within the *Avaritia* (Fox) sub-genus. In mainland Europe within this species distribution the Obsoletus group additionally includes *Culicoides montanus* Shakirzjanova (Rawlings, 1997). *Culicoides pulicaris* s.l. consists of two species (*C. pulicaris* Linnaeus and *C. punctatus* Meigen) and falls within the sub-genus *Culicoides* Latreille, although additional species and rearrangement of this group has been suggested (Gomulski et al., 2006; Pages et al., 2009). The role of *C. obsoletus* s.l. and *C. pulicaris* s.l. in BTV transmission in different areas have been inferred by fine scale temporal and spatial overlap of their distributions with outbreaks (Purse et al., 2007). Virus isolations have also been made from wild-caught, parous, non-engorged adults of both species groups (Savini et al., 2005, 2003;

Caracappa et al., 2003) and from laboratory-based infection studies (Carpenter et al., 2006, 2008a; Jennings and Mellor, 1988).

1.1.1.3 Economic Impact and Control of BTV

The total direct costs of the BTV-8 outbreak to the farming industry is uncertain, but have been estimated at €1.1 million for 2006, and in excess of €150 million in 2007 (Hoogendarn, 2007). It is predicted that these costs will continue to rise as the economic impact of reduced trade and the cost of vaccination and surveillance programmes mount. Hence, it is postulated that this BTV-8 epizootic has caused greater economic damage than any previous single-serotype BT outbreak (Wilson et al., 2008b) and as more serotypes threaten northern Europe the need for successful cost-effective control methods grows.

Current control methods for BTV in epidemic situations are targeted primarily at the host through the use of vaccination, combined with other zoosanitary methods, such as animal movement restrictions and destruction [for review see Oya Alpar et al. (2009)]. Suggested vector control strategies include husbandry modifications, including housing BTV-susceptible livestock during periods of maximum adult vector activity (dawn and dusk), habitat modification for the eradication or alteration of breeding habitats, and vector abatement through repellent application, screening, insecticidal spraying etc. (DEFRA, 2009b; EFSA, 2007). Information regarding control efficacy is limited, however, by a lack of basic entomological research in comparison to other well studied vectors, such as mosquitoes. In addition, many previously tested control methods are now incompatible with current environmental and public health regulations regarding pesticide use [for review see Carpenter et al. (2008b)].

1.2 *Culicoides* Bionomics and its Influence on BTV Transmission Risk

The basic reproduction number (R_0) of a disease is defined as the mean number of secondary cases arising from the introduction of a single infected individual to a susceptible population. A disease is able to spread in a host population only if $R_0>1$ (MacDonald, 1952). By quantifying R_0 an assessment of the level of risk posed by a disease can be made, if the relevant parameters can be accurately measured. Gubbins et al. (2008) found 12 parameters were needed to compute R_0, for one vector and two host species, for BTV in the UK, these included the biting rate, extrinsic incubation period (EIP), vector mortality rate, time interval between blood meals, proportion of bites on cattle, vector host species preference, ratio of vectors to cattle, ratio of vectors to sheep, duration of viremea in sheep, disease induced mortality in sheep, disease induced mortality in cattle, probability of transmission from host to vector and probability of transmission from vector to host. The importance of temperature in determining R_0 reflects the number of temperature-dependent processes involved in the transmission of BTV [the biting rate (Carpenter et al., 2008d; Mullens, 1991; Mullens et al., 2004); the EIP (Gerry and Mullens, 2000; Wittmann et al., 2002; Mullens et al., 2004); vector mortality rate (Gerry and Mullens, 2000; Wittmann et al., 2002)]. Robust estimates of vector:host interaction are hard to obtain, however, as *Culicoides* populations vary spatially and seasonally by several orders of magnitude in response to environmental factors. Efforts to quantify the degree of temporal and spatial variation in *Culicoides* populations, in relation to both habitat, climate and host abundance have, however, received increasing interest over the last decade coincident with the increasing importance of BTV in Europe [for review see Purse et al. (2005, 2008)].

Host abundance data are available for the UK both at a coarse scale, for example the FAO Global Livestock Production and Health Atlas (Clements et al., 2002) and at a finer scale from data collected by Department for Environment Food and Rural Affairs (DEFRA) on a per holding basis (DEFRA, 2008b, 2009c; European

Commission, 2000b, 2003). When incorporating host factors into risk models, consideration must be paid to the high level of both spatial and temporal variability in host abundance at the within-farm scale, which will have a significant effect on the vector-host ratio. The livestock composition of an area will also affect both the number of at-risk animals, and depending on their clinical response to infection the time-frame for detection of virus, due to the possible presence of undetected sub-clinical cases (Verwoerd and Erasmus, 2004). Variation at this local-scale has the most significant impact on transmission in areas where competent vectors occur, studies to define environmental factors driving within-farm variation in abundance are therefore important in understanding local-scale transmission of BTV. To achieve this aim it is necessary to examine the bionomics of *Culicoides* and to review parameters that should be quantified to allow a better understanding of BTV transmission.

1.2.1 *Culicoides* Life History

Culicoides are among the smallest of the haematophagous flies, measuring from 1 mm to 3 mm in body-length (Mellor, 2000). They pass through four life stages, egg, larvae, pupa and adult [see Figure 1.8 on page 15] and as poikilotherms, have life cycles that are mediated by temperature. These range from seven days in the tropics to over seven months in some temperate regions, where individuals are thought to diapause usually as fourth instar larvae over the winter period.

In approximately 96 % of *Culicoides* species investigated to date, adult females are obligate blood feeders (Meiswinkel et al., 2004b). Autogeny, the production of egg batches without taking a blood-meal has, however, been observed in at least 30 species of *Culicoides* (Linley, 1983) including, in the UK populations of *C. impunctatus* Goetghebuer (Boorman and Goddard, 1970; Blackwell et al., 1992). There is no evidence to date that any of the arbovirus vectors, including *C. obsoletus* s.l. and *C. pulicaris* s.l., demonstrate autogeny. Host specificity in *Culicoides* is currently poorly defined (Meiswinkel et al., 1994). *Culicoides obsoletus* s.l. is regarded as a livestock-associated species and is considered to feed primarily on cattle (Bartsch

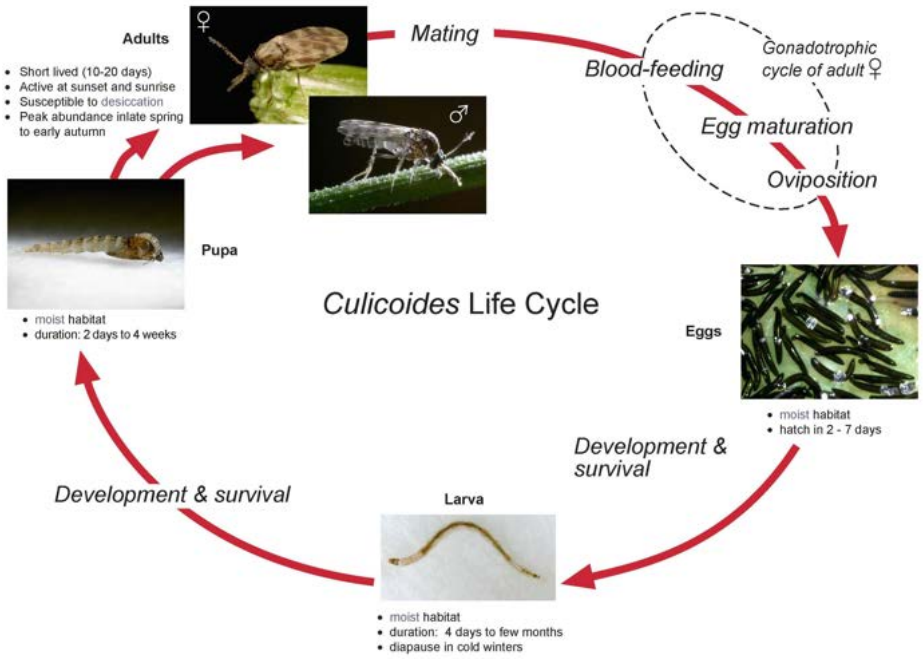


Figure 1.8. ***Culicoides*** **life cycle [reproduced from Purse et al. (2005)]**

et al., 2009; Overgaard Nielsen, 1971), but high biting rates have also been observed on sheep (Carpenter et al., 2008d; Gerry et al., 2009). Evidence has also been provided for *C. obsoletus* s.l. feeding on horses and pigs (Bartsch et al., 2009). The duration of oogenesis after blood feeding varies between species and is substantially influenced by climate, with low temperatures prolonging both oocyte development and subsequent egg deposition (Mullens, 1991; Linley, 1965; Carpenter et al., 2006). Post-oogenesis, female *Culicoides* oviposit from 30 to 250 cylindrical eggs, dependent upon species, size of the female concerned, season and the source of the blood-meal, among other factors. In southern England, the mean duration of the gonadotrophic cycle of field caught *C. obsoletus* s.l. has been found to vary from eight days in April, to five days in May and June (Service, 1968), with eggs laid on average 1 to $2\frac{1}{2}$ weeks after a blood-meal (Hill, 1947; Parker, 1950; Service, 1968). The time interval between blood-meals was found to be a significant in determining the magnitude of R_0 for BTV in the UK (Gubbins et al., 2008). Due to the small size of *Culicoides* and the often vast areas of breeding habitat available observation of oviposition under natural conditions is exceptionally difficult (Kettle, 1977). It is likely, however,

that a wide range of species dependent, exogenous cues, e.g. substrate colour, architecture and olfactory components are used by females to locate suitable oviposition sites that become larval development habitats (Carpenter et al., 2001). In southern England, field-caught *C. obsoletus* s.l. females have been found to oviposit between 14 and 72 eggs in the laboratory (Hill, 1947; Service, 1968). In most species, under favourable temperatures, eggs hatch in a few days, but a few species also over-winter in this stage, including in the UK *C. grisescens* Edwards (Parker, 1950) and *C. vexans* Staegar (Jobling, 1953).

Following hatching, *Culicoides* pass through four semi-aquatic larval instars that utilise a wide diversity of habitats; including marshes, bogs, beaches, swamps, tree holes, soil, animal dung and rotting fruit and other vegetation [for review see Europe: Kettle and Lawson (1952); Nielsen (1964); North America: Jamnback (1965); South and Central America: Mercer et al. (2003); Rowley (1967); Vitale et al. (1981); Australia: Kettle and Elson (1976); Russia: Glukhova (1968, 1969, 1971)]. Although the range of habitats occupied by *Culicoides* larvae is diverse, the preferences of individual species is more restricted, with interrelated environmental factors governing larval presence and abundance within these habitats (Battle and Turner, 1972; Blackwell et al., 1994, 1999; Kardatzke and Rowley, 1971; Schmidtmann et al., 1998). A variety of breeding sites have been identified for British *Culicoides* species [for review see Kettle and Lawson (1952)], but their preferences have generally been very poorly studied in comparison to other vector groups.

The larval developmental time in *Culicoides* varies widely both within and between species, ranging from four to five days, to several months (Kettle, 1984; Meiswinkel, 1989). In contrast to the larval stage, the pupal period for British species appears almost uniformly short, lasting for only two to three days but occasionally three to four weeks, depending on species and ambient temperature (Mellor, 2000; Wittmann, 1999). The seasonal emergence of adults is both species-specific and dependent upon environmental variables. Mating usually occurring 12 to 24 hours after the adults emerge (Downes, 1955; Mair and Blackwell, 1998) and both obligate and faculta-

tive swarming mating behaviour have been observed in *Culicoides* (Downes, 1955; Glukhova and Dubrovskaya, 1974).

Adult lifespan rarely exceeds 20 days (Mellor et al., 2000), but can last for up to 90 days under laboratory conditions at cooler temperatures (Goffredo et al., 2004). Some species in temperate climates and particularly those at the northern edge of their range are univoltine, e.g. *C. biguttatus* Coquillett (Zimmerman and Turner, 1983), while the majority of species have several generations a year. *Culicoides obsoletus* s.l. and *C. pulicaris* s.l. have both been described as bivoltine in Wales (McCall and Trees, 1993) and trivoltine in southern England (Holmes and Boorman, 1987). Habitat use by adult *Culicoides* is driven by their need to find a host, oviposition sites and avoid desiccation, with activity increasing when conditions are warm, still and humid (Carpenter et al., 2008d; Gerry et al., 2009) or in the presence of a host (Kettle, 1951). Adult resting areas, however, remain poorly defined (Bishop et al., 1995).

1.2.2 Biological Factors Underlying BTV Transmission, and their use in Vector and Outbreak Prediction

Pavloskiy (1966) first introduced the concept of nidality (or focality) of vector-borne disease, where pathogens are restricted by climatic and non-climatic factors even within the distributions of their vectors, providing the foundation for the emergence of the contemporary science of landscape epidemiology (Galuzo, 1975). Under this concept, diseases may be associated with distinct landscape features or ecological settings where vector, host and pathogen intersect within a permissive climate [for review see Reisen (2010)]. BTV occurrence, for example, may be delineated spatially by landscape features (Guis et al., 2007), however, outbreaks will only arise where climatic conditions are permissive to both virus replication (Mullens et al., 1995; Wittmann and Baylis, 2000) and vector activity [for review see Mellor et al. (2000)]. Quantification of these constraints would allow accurate predictions of vector abundance and/or distribution to be made, providing critical support for decision making, including the development of a control strategy to minimise exposure risk.

In the case of Lyme disease, for example, risk models have identified those components of landscape structure associated with increased risk of peridomestic contact with ticks, allowing the development of a framework for managing the risk of Lyme disease transmission using landscape and residential planning and design (Jackson et al., 2006; Ward and Brown, 2004).

The climatic and non-climatic drivers of BTV transmission, are common to many other vector-borne disease systems, where key transmission events, such as vector biting rates, are modulated by temperature and moisture availability. Remote sensing techniques in particular lend themselves to defining environmental correlates, over large areas at a range of spatial scales. The application of remote sensing to describing and/or predicting the distribution and abundance of disease vectors generally involves three processes. Firstly, remotely sensed data are used to provide information on land cover, climatic conditions and other environmental characteristics. Secondly, the spatial and/or temporal distribution and abundance of a vector and/or pathogen are quantified in the region of interest. Then, thirdly, these variables are related to identify significant species-environment relationships, which may be used to infer distribution and/or abundance in unsampled areas. One of the first such uses of remotely sensed data was the use of colour and colour-infrared aerial photography to map the larval habitats of *Aedes sollicitans* Walker, a salt-marsh mosquito and a vector of eastern equine encephalitis virus (NASA, 1973). This was possible through the identification of several key indicator plant species, which were identifiable remotely using technology available at that time, to predict the presence of breeding habitats. Since then links between specific climatic/habitat situations and the abundance and distribution of a variety of arthropod vectors have been described [see Table 1.2 on page 22].

While climatic requirements for virus replication are relatively straightforward to define, numerous interactions exist between vectors and their environment. Despite this complexity, the existence of significant species-environment relationships have allowed the presence-absence of *C. imicola* within Europe to be modelled using cli-

matic correlates derived from both ground-based meteorological data (Conte et al., 2003; Sellers and Mellor, 1993; Wittmann et al., 2001) and through remotely-sensed correlates of temperature and moisture availability (Baylis and Mellor, 2001; Purse et al., 2004a, 2006; Tatem et al., 2003). Attempts have also been made to define similar species-environment relationships for the Palaearctic vectors, *C. obsoletus* s.l. (Calvete et al., 2008; Conte et al., 2007a; Purse et al., 2004b) and *C. pulicaris* s.l. (Purse et al., 2004b).

Early in the emergence of BTV across Europe, models driven by climatic correlates were often trained on distributional data for *C. imicola* from individual countries and then used to predict and explain 'continental-scale' patterns in BTV transmission (Tatem et al., 2003; Wittmann et al., 2001). These models performed poorly when validated with independent data from Italy (Pili et al., 2006; Calistri et al., 2003) failing to accurately predict the distribution of *C. imicola* in Sardinia (Pili et al., 2006) and mainland Italy (Calistri et al., 2003), both at the regional and local level. Similarly, the model developed for the Mediterranean Basin by Baylis and Mellor (2001) failed to predict *C. imicola* distribution and abundance in Sicily and the southern regions of mainland Italy, but did achieve a good distributional fit for Sardinia and west-central Italy when compared to independent data (Calistri et al., 2003). Regional level models developed by Conte et al. (2003) successfully predicted *C. imicola* distribution when compared to field-collected data at a 'regional-level' (Pili et al., 2006), but the key variables used to predict the distribution: annual mean daily minimum temperature, annual minimum relative humidity and average altitude; were quite uniform when compared across sub-regions and failed to explain the relative differences in abundance of *C. imicola* observed at this more 'local-level'. This indicates the importance of additional factors not considered within these studies for predicting *Culicoides* abundance at more local-scales.

Culicoides are considered to be habitat generalists, with larval development occurring in a range of moist habitats that are ubiquitous across farmland (Kettle and Lawson, 1952). At a local-scale however, the distribution, abundance, and the specific

composition and arrangement of these moist habitats significantly influences the characteristics of the local vector population. Hence, attempts have been made to define distribution patterns of *Culicoides* and BTV outbreaks using models that incorporate measures describing these influences. These have included ecological correlates of climate, together with landscape structure (Guis et al., 2007), terrain and soil factors (Conte et al., 2007a) and/or host factors (Calvete et al., 2008; Guis et al., 2007).

1.3 How Can the Modelling of Orbivirus Outbreaks be Improved?

One of main limiting factors of the models predicting *Culicoides* abundance and/or distribution is the quality of the entomological data available to train and test them. No amount of statistical manipulation or ecological descriptors can overcome sampling biases or inadequate scale created by poor data collection (Reisen, 2010). Effective sampling protocols require the use of suitable tools to provide representative estimates of vector populations per unit area or per host within an area, and also a method of distributing sampling points in order to encompass a broad range of climatic and environmental conditions (Reisen and Lothrop, 1999). Currently no systematic attempts at sampling *Culicoides* abundance at the within-farm scale have been made. At present, all available *Culicoides* abundance and distribution datasets in Europe are derived from collections made at night using light traps, which are often based on just one or two trap nights at single locations, widely distributed across a country [a 45 km by 45 km sampling grid was recommended by the European Union for surveillance (European Commission, 2000a, 2007)]. Within Europe, the Onderstepoort Veterinary Institute (OVI) 8 w Ultra-Violet (UV) light-suction trap (Agricultural Research Council, South Africa) has been recommended as the 'gold-standard' for *Culicoides* surveillance (EFSA, 2007; Mellor and Hamblin, 2004) and although successful in often capturing large numbers of adult *Culicoides* (Meiswinkel, 1998), OVI traps may provide a biased estimate of the total flying population, or of host-seeking activity of key potential BTV vector species (Carpenter et al., 2008d). Conditions of operation,

such as trap height (Venter et al., 2009a), may also significantly affect the accuracy with which *Culicoides* populations are reflected in trap catches, and in turn have implications for the accuracy of model predictions based on these population estimates. The abundance of host-seeking female *Culicoides* are of particular relevance to estimates of BTV transmission risk, as male *Culicoides* do not take blood meals from vertebrates and consequently have no potential to vector BTV between hosts.

The majority of haematophagous biting flies must locate hosts that are, in many cases, mobile, difficult to find, both in space and time, and have themselves evolved defenses against insect attack (Gibson and Torr, 1999). To achieve this they use a wide variety of visual, olfactory, gustatory and physical stimuli to both locate and select their hosts. The host-orientated behaviour of each species is adapted to the specific surrounding biotic and abiotic conditions [for review see Allan et al. (1987), Birkett et al. (2004) and Gibson and Torr (1999)]. Utilisation of host derived semiochemicals as trap baits would provide an effective method for sampling the host-seeking population in a host-equivalent manner. In comparison to other haematophagous insects, and in particular mosquitoes, the responses of *Culicoides* to host odours are, however, poorly understood and further investigation is required prior to their use for estimating Palaearctic vector populations.

Vector Bionomic	Ecological Variable	Satellite Sensor and Environmental Proxies	Vector Species	Reference
Larval and	Open Water	NOAA-AVHRR: NDVI	*Aedes* and *Culex sp.*	(Linthicum et al., 1990)
Pupal Habitats		SPOT XS: Multispectral mode, Band 3	*Anopheles gambiae*	(Thomson et al., 1996)
		SPOT XS: Supervised Classification	*Anopheles pseuopunctipennis* and *Anophles freeborni*	(Roberts et al., 1996)
	Rice paddies and Waterways	Landsat 7 ETM+: Supervised Classification	*Anopheles* sp.	(Diuk-Wasser et al., 2004; Masuoka et al., 2003; Sithiprasasna et al., 2005; Mushinzimana et al., 2006)
		IKONOS: Supervised Classification	*Anopheles* sp.	(Masuoka et al., 2003; Mushinzimana et al., 2006)
	Water under Mangroves	Side Aperture Radar	*Aedes vigilax*	(Wood et al., 1991a,b, 1992)
	River Margins	SPOT XS: Unsupervised Classification	*Anopheles Albimanus*	(Rejmankova et al., 1995)
	Swamp / Forest	SPOT XS: Unsupervised Classification	*Anopheles vestitipennis* and *Anopheles punctimacula*	(Rejmankova et al., 1998)
	Soil Type	NOAA-AVHRR: NDVI, LST	*Phlebotomus orientalis*	(Thomson et al., 1999)
	Soil Temperature	NOAA-AVHRR: NDVI	*Oestris ovis*	(Flasse et al., 1998)
	Wetlands	ASTER: NDVI, DWSI, EO-1 Hyperion: NDVI, DWSI, CMS	*Anopheles punctipennis*	(Brown et al., 2008)
Nymphal	Broad Leaf Forest / Grassland	Landsat TM: Tassel Cap Transformation Indices	*Ixodes scapularis*	(Dister et al., 1997)
Development	Vegetation Wetness and Greenness	NOAA-AVHRR: NDVI	*Ixodes scapularis*	(Kitron and Kazmierczak, 1997)
Adult	Soil Moisture and	Landsat TM: Band 7	*Glossina pallidipes*	(Kitron et al., 1996)
Abundance	Vegetation Moisture		*Anopheles gambiae*	(Thomson et al., 1996; Thompson et al., 1997)
and/or Distribution	Saturation Deficit	NOAA-AVHRR: NDVI	*Glossina fuscipes*	(Rogers and Randolph, 1991)
			Glossina morsitans	(Rogers and Randolph, 1991)
	Water Table	Landsat TM: Supervised Classification	*Anopheles* and *Culex* sp.	(Sharma and Srivastava, 1997)
	Open Water, Vegetation, Settlements	Landsat TM: Supervised Classification	*Culex*, *Aedes*, *Anopheles* and *Culiseta* sp. etc.	(Moncayo et al., 2000)
	Vegetation	NOAA-AVHRR: NDVI	*Ixodes dammini*	(Kitron and Kazmierczak, 1997)
	Vegetation and Social Factors	MODIS: NDVI and HDI	*Schistosoma mansoni*	(Malone et al., 1997)
	Snail Habitat	NOAA-AVHRR: NDVI	*Phlebotomus papatasi*	(Cross et al., 1996)
		Landsat 5 TM: NDVI and Tasselcap Transformation Indices	*Oncomelania hupensis*	(Jia-Gang et al., 2005)
	Tick Habitat	Landsat 7 ETM+: NDVI and Tasselled Cap Transformation Indices	*Ixodes scapularis*	(Ogden et al., 2006)
	Biotic Factors	NOAA-AVHRR: NDVI, LST, MIR, VPD, DEM, TAIR	*Culicoides imicola* s.s.	(Baylis et al., 1998; Baylis and Rawlings, 1998; Tatem et al., 2003; Purse et al., 2004b,a)

Table 1.2. Examples of remotely sensed environmental variables found to be related to vector bionomics (a list of abbreviations can be found on page xviii)

1.3.1 How do *Culicoides* Locate their Hosts?

By far the most commonly recorded semiochemical used as an olfactory cue in host location is carbon dioxide (CO_2) [for review see Gibson and Torr (1999), Gillies (1980) and Guerenstein and Hildebrand (2008)]. When CO_2 is exhaled in the breath of a host animal, along with other semiochemicals, it forms a filamentous plume whose dispersion pattern is used by many haematophagous insects for host location [for review see Murlis et al. (1992)]. There is also widespread evidence that other host kairomones, such as 1-octen-3-ol and acetone, can have synergistic, and reciprocal, impacts when combined with CO_2, increasing the effective range (i.e. distance) of attraction of CO_2 plumes for haematophagous insects [for example, Tsetse (Torr, 1990) and mosquitoes (Geier et al., 2000)]. In *Culicoides*, Nelson (1965) first described the importance of CO_2 as an attractant. Since then responses to a variety of other semiochemicals have been implicated in host location by *Culicoides* [see Table 1.3 on page 24]. *Culicoides* exhibit both inter- and intra-specific variation in host preference (Kettle, 1977; Schmidtmann et al., 1980a) and in the importance of blood in their diet (Boorman and Goddard, 1970; Blackwell et al., 1992; Linley, 1983). Perhaps as a result of this, the degree of synergism between CO_2 and other semiochemicals given off by hosts differs between *Culicoides* species [see Table 1.4 on page 25].

Historically, techniques to improve the efficiency of light traps have been based on the addition of semiochemical baits, to increase the range and/or number of haematophagous insects collected. Traps baited with semiochemicals, predominantly CO_2, in combination with light or as the sole attractant, have a long history of use with hematophagous insects, particularly in the USA where they are commonly used as part of wider mosquito-borne arbovirus surveillance campaigns [for example West Nile Virus (WNV) (Lukacik et al., 2006; Andreadis et al., 2001)]. In general, semiochemical-baited traps used without a light source have the advantage of collecting only the host-seeking proportion of the adult population. They also collect species with diurnal activity patterns as well as those that are nocturnal or crepuscular and have the potential to be more easily standardised against live animal hosts (Gillies

and Wilkes, 1969, 1970). They, however, tend to be highly selective in species sampled, according to the semiochemical(s) used as bait and how these are representative of preferred vertebrate host species (Takken and Knols, 1999). In comparison to other haematophagous insects, and in particular mosquitoes, the responses of *Culicoides* to host odours are poorly understood, and further investigation is required if they are to be used to improve estimates of Palaearctic vector abundance.

Semiochemical	***Culicoides* Species**	**Reference**
Carbon dioxide	*C. variipennis sonorensis* Wirth and Jones	(Gerry and Mullens, 1998; Mullens, 1995; Nelson, 1965)
	C. impunctatus Goetghebuer	(Bhasin et al., 2000b, 2001)
	C. furens Poey	(Grant and Kline, 2003; Kline et al., 1994)
	C. stellifer Coquillet	(Grant and Kline, 2003)
	C. mississippiensis Hoffman	(Cilek and Kline, 2002; Grant and Kline, 2003)
	C. barbosai Wirth and Blanton	(Cilek and Kline, 2002)
	C. hollensis Melander and Brues	(Kline et al., 1994)
	C. brevitarsis Kieffer	(Bishop et al., 2008)
	C. melleus Coquillet	(Kline et al., 1994)
	C. histrio Johannsen	(Ritchie et al., 1994)
	C. subimmaculatus Lee and Reye	(Ritchie et al., 1994)
1-octen-3-ol	*C. impunctatus* Goetghebuer	(Bhasin et al., 2000a; Blackwell et al., 1996)
	C. nubeculosus Miegen	(Bhasin et al., 2000a)
	C. furens Poey	(Kline et al., 1994)
	C. hollensis Melander and Brues	(Kline et al., 1994)
	C. melleus Coquillet	(Kline et al., 1994)
Lactic acid	*C. impunctatus* Goetghebuer	(Bhasin et al., 2000a)
(2-hydroxypropanoic acid)	*C. imicola* Kieffer	(Sollai et al., 2007)
Acetone	*C. impunctatus* Goetghebuer	(Bhasin et al., 2000a)
(dimethyl ketone; 2-propanone)	*C. nubeculosus* Miegen	(Bhasin et al., 2000a)
Butanone	*C. impunctatus* Goetghebuer	(Bhasin et al., 2000a)
(methyl ethyl ketone)	*C. imicola* Kieffer	(Sollai et al., 2007)
Phenolic components of urine:		
(*i*) 3-methylphenol, 4-methylphenol and 4-ethylphenol	*C. impunctatus* Goetghebuer	(Bhasin et al., 2000a, 2001)
(*ii*) phenol, 3-ethylphenol, 3-*n*-propylphenol	*C. impunctatus* Goetghebuer	(Bhasin et al., 2001)
(*iii*) 3-*n*-propylphenol	*C. impunctatus* Goetghebuer	(Bhasin et al., 2000a)

Table 1.3. Semiochemicals reported as behaviourally active in host location in *Culicoides*

Semiochemical Bait Combination	*Culicoides* Species	Effect on Trap Catches (Compared to Comparison Bait)		Comparison Semiochemical Bait	Reference
		Fold Increase	Decrease		
CO_2 with 1-octen-3-ol					
	C. furens Poey	3.4		CO_2 alone	(Kline et al., 1994)
	C. hollinensis Melander and Brues		Slightly Repellent	CO_2 alone	(Kline et al., 1994)
	C. melleus Melander and Brues		Slightly Repellent	CO_2 alone	(Kline et al., 1994)
	C. brevitarsis Kieffer	6.2		CO_2 alone	(Bishop et al., 2008)
	C. brevitarsis Kieffer	72		1-octen-3-ol alone (zero catch)	(Bishop et al., 2008)
CO_2 ($0.2\,ml{\cdot}h^{-1}$) with:					
Acetone	*C. impunctatus* Goetghebuer	4.7		CO_2 ($0.2\,ml{\cdot}h^{-1}$) alone	(Bhasin et al., 2001)
1-octen-3-ol	*C. impunctatus* Goetghebuer	6.2		CO_2 ($0.2\,ml{\cdot}h^{-1}$) alone	(Bhasin et al., 2001)
Cow urine	*C. impunctatus* Goetghebuer	9.2		CO_2 ($0.2\,ml{\cdot}h^{-1}$) alone	(Bhasin et al., 2001)
CO_2 ($0.2\,ml{\cdot}hour^{-1}$) and phenolic components of urine with:					
Acetone ($23.0\,mg{\cdot}h^{-1}$)	*C. impunctatus* Goetghebuer	22		CO_2 ($0.2\,ml{\cdot}h^{-1}$) alone	Bhasin et al. (2001)
1-octen-3-ol ($0.06\,mg{\cdot}h^{-1}$)	*C. impunctatus* Goetghebuer	24		CO_2 ($0.2\,ml{\cdot}h^{-1}$) alone	Bhasin et al. (2001)
CO_2 ($500\,ml{\cdot}min^{-1}$) with 4:1:8 mix (1-octen-3-ol, 3-*n*-propylphenol, 4-methylphenol) at $8.4\,mg{\cdot}h^{-1}$					
	C. barbosai Wirth and Blanton	100		1-octen-3-ol: phenol 4:1:8 mix ($8.4\,mg{\cdot}h^{-1}$)	(Cilek and Kline, 2002)
	C. mississippiensis Hoffman	100		1-octen-3-ol: phenol 4:1:8 mix ($8.4\,mg{\cdot}h^{-1}$)	(Cilek and Kline, 2002)
	C. melleus Coquillet	25 to 46		1-octen-3-ol: phenol 4:1:8 mix ($8.4\,mg{\cdot}h^{-1}$)	(Cilek and Kline, 2002)
	C. barbosai Wirth and Blanton	3		CO_2 alone ($500\,ml{\cdot}min^{-1}$)	(Cilek and Kline, 2002)
	C. mississippiensis Hoffman	3		CO_2 alone ($500\,ml{\cdot}min^{-1}$)	(Cilek and Kline, 2002)
	C. melleus Coquillet	1.1		CO_2 alone ($500\,ml{\cdot}min^{-1}$)	(Cilek and Kline, 2002)
CO_2 ($200\,ml{\cdot}min^{-1}$) with:					
1-octen-3-ol ($4.0\,mg{\cdot}h^{-1}$)	*C. furens* Poey	3.4 to 11.9		CO_2 alone ($200\,ml{\cdot}min^{-1}$)	(Kline et al., 1994)
1-octen-3-ol ($40.0\,mg{\cdot}h^{-1}$)	*C. furens* Poey	22.8 to 35.7		CO_2 alone ($200\,ml{\cdot}min^{-1}$)	(Kline et al., 1994)

Table 1.4. Evidence for synergistic effects in the responses of *Culicoides* to semiochemicals (where available release rates used are given in parenthesis)

1.4 Summary and Project Aims

BTV transmission in northern Europe, is thought to be carried out primarily via the Palaearctic vector groups, *C. obsoletus* s.l. and *C. pulicaris* s.l., the cryptic nature of these species groups has hampered the acquisition of detailed ecological knowledge required for parameterisation of BTV risk models. New molecular-based techniques for the identification of survey collections of these insects now provide a tool to develop species-specific distribution, abundance and habitat use datasets. Tools to sample accurately the host-seeking segment of a population of livestock-associated *Culicoides* species are, however, still lacking.

Within the climatic range of virus replication, the distribution of BTV epidemics is dependent almost entirely upon the distribution and abundance of vectors, and in particular upon local-scale variation in vector abundance that produces variation in vector-host ratios (Gubbins et al., 2008). A greater understanding of vector-environmental relationships will enable us to relate *Culicoides* demographic rates and carrying capacities of different habitats to the risk of BTV transmission within those habitats. Identification and quantification of the wide spatial variation in abundance of adult and larval life-stages of *Culicoides* vectors at the local within-farm scale, in relation to measurable ecological factors is therefore essential for developing targeted control strategies and for assessing risk of BTV transmission. To achieve this the project will investigate the relationship between a range of ecological correlates (both climatic and non-climatic) and the distribution and abundance of adult and larval *Culicoides* populations at a local 'farm-level' in southern England. In particular the following hypotheses will be investigated:

(a) Predictive mapping techniques for *Culicoides* abundance and BTV outbreak presence-absence at regional-level in mainland Europe can be improved by integrating remotely sensed climatic correlates with non-climatic ecological correlates and the availability of hosts into models.

(b) The larval *Culicoides* habitat characteristics, defined both remotely and directly, within farms in south east England may be used to predict their occurrence and abundance at the within- and between-farm scales.

(c) Semiochemical-based trapping techniques can be used to provide representative estimates of the adult livestock-associated *Culicoides* abundances in south east England.

(d) Adult *Culicoides* distribution and abundance can be explained through remotely sensed and ground-based measures of their ecological and host requirements within-farms in south east England and used to predict their occurrence and abundance at the within- and between-farm scale.

(e) Through understanding those factors driving *Culicoides* abundance at the local-scale, design of population estimation and targeted vector control can be improved.

These hypotheses will be addressed within the project by first investigating the drivers of BTV outbreak occurrence and vector abundance at a regional level (1 km scale) in mainland Europe [Chapter 3]. Then by carrying out detailed, seasonal ecological surveys of immature [Chapter 4] and adult [Chapter 6] *Culicoides* populations, within their farmland habitats in south east England, assessed to species level within the morphologically cryptic Obsoletus species group. This includes the development of a semiochemical-based trapping technique that can be used to sample representatively adult host-seeking *Culicoides* at the within-farm scale [Chapter 5]. Chapter 7 draws these lines of investigation together to assess the potential for any of the identified local-scale drivers of *Culicoides* abundance to be utilised in BTV risk assessments, and in the development of future vector control strategies.

Chapter 2

Materials and Methods

The following methods describe techniques that are common to more than one investigation within this project. Additional material and methods are presented in the individual chapters to which they are relevant.

2.1 Study Area and Farm Sites

Seasonal entomological surveys were conducted on six study farms in south east England (labelled A-F) [see Appendix B on page 258], farms A, B, C, D, E and F were used in Chapter 4, farm A was used in Chapter 5, and farms A, D, E and F were used in Chapter 6. These farms were chosen to encompass the range of the environmental factors and farming practices hypothesised to influence the distribution and abundance of livestock associated *Culicoides* species. One farm kept cattle only (farm E), one farm kept sheep only (farm C) and, on the remaining, four farms both cattle and sheep were kept (farms A, B, D and F) [see Table 2.1 below]. Two of the farms are certified as organic by the Soil Association (Farms D and E).

Farm	**British National Grid**		**Approximate Acreage of**				**Livestock**
ID	OS X	OS Y	Farm Holding	Arable	Grassland	Woodland	
A	450464	179011	2454	1070	670	95	Sheep [60], Cattle [480]
B	456102	192389	740	247	212	247	Sheep [450], Cattle [20]
C	457518	191549	225	201	23	<1	Sheep [470]
D	458180	172593	775	300	325	100	Sheep [950], Cattle [95]
E	487030	200167	365	60	240	60	Cattle [70]
F	458153	153426	5000	3570	460	330	Sheep [800], Cattle [180]

Table 2.1. Farm Characteristics (type and approximate number of livestock of each type present on each farm shown in parenthesis)

2.2 Trapping Protocols for the Collection of *Culicoides*

2.2.1 Collection of Adult *Culicoides* at Light-Baited Traps

Collections of adult *Culicoides* at light in Chapters 3 and 5 were made using OVI type down draught suction motor traps lit with an 8W UV tube (Agricultural Research Council, South Africa) [see Figure 2.1 below]. Traps were suspended at a height of 1.5 m to 2.0 m above the ground. Attracted insects were collected into a 500 ml beaker containing approximatly 100 ml of water with a small drop of detergent, suspended below the trap by a white terylene netting sock with a mesh aperture of <0.25 mm. All samples were collected immediately after the trapping period [generally overnight covering at least one hour before sunset to two hours after sunset, except in Chapter 5 where the trapping period lasted for three hours at dusk (one hour before sunset to two hours after sunset)]. The contents of each collecting pot were passed through a fine mesh sieve (aperture of <0.25 mm). Insects retained in the sieve were washed using 70 % ethanol into 250 ml straight-side wide mouth polypropylene sample jars and topped up with 70 % ethanol sufficient to cover the sample for storage prior to identification.

Figure 2.1. Onderstepoort Veterinary Institute (OVI) 8 w **UV light-suction trap (Agricultural Research Council, South Africa)**

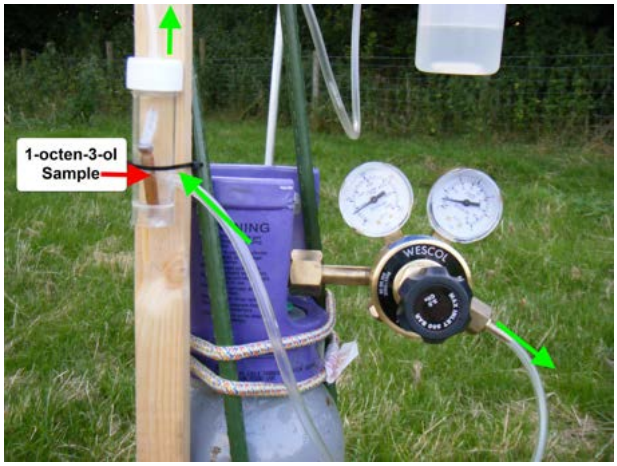
2.2.2 Collection of Adult *Culicoides* using Semiochemical-Baited Traps

Collections of adult *Culicoides* using semiochemical baits in Chapters 5 and 6 were made using miniature Center for Disease Control traps [hereafter referred to as CDC traps] (Model 512, J.W. Hock, Gainsville, Florida). Traps were used unlit (i.e. without the use of the incandescent bulb) powered by D-cell battery adapters (J.W. Hock, Gainsville, Florida) with 1-octen-3-ol and/or CO_2 combinations. Traps were suspended approximately 0.8 m above the ground using metal crooks (Gardman, Spalding, UK). Insects were collected into approximately 100 ml of water containing a small drop of detergent in a 500 ml collecting jar suspended below the trap by a metal mesh sleeve (mesh aperture <0.25 mm) (J.W. Hock, Gainsville, Florida).

CO_2 was supplied to each trap from either 14.5 kg compressed gas cylinders (Air liquide UK, Birmingham, UK) or from 6.5 kg compressed gas cylinders (BOC Sureserve, Manchester, UK), fitted with 4 bar two stage CO_2 regulators (C.S. Milne, Leicester, UK), through Tygon R3603 laboratory tubing with a 4.8 mm internal diameter (i.d.) (Saint-Gobain Performance Plastics, USA). The CO_2 was passed via a sealed polypropylene universal [height: 9.0 cm, diameter: 2.3 cm] containing the 1-octen-3-ol sample. The 1-octen-3-ol samples were held in 0.8 ml amber borosilicate vials (Chromacol, Welwyn Garden City, UK). Each vial had a 40 mm cotton pipe cleaner wick fitted through a 1 mm holed cap, with 20 mm of wick exposed [see Figure 2.2c on page 31]. The flow rate was controlled after CO_2 had been combined with any other semiochemical(s) used in the universal described above. The final release rate of the CO_2 was controlled using a Planton valved flow meter (Roxspur, Sheffield, UK), using models GRV13 (flow rate range 100 $cm^{-3} \cdot min^{-1}$ to 1000 $cm^{-3} \cdot min^{-1}$) and GRV14 (flow rate range range 250 $cm^{-3} \cdot min^{-1}$ to 2500 $cm^{-3} \cdot min^{-1}$). A correction factor of 0.811 was applied to the flow rate readings, since the flow meters are calibrated for air rather than CO_2. The final release point of the odour stream was positioned approximately 10 cm away from the trap entrance on the underside of the rain cover of the trap [see Figure 2.2 on page 31]. All samples were collected immediately after the trapping period had finished. The contents of each collecting pot were passed

through a fine mesh sieve (aperture of <0.25 mm), insects retained in the sieve were washed using 70 % ethanol into 250 ml straight-side wide mouth polypropylene sample jars and topped up with 70 % ethanol sufficient to cover the sample for storage prior to identification.

(a). **Trap Connections** (b). **Odour Stream Release Point**



(c). **CO_2 Multistage Regulator and Odour Mixing Chamber**

Figure 2.2. Trap design for the collection of adult *Culicoides* using semiochemical-baited CDC traps (green arrows in (c) indicate direction of CO_2 flow)

2.2.3 Collection of Adult *Culicoides* using a Sheep-Baited Drop Trap

A host baited drop trap was used in Chapter 5 for the collection of adult *Culicoides* attracted to and in the immediate vicinity of a host. Traps were baited with 30 kg to 40 kg Poll Dorset sheep, a dual mutton and fine wool breed. The drop trap was based on a design modified from Carpenter et al. (2008d) [see Figure 2.3 on page 33]. The drop trap cage had a rectangular cross section, measuring 3.5 m long by 2.5 m wide by 2.0 m high, constructed from timber and covered by white fabric netting, with a mesh aperture of <0.25 mm. The net roof of the cage was fixed, but the sides could be raised and lowered. The bottom edge of each side was weighted with 22 mm copper pipe to help secure the netting to the ground once the sides had been lowered. When raised the sides were secured using 20 mm wide Velcro® straps (Velcro, UK). A second smaller 'sampling' corral within the netted frame was used to confine the sheep (2.2 m long by 1.5 m wide) and connected to the large (approximately 170m^2) 'holding' corral by a gate [see Figure 2.3 on page 33].

On each sampling day, 10 sheep were randomly selected, from a flock of 32, and herded into the 'holding' corral. Selected sheep were allowed to acclimatize within the 'holding' coral for 10 minutes prior to the first sampling period. A single sheep was selected at random from the 10 in the holding corral and moved to the sampling coral (with the netted sides of the cage raised) [see Figure 2.3c on page 33]. The sheep was free to move within the confines of the sampling corral for 10 minutes (the exposure period). During this period all humans moved >100 m from the cage to prevent any interaction/competition between sheep and human odours. All other sheep in the field were >150 m from the cage and corrals throughout the experiment. After 10 minutes the netted sides of the trap were dropped [see Figure 2.3d on page 33], trapping any *Culicoides* present on, or in close proximity to, the sheep. All visible *Culicoides* in the cage were collected and placed into pillboxes (Watkins and Doncaster, UK) using an aspirator, with a maximum collection time of 15 minutes. After this time the cage sides were raised and the sheep returned to the holding coral. The netting was shaken to remove remaining insects and, if required, the underside

of the netted roof was swept. At least 20 minutes passed before replicates of the exposure period could take place. Exposure periods were limited to the dusk period [approximately 6.30pm to 8.30pm] when *Culicoides* were active and could be seen by the collector.

(a). **Drop Trap Setup**

(b). **Holding Corral**

(c). **Sampling Corral - Sides Up**

(d). **Sampling Corral - Sides Down**

Figure 2.3. Sheep baited drop trap setup

Pillboxes containing sampled *Culicoides* were placed within a sealed 2-litre leak-proof polypropylene container with an 'O' ring lid (DGP PATHOPAK Bio-Bottle, York, UK). This contained an unsealed 20 ml universal tube enclosing a cotton wool ball soaked in chloroform. Pillboxes were left in the container for a minimum of 20 minutes to kill the *Culicoides*. The contents of each pillbox were then transferred into 250 ml straight-side wide mouth polypropylene sample jars and filled with sufficient 70 % ethanol to cover the sample for storage prior to identification.

2.2.4 Collection of Larval *Culicoides*

Within Chapter 4 substrates were examined directly for the presence of *Culicoides* larvae by removing a randomly selected 10 cm by 10 cm sample of substrate using a shallow scoop trowel with a depth of 10 cm where possible. Extraction of substrate at sampling points was randomised by using a 50 cm by 50 cm quadrant divided into 25 equal (10 cm by 10 cm) divisions (Model Q2, Alana Ecology, Bishop's Castle, UK) and one square selected for sampling from the quadrant using a randomly generated number list. Substrate samples were transported from the field to the laboratory in individual, sealed polypropylene bags, and processed on the day of collection to limit external influence on the substrate sample, such as moisture loss, and maximise larval survival.

Larvae were extracted from substrate samples using the combined sieving and sugar flotation technique described by Kettle and Lawson (1952). This technique was deemed the most suitable method (over alternative methods such as sieving alone, salt-flotation, Berlese funnel-extraction, light extraction, sand extraction, decanting, and carbon dioxide flotation [for review see Hribar (1990), Kline and Axtell (1975) and Jones (1978)]), since it could be used to extract larvae successfully from a variety of different substrates, including those with a high organic content, in a timely and accessible manner.

Small sections of substrate were initially gently broken up in water, then poured and gently washed through three consecutive brass laboratory sieves (mesh size gradient 2.00 mm; 0.355 mm; 0.045 mm) (W.S Tyler, USA). Fine particles of substrate and larvae were retained in the lower sieve and the contents of this sieve was washed using saturated sugar solution into a 15 cm by 30 cm dark coloured tray. Illumination of the tray using two swan lamps, allowed larvae to be removed using a hooked 20 gauge needle. Extraction times were standardised at a maximum of 30 minutes per tray processed, however, if no larvae were found for five minutes within this period, the contents of the tray was be considered to be devoid of larvae and discarded, and the next section of the sample was processed.

2.2.5 Collection of Adult *Culicoides* on Emergence

Within Chapter 4 adult *Culicoides* emerging from their larval development sites were collected using tent emergence traps, constructed based on a design modified from Pajor (1987). This design was modified to include a medium gauge wire mesh frame, producing a more rigid trap with a constant sampling area. The wire frame of each trap was covered in fine white terylene netting, with a mesh aperture of <0.25 mm [see Figure 2.4 on page 36]. The area of substrate sampled by each trap was approximately 0.25m^2. The lower part of the trap consisted of a 5 cm wide skirt reinforced by water resistant vinyl, fabric-reinforced tape (Henkel Consumer Adhesives, Inc., US), through which the frame was secured to the ground with eight 17.8 cm steel tent pegs. Each peg passed through eyelet holes in the skirt, one at each corner and one midway along each side, keeping the trap flush to the ground and minimising the possibility of escape of emerged *Culicoides*.

An opening at the top of the tent was fitted with a 5.0 cm length of 6.8 cm diameter unplasticised polyvinyl chloride (PVCu) pipe (FloPlast, Sittingbourne, UK). The pipe guided emerging insects from the mesh covered area, up into a wide-necked, 1-litre volume polypropylene collecting pot (Naglene Labware, Thermo Fisher Scientific, Roskilde, Denmark), containing approximately 250 ml of 70 % ethanol [See Figure 2.4 on page 36]. The vertical pipe was pushed through a 6.8 cm hole drilled in one side of the pot, protruding 2.0 cm inside the pot, and secured with silicone sealant (Henkel Unibond, Inc., US). The tape that had been used to secure the skirt of the mesh cover was again used to secure the mesh around the wire frame and to the collection pipe at the top of the trap.

After collection insects were passed through a fine mesh sieve (aperture of <0.25 mm). Those retained in the sieve were then washed using fresh 70 % ethanol into 250ml straight-side wide mouth polypropylene sample jars and topped up with sufficient 70 % ethanol to cover the sample for storage prior to identification.

(a). **Unsecured Trap** (b). ***in situ***

Figure 2.4. Emergence trap design used for the collection of newly emerged adult *Culicoides* directly above their larval development sites

2.3 Morphological Identification of *Culicoides*

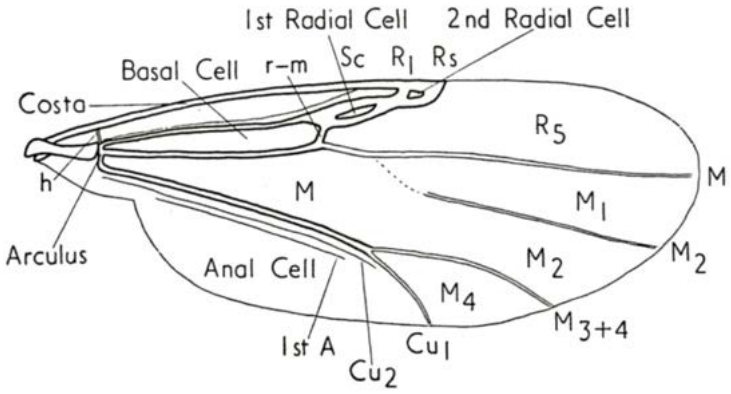
2.3.1 Larval *Culicoides* Identification

Culicoides larvae, collected in Chapter 4, were separated from other genera under a stereomicroscope (10 - 40 x) using the behavioural descriptions and key of Kettle and Lawson (1952). *Culicoides* larvae were differentiated from other Diptera by their segmented body with a conic head structure and when collected into fluid, 3^{rd} and 4^{th} instar *Culicoides* larvae are readily recognised by their active snake-like movements. Earlier instars tended to be more quiescent and lay coiled on the surface of fluid (Kettle and Lawson, 1952). The head of 3^{rd} and 4^{th} instar *Culicoides* larvae is characteristic for all species with most having either pale yellow heads or amber heads (Kettle and Lawson, 1952). Fourth instar larvae were identified by their size and the characteristics of the abdominal fat body, which is very strongly developed in 4^{th} instar larvae, such that the opaque gray abdomen contrasts markedly with the translucent thorax (Kettle and Lawson, 1952).

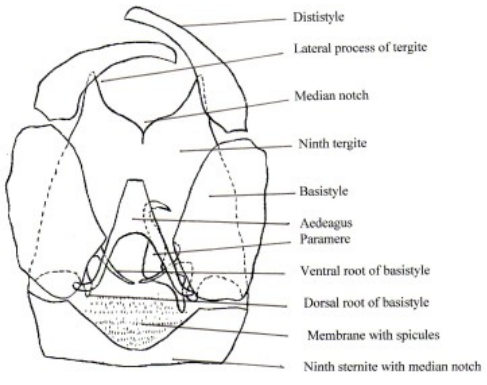
The key of Kettle and Lawson (1952) for morphological identification of *Culicoides* larvae to species can only be applied to 4^{th} instar larvae, is time consuming to follow and has a high potential error rate, since it requires detailed study of an individual's pharyngeal skeleton and thoracic pigmentation. It is now also of questionable accuracy, since many of the attributes are based on subjective measures, such as head capsule colouration. The key was originally based on relatively small sample sizes of wild-caught larvae, reared and identified as adults, with limited allowance for inter-specific variation. Tools for molecular identification of adult specimens of cryptic species groups, including *C. obsoletus* s.l. and *C. pulicaris* s.l. (Nolan et al., 2007) have recently been optimised for identification of larval specimens (Schwenkenbecher et al., 2009). Molecular techniques were therefore employed in this project for the identification of sampled larvae [see section 2.4 on page 40]. *Culicoides pulicaris* s.l. specimens were not analysed to species level due to on-going concerns over the identity of the species within this group (Gomulski et al., 2006; Pages et al., 2009).

2.3.2 Adult *Culicoides* Identification

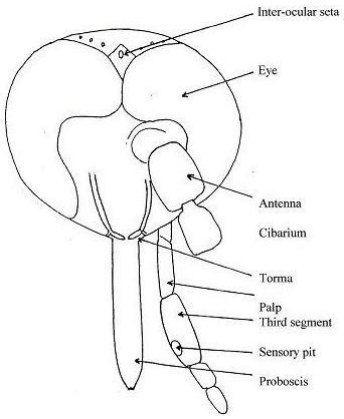
Adult insects, collected in Chapters 4, 5 and 6, were first sorted under a stereomicroscope (10 - 40 x), and individuals of non-*Culicoides* genera removed and discarded. *Culicoides* were identified to species level where possible based on wing morphology and using the key of Campbell and Pelham-Clinton (1960) and Delecolle (1985). The morphological features that allow adult *Culicoides* to be distinguished from all other Diptera are as follows: a well developed radial cells but they lack an R4 and R5 region, a 15 segmented antenna, 13 antennal flagellomeres, a short anepisternal suture and a short anal cerci [see Figure 2.5 below and Figure 2.6 on page 39]. The majority of species also have distinctively pattered wings (Borkent, 2005; Campbell and Pelham-Clinton, 1960).



(a). **Wing Venation**



(b). **Male Genitalia**

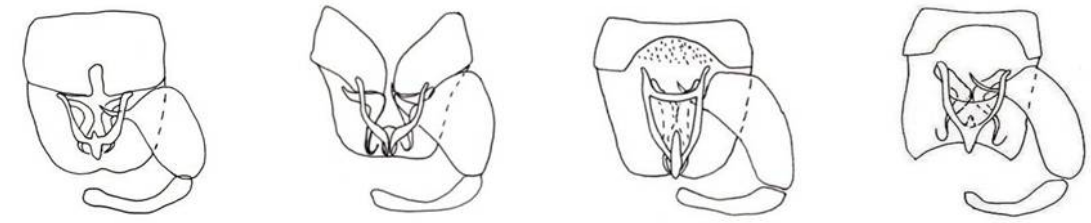


(c). **Head**

Figure 2.5. Standard notation for *Culicoides* morphology [drawings provided by Dr. John Boorman]

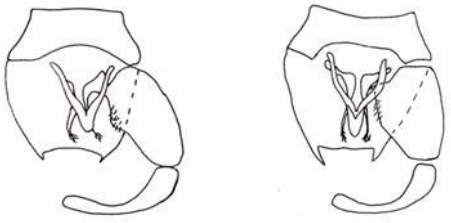
Figure 2.6. Female *C. obsoletus* s.l. [photograph provided by E. Denison and S. Archibald, ©Institute for Animal Health]

For the Obsoletus and Pulicaris group species adult females were identified by morphology to group level only, while males of these two species groups were identified to species level based on the morphology of their genitalia as described in the key of Campbell and Pelham-Clinton (1960) and Delecolle (1985) [see Figure 2.5b on page 38 and Figures 2.7 and 2.8 below]. Within their species, or species groups, the parity stage of females were identified as either, nulliparous, parous, blood-fed or gravid based on abdominal pigmentation (Dyce, 1969). All samples were labelled, catalogued and stored in 70 % ethanol in 7 ml glass bijou's.



(a). ***C. obsoletus* s.s.** (b). ***C. scoticus*** (c). ***C. dewulfi*** (d). ***C. chiopterus***

Figure 2.7. Morphological characteristics of male *C. obsoletus* s.l. species [reproduced from Campbell and Pelham-Clinton (1960)]



(a). ***C. pulicaris*** (b). ***C. punctatus***

Figure 2.8. Morphological characteristics of male *C. pulicaris* s.l. species [reproduced from Campbell and Pelham-Clinton (1960)]

2.4 Molecular Identification of *Culicoides*

The molecular studies described below were undertaken on my behalf as part of a collaborative project. Adult identification, by PCR, of *C. obsoletus* group specimens were carried at the Institute for Animal Health, Pirbright Laboratory, according to the methodology developed by Veronesi et al. (2008) for homogenization of individual *Culicoides*, and Nolan et al. (2007) and Schwenkenbecher et al. (2009) for multiplex PCR assays based on DNA sequence variation at the mitochondrial Cytochrome C oxidase I gene.

Larval identification of *Culicoides* specimens was carried out at the University of Aberdeen using the methodology from Schwenkenbecher et al. (2009) based on the assay developed by Nolan et al. (2007).

2.5 Derivation of Remotely-Sensed Environmental Variables

Remotely-sensed ecological correlates of environmental conditions within the study area described in Section 2.1 (page 28) were derived from Landsat 7 ETM+ imagery of the area. The Landsat series of satellites have repetitive sun-synchronous near-polar orbits and provide full coverage between 81°N and 81°S. The main instrument on board Landsat 7 is the Enhanced Thematic Mapper Plus (ETM+), an apto-mechanical fixed 'whisk-broom', eight-band sensor [see Table 2.2 on page 41] which collects images from the Earth in a 183 km wide swath. An ETM+ scene has an Instantaneous Field Of View (IFOV) (i.e. on the ground resolution) of 30 m in bands 1-5 and 7, while band-6 has an IFOV of 60 m, and band-8 an IFOV of 15 m. Approximate scene size is 170 km north-south by 183 km east-west. The Landsat ETM+ acquires spectral measurements in all major portions of the solar electromagnetic spectrum allowing the development of spectral vegetation indices and correlates of surface temperature [for review Cohen and Goward (2004)] at resolution high enough (15m for pansharpened multispectral scenes) to allow the differentiation between within-farm habitats (Cohen and Shoshany, 2002; Alexandridis et al., 2008).

ETM+ Band	Spectral Bandwidth (nm)	Band Description	Sensor Resolution (m)
1	450-515	Blue	30
2	525-605	Green	30
3	630-690	Red	30
4	775-900	Near Infra-Red	30
5	1550-1750	Mid Infra-Red	30
6a	1040-1250	Thermal High Gain	60
6b	1040-1250	Thermal Low Gain	60
7	2090-2350	Mid Infra-Red	30
8	520-900	Panchromatic	15

Table 2.2. Landsat 7 ETM+ sensor characteristics

Manipulation of all remotely-sensed satellite imagery was conducted using ERDAS Imagine version 9.3 (ERDAS, 2008).

2.5.1 Image Acquisition

From the 31^{st} May 2003 to date, full ETM+ scenes are not available due to a failure of the Scan Line Corrector (SLC) on-board Landsat 7, such that the ETM+ line of sight now traces a zig-zag pattern along the satellite ground track (NASA, 2003; NASA, 2009). The total estimated image loss is approximated to be 22 % over any given scene (Letham, 2004). This image loss resulted in substantial areas of the study area not been scanned, therefore, only Landsat 7 ETM+ scenes acquired prior to the SLC failure were used in this study. The most up to date cloud-free Landsat 7 ETM+ imagery freely available for the study area (Landsat WRS-2 path 202 row 024) prior to SLC failure were acquired on the 16^{th} April 2003 [representing spring 'leaf-on' conditions] and on the 22^{nd} December 2001 [representing winter 'leaf-off' conditions]. The date of both acquisitions was reasonably close to the period of field study they were to be compared to. This imagery was acquired pre-processed to level 1T, which includes systematic radiometric and geometric corrections, from the United States Geological Survey (USGS) via the USGS Global Visualization Viewer (USGS, 2009) in GeoTiff format.

2.5.2 Geometric Corrections

The image supplied had been projected, using nearest neighbour resampling, to the WGS1984 datum, Universal Transverse Mercator (UTM) zone 30, with an approximate accuracy of 250 m. Both the leaf-on and leaf-off images were geocorrected and reprojected to match other GIS layers of environmental factors in the Ordnance Survey Great Britain (OSGB) 1936 projection (Transverse Mercator projected on the Airy spheroid). The higher spatial resolution (15 m) Landsat 7 ETM+ panchromatic band is inherently co-registered with the lower resolution multispectral (30 m) and thermal (60 m) bands. The geometric model (see below) used for geometric correction of the panchromatic bands for both the leaf-on and leaf-off scenes to sub-pixel accuracy was therefore used for geometric correction of the multispectral and thermal bands.

The panchromatic band (band-8) scene was geometrically corrected using a 1^{st} order polynomial geometric model. Image to map rectification was conducted using 1:50,000 OS maps (20 km by 20 km 5 m resolution edge-matched OS colour raster tiles downloaded from Edina Digimap (EDINA, 2002) and mosaiced using ArcInfo Workstation (ESRI, 2006a) as the reference map. Fifty ground control points (GCPs) were based on those major road and rail intersections that could clearly be identified on both the reference map and the remotely sensed image. These were distributed as evenly as possible across the image. Verification of the accuracy of the rectification was conducted through the selection of 20 independent check points distributed across the image. An overall root mean squared error (RMSE), as defined by Equation (2.1), of less than 0.2 pixels (3 m) was achieved for both control and check points [see Table 2.3 on page 43] using the transformation coefficients shown in Table 2.4 on page 43.

$$RMSE = \sqrt{(x' - x_{orig})^2 + (y' - y_{orig})^2} \tag{2.1}$$

Where x_{orig} and y_{orig} are the original row and column coordinates of the GCP, and $x$and y are the estimated coordinates in the rectified image.

The nearest neighbour resampling algorithm, using the transformation coefficients defined by the selected control GCP's [see Table 2.4 below] was used to complete the geometric correction. The nearest neighbour resampling method allows the preservation of the images original radiance values (the radiometric value of the output pixel is set equal to the value of the nearest input pixel in the original geometry). Accurate feature alignment across the geocorrected image was then visually assessed by geo-linking the reference map and geocorrected scene viewers within Erdas.

Scene	**Image Acquisition Date**	**RMSE Error Control**			**RMSE Error Check**		
		X	**Y**	**Total**	**X**	**Y**	**Total**
Leaf-On	16/04/2003	0.0133	0.1478	0.1991	0.1553	0.0812	0.1752
Leaf-Off	22/12/2001	0.1328	0.1075	0.1708	0.1521	0.0943	0.1789

Table 2.3. RMSE for geometric correction of Landsat 7 ETM+ scenes

	Leaf-On		Leaf-Off	
	X"	*Y"*	*X"*	*Y"*
Constant	171692	5.52E+06	171692	5.52E+06
X	1.00014	0.01386	1.00014	0.013859
Y	-0.01377	1.00027	-0.01377	1.00027

Table 2.4. Transformation coefficients for geometric correction of Landsat 7 ETM+ scenes

2.5.3 Image Subset

Prior to further analysis/enhancement all Landsat 7 ETM+ leaf-on and leaf-off individual band scenes were subset to the area of interest (AOI) surrounding the study farms [British national grid coordinates - top left: 430000.00, 210000.00; bottom right: 5000000.00, 140000.00]

2.5.4 Image Enhancement

The Ehlers Fusion method was used to pansharpen the subset non-thermal Landsat 7 ETM+ bands. The Ehlers fusion algorithm is based on an intensity hue saturation (IHS) transform coupled with adaptive filtering in the Fourier domain (Ehler, 2004). This method preserves the spectral characteristics of multispectral images while keeping the spatial resolution of the panchromatic image allowing for an optimum spatial enhancement of selected geo-objects e.g. houses, parcel or field boundaries without compromising the spectral characteristics of the multispectral image. The maintenance of which is vital if the image is to be used for the accurate generation of vegetation indices and other spectrally derived products (Ehler, 2004).

2.5.5 Digital Number to Reflectance Transformation

The 8-bit digital number (QCAL) values of the pansharpened multispectral bands (1-5, 7) and the thermal bands (6_H and 6_L) were converted into spectral radiance (in $\mathrm{W{\cdot}m^{-2}{\cdot}st^{-1}{\cdot}\mu{\cdot}m^{-1}}$), as defined in Equation (2.2) (Markham and Barker, 1986; Irish, 2000). The calculated spectral radiances for the multispectral bands were normalised for both solar elevation angle [see Table 2.5] and atmospheric correction [see Table 2.6 see below] using the COST method (Chavez, 1996) as defined in Equation (2.3) to give the exoatmospheric radiance. For the multispectral bands both Equation (2.2) and (2.3) were completed on a layer stack of the multispectral bands using the Landsat 7 Reflectance conversion tool. For the thermal bands Equation (2.2) was completed on both bands using a model defined in Erdas Imagine Model Maker.

Scene	**Acquisition Date**	**Day of the Year**	**Solar Distance (in astronomical units)** d	**Solar Elevation (in degrees)** $ESUN_\lambda$
Leaf-On	16/04/2003	106	1.00353	45.5039542
Leaf-Off	22/12/2001	356	0.98370	13.0498801

Table 2.5. Solar elevation and distance parameters for leaf-on and leaf-off Landsat 7 ETM+ scenes

$$SpectralRadiance = gain \cdot DN + offset$$

$$L_\lambda = \frac{L_{MAX} - L_{MIN}}{QCAL_{MAX} - QCAL_{MIN}} \cdot (QCAL - QCAL_{MIN}) + L_{MIN} \tag{2.2}$$

Where L_{MIN} and L_{MAX} are the spectral radiances for each band at the minimum and the maximum digital numbers $QCAL_{MIN}$ (1) and $QCAL_{MAX}$ (255) respectively [see Table 2.6 below].

$$\rho_p = \frac{\pi \cdot L_\lambda \cdot d^2}{ESUN_\lambda \cdot cos\theta_s} \tag{2.3}$$

Where:

ρ_p	=	Unit-less planetary reflectance
L_λ	=	Spectral radiance at the sensors aperture [see Eq. (2.2)]
d	=	Earth-Sun distance in astronomical units [see Table 2.5]
$ESUN_\lambda$	=	Mean solar exoatmospheric irradiance [see Table 2.6]
θ_S	=	Solar zenith angle in degrees [see Table 2.5]

Band	**Leaf-On**		**Leaf-Off**		**Mean Solar**
	$\mathbf{L}_{MIN}$	$\mathbf{L}_{MAX}$	$\mathbf{L}_{MIN}$	$\mathbf{L}_{MAX}$	**Exothermal Irradiance [ESUN$_\lambda$]** (Markham and Barker, 1986)
1	-6.200	191.600	-0.250	191.600	1969.000
2	-6.400	196.500	-6.400	196.500	1840.000
3	-5.000	152.900	-5.000	152.900	1551.000
4	-5.100	241.100	-5.100	157.400	1044.000
5	-1.000	31.060	-1.000	31.060	225.700
6a	0.000	17.040	0.000	17.040	-
6b	3.200	12.650	3.200	12.650	-
7	-0.350	10.800	-0.350	10.800	82.070

Table 2.6. Radiance parameters for leaf-on and leaf-off Landsat 7 ETM+ scenes, multispectral and thermal bands

2.5.6 Tassel Cap Transformation

An at-satellite reflectance based tasseled cap transformation (Crist and Cicone, 1984) was used to generate 'wetness' (TC-WET) [1^{st} component], 'greeness' (TC-GREEN) [2^{nd} component] and 'brightness' (TC-BRIGHT) [3^{rd} component] indices, from the at-satellite reflectance scenes [Section 2.5.5 on page 44] and the coefficients shown in Table 2.7 (see below), based on the methodology of Huang et al. (2002a) and the Erdas model of Huang et al. (2002b).

The tasseled cap transformation is a special case of principle component analysis and recombines the spectral information of the six multispectral ETM+ bands (bands 1-5, and 7) into six principal view components, the first three of which are associated with brightness, greenness, and wetness respectively, through the use of coefficients derived by sampling known land cover spectral characteristics (Richards and Jia, 2006). Tasseled cap indices derived from Landsat imagery have previously been useful in predicting tick (*Ixodes scapularis* Say) abundance (Rogers and Mather, 2006) and survival (Ogden et al., 2006) in relation to Lyme disease (*Borrelia* sp.) transmission, predicting risk of *Aedes aegypti* Linnaeus transmitted dengue (Rotela et al., 2007) and for mapping *Culex tarsalis* Coquillett larval habitats in relation to WNV transmission risk (Zou et al., 2006).

Index	Brightness	Greenness	Wetness	Fourth	Fifth	Sixth
Band-1	0.3561	-0.3344	0.2626	0.0805	-0.7252	0.4000
Band-2	0.3972	-0.3544	0.2141	-0.0498	-0.0202	-0.8172
Band-3	0.3904	-0.4556	0.0926	0.1950	0.6683	0.3832
Band-4	0.6966	0.6966	0.0656	-0.1327	0.0631	0.0602
Band-5	0.2286	-0.0242	-0.7629	0.5752	-0.1494	-0.1095
Band-7	0.1596	-0.2630	-0.5388	-0.7775	-0.0274	0.0985

Table 2.7. Tasseled cap coefficients for Landsat 7 ETM+ at-satellite reflectance

2.5.7 Land Surface Temperature

Landsat 7 produces two thermal images, one acquired using a low gain setting (band-6_L) saturating at 347.5 K and the second using a high gain setting (band-6_H) saturating at 322 K. The information from band-6_H was used in this investigation as it is more sensitive to most land targets, especially vegetated areas. Band-6_L is of more use in areas of interest covered by deserts, sand beaches and impervious surfaces where the temperatures can be higher than 322 K (the saturation temperature for band-6_L). Land surface temperature (LST) derived from Landsat images has previously been useful in predicting the distribution of *Oncomelania hupensis* sp. in relation to *Schistosoma japonicum* transmission (Zhi-Ying et al., 2005)

The thermal radiance values calculated from the digital numbers of the band-6_H images [Section 2.5.5] were converted using a simplified plank function as defined in Equation (2.4) (Markham and Barker, 1986) to surface temperatures using the pre-launch calibration constants (NASA, 2009; Schott, 1988) and assuming unity emissivity.

$$T_{ls} = \frac{K_2}{1n(\frac{K_1}{L_\lambda} + 1)} \tag{2.4}$$

Where Landsat 7 ETM+ thermal band calibration constants (NASA, 2009; Schott, 1988) are:

T_{ls}	=	Effective at-satellite temperature in Kelvin		
K_1	=	First Calibration Constant in $W{\cdot}m^{-2}ster^{-1}\mu{\cdot}m^{-1}$	=	666.09
K_2	=	First Calibration Constant in Kelvin	=	1282.71
L_λ	=	Spectral Radiance in $Wm^{-2}{\cdot}sr^{-1}\mu{\cdot}m^{-1}$ as calculated in Eq. (2.2)		

2.5.8 Enhanced Vegetation Index

The Enhanced Vegetation Index (EVI) as defined by Equation (2.5), based on the at-satellite reflectance scenes [Section 2.5.5 on page 44], was calculated for both leaf-on and leaf-off images using the Erdas Model of Rodriguez (2004). EVI, is a modification to the Normalized Difference Vegetation Index (NDVI) and is an optical measure of surface vegetation conditions (Tucker et al., 2005) i.e. the vegetation

canopy 'greenness', a composite property of leaf chlorophyll, leaf area, canopy cover and canopy architecture (Jiang et al., 2008). NDVI derived from Landsat imagery, has been previously been useful in predicting WNV disease occurrence (Ward, 1996) and in forecasting habitat suitability for ticks (*Boophilus microplus* Canestrini and *Boophilus annulatus* Say) in relation to *Babesia* sp. transmission (Estrada-Pena and Venzal, 2006). EVI was developed to optimize the vegetation signal with improved sensitivity in high biomass regions and improved vegetation monitoring through a decoupling of the canopy background signal and a reduction in atmosphere influences (Huete et al., 2002) and has been correlated with levels of soil moisture (Chen et al., 2006; Waring et al., 2006). In comparison to NDVI, EVI has been found to be more linearly correlated with green leaf area index (LAI) in crop fields (Boegh et al., 2002), is minimally sensitive to residual aerosol contamination (Miura et al., 1998; Xiao et al., 2003) and is less prone to saturation in temperate and tropical forests (Huete et al., 2006; Xiao et al., 2004) particularly temperate broad-leaf deciduous forests (Rahman et al., 2005; Sims et al., 2006).

$$EVI = G \cdot \frac{\rho NIR - \rho R}{\rho NIR + C_1 \cdot \rho R - C_2 \cdot \rho B + L} \tag{2.5}$$

Where:

ρNIR	=	Reflectance factor values for near infrared band (band-4)
ρR	=	Reflectance factor values for the red band (band-3)
ρB	=	Reflectance factor values for the blue band (band-1)

with EVI algorithm coefficients (Huete and Justice, 1999) for Landsat 7 ETM+ scenes of:

L	=	1	(Canopy Background Adjustment Term)
G	=	2.5	(Gain Factor)
C_1	=	6	(First Coefficient of the Aerosol Resistance Term)
C_2	=	7.5	(Second Coefficient of the Aerosol Resistance Term)

2.6 Statistical Analysis Software

Unless stated, all statistical analysis was implemented in R version 2.9.1 (R Development Core Team, 2009). Packages used in addition to the base distribution of R are cited where appropriate.

Chapter 3

Ecological Correlates of BTV Outbreak Occurrence and Vector Abundance in Mainland Europe

3.1 Introduction

Spatial patterns in the abundance of *Culicoides* within and between farms is influenced to varying degrees by the abundance and proximity of a range of resources, namely hosts, oviposition and resting sites. Environmental conditions will influence the distribution these resources within the landscape and in addition will have direct impacts on demographic rates, such as births and deaths, within the *Culicoides* population.

Species distribution models are numerical tools that combine observations of a species occurrence and abundance with environmental predictors and/or spatial characteristics of a location [for review see Elith and Leathwick (2009)]. These allow species-environment relationships to be derived by matching species' distributions with spatial and/or temporal patterns in environmental factors and can then be used to map habitat suitability for vectors and diseases across unsampled areas (Rogers and Randolph, 2003). Models of haematophagous insect distribution and abundance are, however, complicated by trap catch data only providing a relative rather than an absolute measure of abundance for an area [for review see Southwood and Henderson (2000)]. The accuracy of this estimate will vary with both sampling effort and trap efficiency, species sampled and trap type (if more than one is used). Variations in seasonality of species sampled and the timing of collections will also influence estimates of relative abundance. If only a few collections are made through the year significant errors in the recorded presence-absence of a species at a location may occur if that species is temporally absent, or present at very low numbers, during the sampling period. Phototropism in many species of insects allows their collection, often in very

large numbers at light. In haematophagous insects how the attraction to and collection at light traps relates to the numbers and species composition attracted to hosts has received limited attention for many important disease vectors, including *Culicoides* (Carpenter et al., 2008d). Discrepancy in the abundance or occurrence of a species observed in trap catches compared to those observed on susceptible hosts will have implications for models describing the spatial and temporal distribution of vector-borne diseases.

Given well designed survey data and functionally relevant predictors, species distribution may be modeled both in terms of an individual species relationship to environmental conditions and/or how environmental conditions can predict community-level features such as species composition and species turnover or richness [for review see Ferrier and Guisan (2006) and Franklin (2009)]. Predictions of species distribution/abundance may also be incorporated as a predictor of risk within spatial distribution models of vector-borne disease. Distinction, however, has to be made between models of disease occurrence, i.e. models based on occurrence data derived from records of clinical presentation of for example BT, and those which seek to model the distribution of the pathogen, for example models using records of both clinical and sub-clinical presentation of BT providing information on the distribution of BTV. A wide range of statistical techniques are now available for the modeling of both species distribution and abundance and disease outbreak risk, allowing the incorporation of both linear and non-linear responses to predictors. Some approaches also allow for effects such as spatial autocorrelation to be explicitly included within the models (Legendre, 1993; Rangel et al., 2006). Alongside these models geographical information systems allow the storage and evaluation of large amounts of environmental data, aiding in the mapping of vectors and diseases across unsampled areas (Rogers and Randolph, 2003).

3.1.1 The Current State of Modelling for *Culicoides* Species-Environment Relationships in Europe

As awareness of the role of Palaearctic vector groups in transmission within Europe increased, the importance of developing habitat suitability models for these groups, in addition to *C. imicola*, has been realised along with the need to account for the different ranges and habitat preferences of their constituent species (Calvete et al., 2008, 2009; Conte et al., 2007a; Purse et al., 2006). Across Europe, *C. imicola* and the Palaearctic vector groups have been shown to prefer different, but overlapping, ranges of environmental conditions (Purse et al., 2007). *Culicoides imicola* prefers warmer (annual mean 12 ℃ to 20 ℃) more thermally stable locations that are dry in summer (<400mm precipitation) than *C. obsoletus* s.l. or *C. pulicaris* s.l., whose distributions extend into areas that were much cooler (average annual temperature 7 ℃ to 8 ℃) and wetter (summer precipitation up to 600 mm) (Purse et al., 2007). The overlap in the distribution of *C. obsoletus* s.l. and *C. pulicaris* s.l. with *C. imicola* is thought to have facilitated the spread of BTV into cooler, wetter regions of Europe (Purse et al., 2007), where *C. imicola* is absent, via the so called 'baton' effect (Mellor and Boorman, 1995).

Despite *C. imicola* and the Palaearctic vectors having been shown to have overlapping environmental requirements (Purse et al., 2007), the largest catches of *C. imicola* have been found not to occur in the same areas as the largest catches of *C. obsoletus* s.l. in Italy and Spain (Conte et al., 2007a; Ortega et al., 1998). This suggests that, in Italy, and probably elsewhere, *C. imicola* and the Obsoletus group generally do not share a 'common habitat'. Patakakis et al. (2009) observed that, in Greece, sites capable of supporting high densities of both *C. imicola* and *C. obsoletus* s.l. were rare, but that sites where the two overlapped at lower densities were considerably more common. The environmental envelope analysis of Purse et al. (2007), that compared the overlap of BTV transmission with each major vector group, indicated that the Palaearctic groups are playing some role in transmission even within the distribution of *C. imicola*. Overall, these studies suggest that the Obsoletus and Pulicaris species

groups share some of the same basic drivers governing their distribution in these regions, but that different sets of ecological conditions are required to attain high abundances and facilitate transmission.

Environmental relationships for *C. pulicaris* s.l. have received limited attention in comparison to *C. obsoletus* s.l. Within Sicily, Purse et al. (2004a) found the presence of *C. pulicaris* s.l. was determined mainly by moisture and vegetation-related variables (six out of ten variables in the final distribution model were related to NDVI), in contrast to *C. obsoletus* s.l., whose presence was primarily related to temperature variables (seven out of ten variables related to LST or Air Temperature (TAIR)). Within Switzerland, *C. pulicaris* s.l. has been found to become more prevalent in trap catches with increasing altitude (1200 m to 2000 m above sea level) (Tschuor et al., 2009). These finding suggest that although their environmental envelopes overlap these two species groups find their optimum in different positions along the key environmental gradients of elevation, temperature and moisture.

To date, models concerning the Palaearctic BTV vectors (Conte et al., 2007a,b; Purse et al., 2004a) have focused on links to climatic conditions within areas towards the southern limit of these vectors range. In contrast to *C. imicola*, the Palaearctic BTV vector groups, *C. obsoletus* s.l. and *C. pulicaris* s.l., are widely distributed across the temperate regions of Europe. Thus, the links identified, within the Mediterranean Basin, to climatic correlates, between *C. obsoletus* s.l. distribution and temperature (Calvete et al., 2008; Purse et al., 2004a, 2007) and indicators of moisture availability (NDVI and seasonality in precipitation) (Calvete et al., 2008; Conte et al., 2007a; Purse et al., 2004a, 2007) may not accurately portray these species' preferences within the temperate regions of their distribution.

3.1.1.1 Improvements to Climate Based Distribution Modelling

It is probable that within climatically suitable areas, non-climatic factors such as soil type (Baylis et al., 1999), host abundance (Calvete et al., 2008; Guis et al., 2007), landscape structure (Guis et al., 2007), terrain (Conte et al., 2007a; Guis et al., 2007)

and farm husbandry methods (Meiswinkel et al., 2000) determine the distribution and abundance of vector populations at a finer spatial scale. The inclusion of such factors in addition to climatic correlates may therefore improve prediction of areas at-risk of BTV transmission.

Currently, only four European based investigations into *Culicoides* abundance or BTV transmission have incorporate non-climatic ecological variables (Calvete et al., 2009; Conte et al., 2007a,b; Guis et al., 2007) and only one has done so for the Palaearctic group species (Conte et al., 2007a). These models, however, indicate the potential for non-climatic variables to explain variation in *Culicoides* abundance in addition to that currently explainable by purely climate based models (Pili et al., 2006; Calistri et al., 2003).

Within Corsica, where the primary vector of BTV is considered to be *C. imicola*, Guis et al. (2007) highlighted the importance of landscape features, in particular the edge metrics, in determining the risk of BTV outbreak occurrence. Landscapes containing highly fragmented areas of woodland and pastures were linked to a higher risk of BT occurrence and interpreted as providing an increased number of meeting points between hosts and vectors, the latter moving from woodland larval development sites to pastures in search of hosts. Calvete et al. (2009) found the inclusion of additional host availability variables to climatic variables in a distribution model for BTV-4 led to an improved model fit, with transmission risk positively associated with small ruminant farm density (sheep and/or goat farms) and cattle density, however, negatively associated with cattle farm density. In addition Calvete et al. (2009) found BTV outbreak occurrence (BTV-4) to be positively impacted by an in increase seasonality and increasing annual mean levels of vegetation activity (NDVI), in contrast outbreak risk increased in areas where precipitation levels were lower but seasonally more variable.

In a model combing both climatic and landscape factors, Conte et al. (2007a) found that, in Italy, dominant populations of *C. imicola* were linked to sparsely vegetated habitats, with low NDVI, i.e. areas of summer-arid sclerophyllous vegetation, that are

more open and exposed to full sunlight. In contrast, dominance of *C. obsoletus* s.l. in populations was linked to more shaded forest habitat, with increased green leaf density (higher NDVI levels on average). By combing 'abiotic' (minimum temperature, aridity index, terrain slope and altitude) and 'biotic' (percentage of forest and NDVI) factors, 84 % of the observed variation in *C. obsoletus* s.l. and *C. imicola* populations could be explained, with 87.5 % of municipalities being classified correctly as to their 'dominant' vector species / species group (Conte et al., 2007a). The addition of soil factors (percentage of soil organic material, percentage of clay, slit, sand and soil water content, distance from fine texture soils) enabled the presence-absence of *C. imicola* to be correctly identified at 89.5 % of sites (Conte et al., 2007b). The methodological approach of Conte et al. (2007a,b), however, would have lead to a large underestimate of the range and overlap of conditions preferred by these taxa, since it focused on the analysis of only the 100 largest collections of *C. imicola* and of *C. obsoletus* s.l. (out of 38,000 and 3,000 light trap catches of *C. imicola* and *C. obsoletus* s.l. respectively). How the relationships identified at these 'super-abundance' sites relates to the species groups abundance across the length of their distribution within an environmental gradient is undetermined.

Calvete et al. (2008) have developed risk maps for *C. imicola* and *C. obsoletus* s.l. occurrence, in Spain, based on these species/species groups relationship to climatic predictors, including sun index, mean NDVI, seasonality of NDVI, annual precipitation, seasonality of precipitation, mean temperature and seasonality in temperature, although these models were found to under-estimate *C. obsoletus* s.l. occurrence and over-estimate *C. imicola* occurrence. The occurrence of *C. imicola* and *C. obsoletus* s.l., have also been related to the spatial occurrence of BTV in Spain, in which the BTV-4 outbreak distribution was determined to be mainly driven by the spatial distribution and population dynamics of *C. imicola* rather than the Obsoletus group species (Calvete et al., 2009). Identification of significant species-environment relationships through GIS-mapping techniques combined with statistical and mathematical models can help improve disease surveillance and control methods by provid-

ing a basis for targeting of both monitoring efforts and vector control measures. The identification of productive habitat types, for example, have previously enabled more targeted, and therefore cost-effective, control strategies to be developed for *Anopheline* mosquitoes in relation to malaria transmission (Protopopoff et al., 2007, 2008). If utilised correctly risk-based targeting of resources can result in reduced operational costs (Hay et al., 1998). With regards to BTV transmission, Switzerland for example, has implemented a risk-based sampling scheme for BTV occurrence (Racloz et al., 2006) based on identifying geographical areas which matched habitat criteria for the presence, survival and establishment of the different vector species (Casati et al., 2010; Racloz et al., 2006). These criteria were based on vector habitat suitability maps created from thematic maps of temperature, humidity and altitude to which relationships to vector activity had previously been described (Racloz et al., 2007), combined with monthly R_0 risk maps (Racloz et al., 2006).

The usefulness of climatic variables as predictors for BTV outbreak occurrence has been confirmed repeatedly (Baylis et al., 2001; Purse et al., 2004a,b; Tatem et al., 2003). In efforts to improve more local-level predictions Calvete et al. (2009) were the first to combine host information with climatic predictors for the spatial occurrence of BTV and Guis et al. (2007) the first to utilise landscape factors for predicting BT occurrence. To date, however, no attempts have been made to combine all these different ecological factors to improve BTV risk assessment or to explain the environmental relationships of the different BTV vector species. Risk maps for BTV transmission, however, are currently limited to the assumption that the vectorial capacity of the Obsoletus and Pulicaris group species are equal and until further evidence of divergence in the vectorial capacity and/or environmental preferences the constituent species of these groups are established this limitation will remain.

3.1.2 Justification for Research

To date, models driven solely by vector species' relationships to climatic conditions do not explain the high level of local-scale spatial variation observed in BTV vector abundance (Calistri et al., 2003; Pili et al., 2006). In addition, the differing relative importance of climatic variables as determinants of species occurrence at the presence-absence level, indicate that the Palaearctic vectors, *C. obsoletus* s.l. and *C. pulicaris* s.l., have differing environmental requirements to *C. imicola*. Thus, predictive BTV risk maps derived entirely from *C. imicola* distributional data will omit extensive at-risk areas. Similarly, within regions where transmission occurs primarily through the Palaearctic vectors variation in the environmental requirements of *C. obsoletus* s.l. compared to *C. pulicaris* s.l. will lead to errors in the prediction of risk within these regions.

By analyzing patterns in BTV vector abundance and BTV outbreak distribution in relation to both climatic and non-climatic ecological correlates in the same region, we hope to gain some understanding of the biological mechanisms underlying the differential sensitivity of the different BTV vector species groups to particular environmental factors. Examination of the relative importance of climatic and non-climatic ecological correlates in promoting/depressing abundance within these species, and how these relate to the ecological correlates of BTV outbreak occurrence will potentially lead to improvements in BTV risk assessment through the explanation of a greater degree of the variation observed in *Culicoides* abundance.

This study, therefore, aims to address the hypothesis that predictive mapping techniques for *Culicoides* abundance and BTV outbreak presence-absence, at regional-level in mainland Europe, can be improved via integrating remotely-sensed climatic correlates with non-climatic ecological correlates and the availability of hosts. Testing the hypothesis that additional ecological correlates are required to determine BTV vector abundance compared to those required to determine the spatial occurrence of BTV outbreaks. The climatic and non-climatic constraints on BTV vector abundance were also investigated testing the hypothesis that *C. imicola*, *C. obsoletus* s.l. and

C. pulicaris s.l. abundance show differing relationships to environmental conditions. To address this hypothesis this study focuses on the prediction of BTV outbreak occurrence and vector abundance within Greece and Bulgaria, from which large datasets of BTV outbreak presence-absence and vector abundance, covering a wide range of environmental conditions, are currently available.

Epidemiologically Greece and Bulgaria are of particular interest, as all three of the European BTV vector species/species groups are implicated in transmission in the region (Purse et al., 2006, 2007; Patakakis et al., 2009). Both countries also contain significant areas where *C. imicola* is absent, indicating that transmission is primarily, if not entirely, carried out by the Palaearctic vector groups within these areas (Purse et al., 2007). The area also contains a wide diversity of climatic and landscape conditions characteristic of both the Mediterranean BTV episystem in the south, and temperate regions in the north, bridging the transition between the two. This region may therefore provide a baseline for indicating what ecological drivers are important for determining BTV outbreak risk and vector abundance, and whether combining host, landscape, climate and terrain predictors can improve models of BTV outbreak distribution and vector abundance.

3.2 Materials and Methods

3.2.1 Study Area

Greece is formed from a mountainous, peninsular mainland jutting out into the sea at the southern end of the Balkans, with over 2,000 associated islands (227 of which are inhabited) scattered across the Aegean sea between the mainland of Greece and Turkey. The Greek territories can be subdivided into six ecoregions (WWF, 2009): Aegean and western Turkey sclerophyllous and mixed forests, Crete Mediterranean Forests, Pindos mountains mixed forests, Illyrian deciduous forests, Rodope montane mixed forests and Balkan mixed forests [see Figure 3.1 on page 59]. Climatic conditions are similarly diverse and are categorised into three types: Mediterranean, Alpine, and Temperate. The temperate northern regions of Greece share a land border with Bulgaria, which has a temperate climate, with cold winters and hot summers. Northern Bulgaria experiences lower temperatures and receives more rain than the southern lowlands. Bulgaria spans three main ecoregions (WWF, 2009), including: Balkan mixed forests, East European forest steppe and Rodope montane mixed forest [see Figure 3.1 on page 59]. The wide range of climatic and topographic conditions in Greece and Bulgaria allow for a variety of crops and livestock products to be produced, with animals and animal production constituting a significant proportion of both Greece and Bulgaria's agricultural output (EU, 2009).

3.2.2 Sero-Surveillance and Vector Data

From an EU project 'Bluetongue and other *Culicoides*-borne diseases threatening the EU: identification of vulnerable areas by surveillance and GIS modelling to aid risk assessments' (contract no. QLK2-2000-00611), georeferenced locations were available for (i) 400 presence sites - where BT outbreaks or seropositive animals had occurred in Greece and Bulgaria between 1999 and 2003 (presence sites) and (ii) 132 absence sites where no BTV antibodies had been detected during sentinel surveillance in the same years [see Figure 3.2 on page 59]. The mean minimum distance to the nearest neighbouring BTV outbreak presence-absence sampling site was 6.23 km.

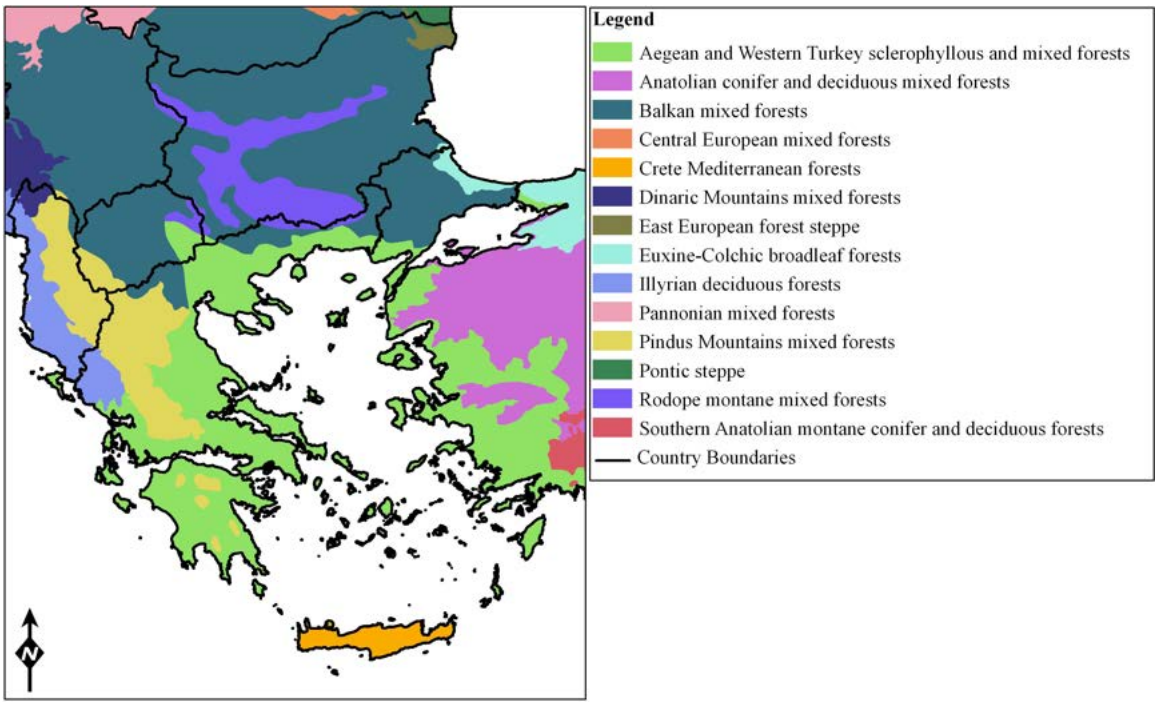


Figure 3.1. Spatial distribution of ecoregions within Greece and Bulgaria derived from the World Wildlife Fund terrestrial ecoregions database (WWF, 2009)

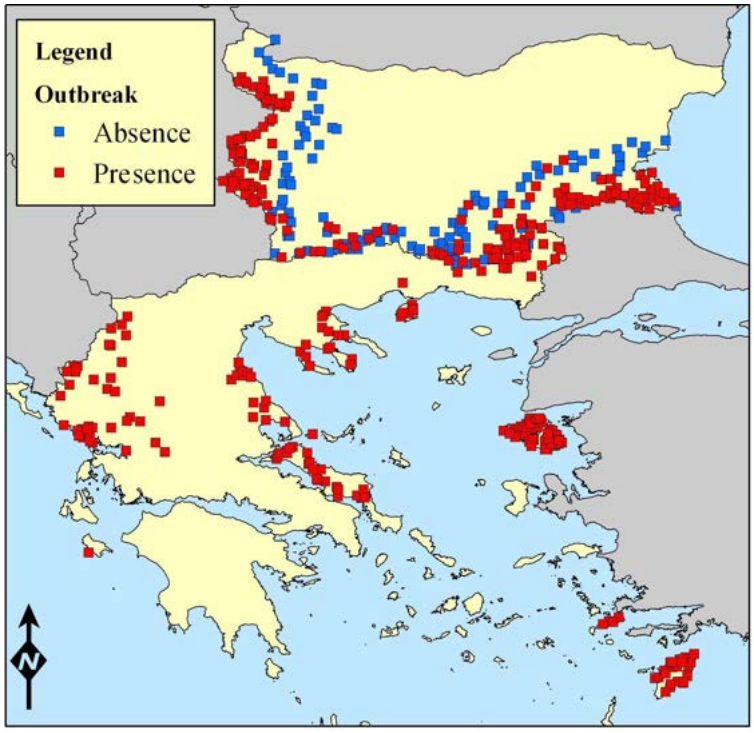


Figure 3.2. Spatial distribution of sites sampled for BTV outbreak presence-absence in Greece and Bulgaria

From the same project, georeferenced entomological data collected between 1999 and 2003, recorded the presence of BTV vectors at 185 sites across Greece and Bulgaria. The mean minimum distance to the nearest neighbouring trapping site was 16.04 km. All three vector species / species groups currently implicated in BTV transmission in Europe were recorded, the main Afro-Asiatic vector of BTV, *C. imicola*, and the two Palaearctic species groups *C. pulicaris* s.l. and *C. obsoletus* s.l. (species-specific identification of individuals for the Palaearctic vector group samples were not made). For each of these vector species/species groups, measures of abundance at each site, were taken as the maximum catch of females out of two light trap collection made on two consecutive nights in summer, between June and October. Maximum catches at this time have been shown in previous studies to be consistently related to the annual abundance of *Culicoides* (Baylis et al., 1997). Only female *Culicoides* were considered in this analysis, as male *Culicoides* do not take blood meals and consequently have no potential to vector BTV between hosts.

All entomological data were collected using OVI type, 8W UV down-draught suction traps (Agricultural Research Council, South Africa). Traps were run from dusk until dawn to coincide with the crepuscular peaks in *Culicoides* activity (Hill, 1947; Kettle, 1957; Parker, 1949; Service, 1969) [see Section 2.2.1 and Purse et al. (2006) for further details of trapping protocols used].

3.2.3 Quantifying Environmental Conditions Surrounding Sample Sites

Variables quantifying environmental conditions surrounding sampling sites were selected from four suites (host, landscape, climate and terrain/soil) based on their hypothesised influence on the abundance and distribution of BTV vectors and their usefulness as predictors of patterns in BTV vector and/or outbreak distribution within previous regional scale studies; [*A* - BTV hosts (Calvete et al., 2009; Guis et al., 2007); *B* - landscape features (Guis et al., 2007; Conte et al., 2003, 2007a); *C* - climatic conditions (Baylis and Mellor, 2001; Conte et al., 2003, 2007a; Purse et al., 2004a,b, 2007; Tatem et al., 2003; Wittmann et al., 2001), and *D* - terrain and soil characteristics (Conte et al., 2007a,b; Guis et al., 2007)].

Variables contained within the four factor suites were as follows:

(A) **Host Variables** - To capture details of the availability of BTV susceptible livestock within the area surrounding sample sites, information on cattle (CATTLE), sheep (SHEEP) and goat (GOATS) populations were derived from the FAO Global Livestock Maps data (5 km^2 grid square resolution) (FAO, 2005).

(B) **Landscape Variables** - Landscape structure was characterised based on circular buffers around sampling points from land cover data derived from the CORINE Land Cover Map 2000 (100 m grid square resolution) (CLC2000) (EEA, 2004). CLC2000 is a thematic classification of satellite imagery with a minimum mappable areas of 25 hectares. It records 44 land cover and land use classes which represent the major surface types across Europe (European Commission, 1993).

Landscape structure within buffer regions was quantified using the spatial pattern analysis program 'Fragstats' (McGarigal and Marks, 2002). Metrics were calculated at two levels: one for selected land-cover classes found in a buffer (class-level metrics) and the other for the whole landscape, i.e. for the entire buffer regardless of the class (landscape-level metrics). Four landscape level metrics: landscape shape index (LSI), landscape patch index (LPI), mean radius of gyration distribution (MEAN.GYRATE) and patch richness density (PRD) were calculated [see Table 3.2 on page 65 for metric descriptions], and four class level metrics (per selected land cover class): percentage of landscape covered by patches of class (PLAND), patch density of class (PD), edge density of class (ED) and cohesion of class (COHESION) [see Table 3.1 on page 64 for metric descriptions]. Class level metrics were calculated for selected agriculture associated land cover types, which were abundant within the three buffer scales i.e. formed >1 % of the buffer landscape [see Figure 3.3 and 3.4 on page 63] and were expected *a priori* to influence the resource requirements of *Culicoides*. Land cover classes selected included: non-irrigated arable land (class 12), pastures (class 18), complex cultivation patterns (class 20), land

principally occupied by agriculture, with significant areas of natural vegetation (class 21), broad-leaved forest (class 23), mixed forest (class 25) and natural grasslands (class 26), and additionally for the vector abundance analysis only, fruit trees and berry plantations (class 16) [see Figure 3.3 and 3.4 on page 63]. Selected metrics provide measures of the degree of coverage, fragmentation and cohesion within- and between- different land cover classes, and have previously been useful in explaining patterns in the occurrence of BT (Guis et al., 2007) and other vector-borne diseases (Brownstein et al., 2005; Danson et al., 2004; Gleiser et al., 2002; Graham et al., 2004; Jackson et al., 2006).

Landscape structure was summarized at multiple spatial scales, as assessment of landscape structure is sensitive to the scale at which it is measured [for review see (Klopatek and Gardner, 1999)]. Guis et al. (2007) previously found 1 km and 2 km buffer zones to offer good predictive value for distinguishing areas of BT occurrence at the presence-absence level in Corsica. Selected buffer sizes reflect the findings of Guis et al. (2007), but with the addition of a 5 km buffer size, to reflect the uncertainty in the dispersal range of *Culicoides*. The 5km buffer size was used to reflect the potential dispersal range of the vectors identified in the literature (Lillie et al., 1981, 1985). Mark recapture studies indicate that *C. sonorensis* Wirth and Jones were capable of dispersing up to 4 km (Lillie et al., 1981) and *C. mississippiensis* Hoffman up to 3.2 km from the release point (Lillie et al., 1985). These mark-recapture based estimates should be treated with caution given the very low marking and recapture rates for *Culicoides*, which may be partly due the to potential for dispersal in any direction from the release point. Lillie et al. (1981) recaptured just 0.49 % of 82,800 individuals released.

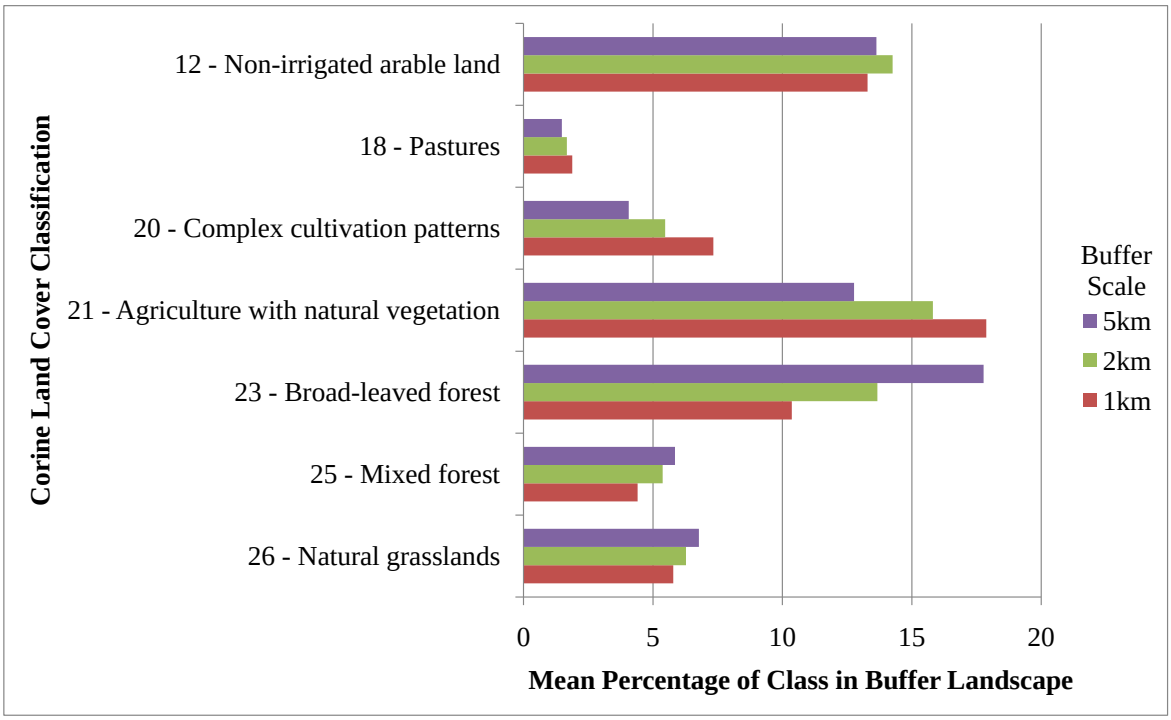


Figure 3.3. Mean percentage of buffer landscape, surrounding sites of recorded BTV outbreak presence-absence, covered by selected land cover classes

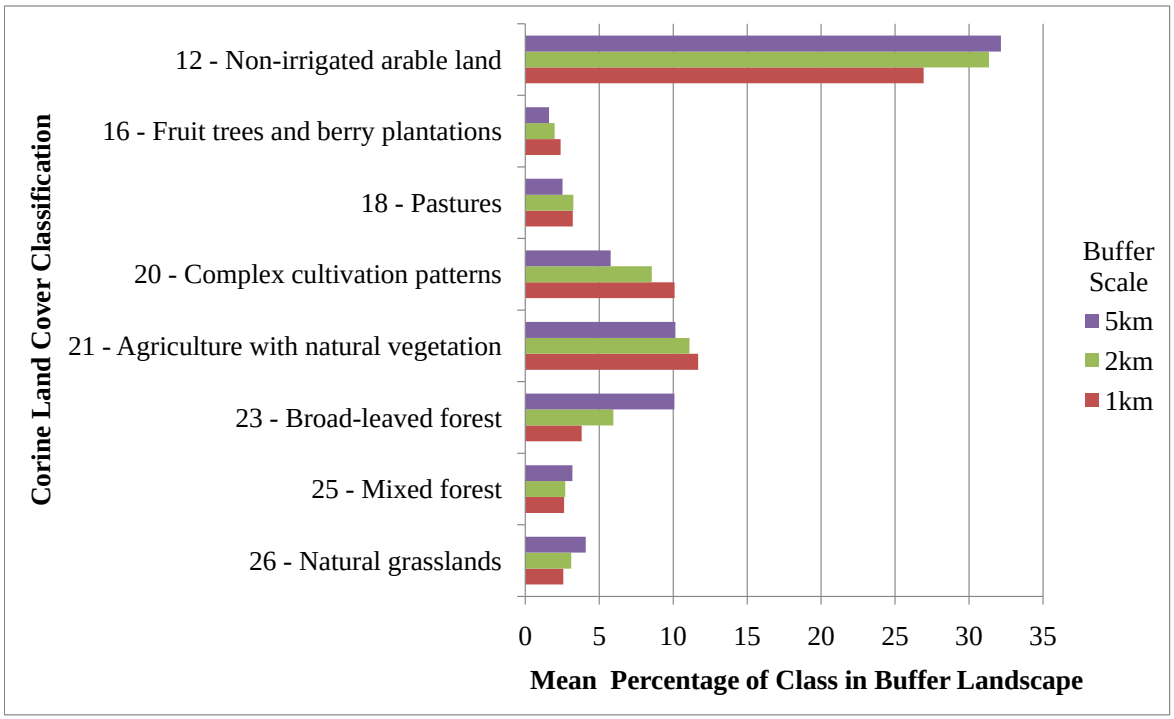


Figure 3.4. Mean percentage of buffer landscape, surrounding sites of recorded BTV vector occurrence, covered by selected land cover classes

Variable	Name *(Metric Level; Metric Type)*	Definition
PLAND.X*	**Percentage of Landscape covered by patches of class X*** *(Class; Area-Density-Edge Metrics)*	Quantifies the proportional abundance (in %) of each land cover type (X) in the landscape.
PD.X*	**Patch Density of Class X*** *(Class; Area-Density-Edge Metrics)*	A simple measure of the extent of subdivision or fragmentation of land cover type X within the landscape.
ED.X*	**Edge Density of Class X*** *(Class; Area-Density-Edge Metrics)*	An absolute measure of total edge length of class type/land cover type X, expressed on a per unit area basis (metres per hectare).
COHESION.X*	**Patch Cohesion Index of Class X*** *(Class; Connectivity Metrics)*	A measure of the physical connectedness of land cover type X. Patch cohesion increases as the patch type becomes more clumped or aggregated in its distribution; hence, more physically connected

* X represents each of the land cover classes used in the analysis

Table 3.1. Selected class level metrics calculated using the Fragstats landscape pattern analysis software (McGarigal and Marks, 2002)

Variable	Name *(Metric Level; Metric Type)*	Definition
LSI	**Landscape Shape Index** *(Landscape; Area-Density-Edge Metrics)*	Provides a standardized measure of total edge or edge density that adjusts for the size of the landscape. LSI can also be interpreted as a measure of patch aggregation or disaggregation. Specifically, as LSI increases, the patches in the landscape are increasingly disaggregated.
LPI	**Landscape Patch Index** *(Landscape; Area-Density-Edge Metrics)*	Quantifies the percentage of total landscape area comprised by the largest patch. As such, it is a simple measure of dominance. LPI approaches zero when the largest patch in the landscape is increasingly small. LPI = 100 when the entire landscape consists of a single patch; that is, when the largest patch comprises 100 % of the landscape.
MEAN.GYRATE	**Mean Radius of Gyration Distribution** *(Landscape; Area-Density-Edge Metrics)*	Reflects the mean patch extent (in metres) across the entire landscape
PRD	**Patch Richness Density** *(Landscape; Diversity Metrics)*	The number of different patch types present standardizes richness to a per area (mean no. of patches per 100 hectares) within the landscape boundary

Table 3.2. Selected landscape level metrics calculated using Fragstats (McGarigal and Marks, 2002)

(C) **Climate Variables** - *Culicoides* life cycles are highly dependent on seasonal conditions of temperature and soil moisture (Wittmann and Baylis, 2000). Seasonal variation in vegetation activity temperature and moisture levels were summarised at sample sites using Fourier processing of low resolution moderate-resolution imaging spectroradiometer (MODIS) satellite imagery to derive nine measures of the Fourier harmonics corresponding to the frequencies of the fitted signal and one measure of the variance of the original signal (V) of the following

four variables: day-time Land Surface Temperature (dLST), night-time Land Surface Temperature (nLST), Enhanced Vegetation Index (EVI) and Middle Infrared Reflectance (MIR) [see Table 3.3 below]. These Fourier-derived variables were obtained from a global archive, of temporal Fourier processed MODIS sensor imagery (2001-2005) at a $1\,km^2$ grid square resolution, at the Spatial Ecology and Epidemiology Group of the Department of Zoology, University of Oxford. A complete description of the processing of MODIS satellite data to derive seasonal Fourier variables is provided by Scharlemann et al. (2008). Temporal Fourier processing produces a set of orthogonal (i.e. uncorrelated) variables that capture the seasonality of natural habitats and is thus ideal for describing seasonal processes that are often critical for vector-borne disease transmission (Rogers, 2000). The satellite signal for any channel is split into annual, biannual or triannual sinusoidal components, each with a characteristic amplitude and phase. Seasonal climatic variables of this type have been shown to be good predictors of patterns in a range of vectors and vector-borne diseases (Fichet-Calvet and Rogers, 2009; Guerra et al., 2008; Randolph, 2000; Rogers, 2000; Rogers et al., 2002) including BTV outbreaks and vectors (Purse et al., 2007; Tatem et al., 2003).

Variable	**Description**
MIN	Minimum
MAX	Maximum
MEAN	Mean
A1	Annual Amplitude
A2	Biannual Amplitude
A3	Triannual Amplitude
P1	Annual Phase
P2	Biannual Phase
P3	Triannual Phase
V	Variance

Table 3.3. Measures of seasonality derived from seasonal Fourier variables provided by Scharlemann et al. (2008)

(D) **Terrain/Soil Variables** - The altitude, aspect and slope at sample sites were derived from digital elevation data from the USGS/NASA Shuttle Radar Topography Mission (SRTM) [Global 30 Arc-Second Elevation Data Set (GTOPO30)] (USGS, 2007). From this aspect and slope rasters were created using ESRI Spatial Analyst (ESRI, 2009). Aspect is a circular variable, with the constraint that 0° and 360° represent the same direction. The aspect at each sampling point was therefore calculated using the ArcTangent2 function in Excel (Microsoft Corporation, 2003), according to the mean angle trigonometric approach that transforms aspect from cosine (northness) and sine (eastness) variables. Variables describing the slope and altitude of areas have has previously been found to be useful in predicting adult *C. imicola* and *C. obsoletus* s.l. abundance in Italy (Conte et al., 2007a,b).

The soil characteristics at sampling sites were derived from the EU-JRC European Soil Database (EU-JRC, 2009), from which the dominant surface textural class and the dominant annual average soil water profile were derived and considered to be biologically relevant to *Culicoides* abundance and occurrence with regards to the distribution and abundance of suitable larval development sites. Soil type has previously been found to be useful in predicting adult *C. imicola* and *C. obsoletus* s.l. abundance in Italy (Conte et al., 2007a). The dominant surface textural class summarises the top soil horizon into one of six classes [see Table 3.4 below] the first five of which were present at study sites:

Factor Level	**Description**
TEXTURE 1	No mineral texture (peat soils)
TEXTURE 2	Coarse (18 % < clay and > 65% sand)
TEXTURE 3	Medium (18 % < clay < 35 % and ≥ 15 % sand, or 18 % < clay and 15 % < sand < 65 %)
TEXTURE 4	Medium fine (< 35 % clay and < 15 % sand)
TEXTURE 5	Fine (35 % clay < 60 %)
TEXTURE 6	Very fine (clay > 60 %)

Table 3.4. SOIL.TEXTURE factor levels and descriptions

The dominant annual average soil water profile summarises the top soil horizon into one of four classes [see table 3.5 below] all of four of the classes were present at study sites:

Factor Level	Description
PROFILE 1	Not wet within 80 cm for over 3 months, nor wet within 40 cm for over 1 month
PROFILE 2	Wet within 80 cm for 3 to 6 months, but not wet within 40 cm for over 1 month
PROFILE 3	Wet within 80 cm for over 6 months, but not wet within 40 cm for over 11 months
PROFILE 4	Wet within 40 cm depth for over 11 months

Table 3.5. SOIL.PROFILE factor levels and descriptions

3.2.4 Approach to Modelling BTV Outbreak Distribution and Vector Abundance

The importance of different predictors amongst the host, landscape, climate and terrain/soil variable suites in discriminating between areas of BTV outbreak presence-absence, was assessed using generalized linear modelling with a logistic link function assuming a binomial error distribution and a hierarchical variable selection approach. Logistic regression estimates the odds of a certain event occurring as the logistic function is bounded by 0 and 1, i.e. between the probability of the event never occurring and definitely occurring. Logistic regression does not assume linearity of relationship between the independent variables and the dependent, does not require normally distributed variables, and does not assume homoscedasticity, although linearity in the logit is required.

For *C. imicola*, *C. obsoletus* s.l. and *C. pulicaris* s.l. log transformed abundance was related to environmental variables from the four variable suites (host, landscape climate and terrain/soil) using generalized additive models (GAMs). GAMs are semi-parametric extensions of generalized linear models (GLMs) and permit both linear and more complex response functions between individual species and predictor variables to be fitted, as well as a combination of the two within the same model (Hastie and

Tibshirani, 1987).

Within spatial data sets, observations that are close in space tend to be more similar than observations that are far apart, i.e. data are spatially correlated. Calvete et al. (2009) previously found that spatial autocorrelation significantly influenced patterns in BTV-4 outbreak occurrence in Spain. It was expected that BTV outbreak occurence and vector abundance in this study would also be spatially autocorrelated. Hence, a Moran's I test was performed on the residuals of the final model for each species/species group and the BTV outbreak occurrence data [R package 'ape' (Paradis et al., 2004)], using neighbourhood sizes of 50 km, 100 km and 200 km. Values of the Moran's I are assessed by a test statistic (the Moran's I standard deviate) which indicated the statistical significance of spatial autocorrelation in the model residuals compared to the expected similarity between sites in the absence of spatial autocorrelation [expected $I = \frac{1}{(n-1)}$, where n is the sample size] (Moran, 1950).

Significant collinearity (correlation between variables) was expected within variable suites. Failure to deal with collinearity leads to an increase in type II errors i.e. the failure to reject the null hypothesis when it is untrue. Variance Inflation Factors (VIF) of over 10 were used as a rule of thumb to indicate collinearity in the data (Montgomery and Peck, 1992). To reduce the initial predictor set to variables that were uncorrelated with each other, correlation both within and between explanatory variable suites was assessed using VIF's [R package 'car' (Fox, 2009)]. Where correlation between predictor variables was detected, the covariate with the highest VIF was dropped and the VIFs recalculated. This process repeated until all VIF's were smaller than 10.

3.2.4.1 Approach to Modelling BTV Outbreak Occurrence

Following the removal of correlated variables (see results Table 3.6 on page 75), remaining variables from the four variable suites (host, landscape, climate and terrain/soil) were incorporated into the outbreak model using a hierarchical modelling approach. Mann-Whitney, Pearson chi-square or Fisher exact tests were first used to

test for significant ($P \leq 0.05$) associations between BTV outbreak occurrence status and predictor variables within the four suites (host, landscape, climate, terrain/soil). For the landscape suite, variables were included in the next step if a variable had a significant relationship in at least one buffer scale. Non-significant variables were excluded from further analyses. Following these steps binary logistic regression GLMs were then fitted using maximum likelihood estimation [R package 'RMS' (Harrell, 2009)]. First, to asses which landscape buffer scale would best describe BTV outbreak risk, remaining landscape variables were assessed, one model per buffer scale, [$\text{Landscape}_{1\,\text{km}}$; $\text{Landscape}_{2\,\text{km}}$; $\text{Landscape}_{5\,\text{km}}$]. Following this, all variables from the most predictive, highest bias-corrected D_{xy} (see below), landscape buffer scale were then used again in a global model combined with variables from the other three variable suites (host, climate, terrain/soil) [see results Table 3.6 on page 75].

The Wald test for significance [R package 'RMS' (Harrell, 2009)] was used to identify variables whose relationship showed significant deviations from a linear trend, and therefore invalidated the assumption of linearity in the logit required for logistic regression. Predictors were kept as quantitative variables wherever possible, and were coded using two tertiles into low, medium and high values when necessary with the lowest tertile serving as the reference group [see results Table 3.6 on page 75].

Variable selection within each model was achieved using a backwards-stepwise selection procedure on the basis of Akaike's Information Criterion (AIC) [R package 'RMS' (Harrell, 2009)]. AIC is defined as the *deviance+2*p*, where p is the number of parameters of the model (Akaike, 1973). Variables that did not contribute significantly to predicting outbreak occurrence were successively eliminated, until a minimum adequate model was produced (Crawley, 2002).

To account for over-optimistic classification measures when validating the model on the training data (Miller et al., 1991), the bootstrap resampling procedure was used for model validation to obtain bias-corrected (i.e. overfitting-corrected) estimates of D_{xy} rank correlation (Somers, 1962) (10,0000 bootstrap samples) which is indicative of the models discriminative ability. D_{xy} is defined as *2*(C-0.4)*, where C

is the concordance coefficient. The concordance coefficient describes the probability of concordance between predicted and observed occurence, with C = 0.5 for random predictions and C = 1 for a perfectly discriminating model (Harrell, 2001). D_{xy} ranges from -1 (indicating perfect status misclassification, where all absences are classified as present or vice versa) to 1 (perfect classification); values >0.6 indicate good discrimination (Manel et al., 1999). The bias-corrected D_{xy} produces a more conservative, estimate of the models discriminative ability (Harrell, 2001, 2009) by correcting the D_{xy} of the original model by a measure of the optimism in the variable selection, i.e. as a measure of model overfitting caused during stepwise selection of variables. Nagelkerke generalization of R^2 for maximum likelihood-based models (Nagelkerke, 1991) was also used to describe the variance explained by the model. Pseudo R^2 indexes generally do not take on a full 0-1 range as the Nagelkerke R^2 index is adjusted relative to the Cox and Snell R^2 index, so that it can attain a maximum value of 1, allowing it to become an appropriate measure of goodness-of-fit. The predictive accuracy of the final models were additionally assessed using the Receiver Operating Characteristic (ROC) Area Under the Curve (AUC) and its associated sensitivity and specificity [R package 'epi' (Carstensen et al., 2009)]. ROC and AUC are based on the concept of class-dependent accuracy, which may be tabulated through a confusion matrix indicating the true positive (TP), false positive (FP), false negative (FN), and true negative (TN) predictions. Given a model M(h) and a parameter h, the points on the ROC curve are defined, at different values of h, by the sensitivity, or true positive rate [TP/(TP + FN)], obtained as a function of the 1-specificity indicator, or false positive rate [FP/(FP + TN)]. The AUC is a measure of the area under this curve, ranging from 0.5 (random accuracy) to a maximum value of 1, that represents the most accurate model theoretically achievable. ROC analysis assess the trade off between the probability of the false positive fraction against the probability of the false negative fraction. The greater the AUC the more discriminating the model is and the closer the predictions are to the observed data.

The relative effects of the predictor variables in the final model selected by back-

wards stepwise selection were assessed using Odds Ratios (OR) and their 95 % confidence intervals (OR <1 negative effect OR >1 positive effect). A hierarchical partitioning analysis using log-likelihood as the goodness-of-fit measure on the final selected model was also conducted [R package 'heir.part' (Mac Nally and Walsh, 2004)] to estimate the independent explanatory power of each selected predictor variable and the influence of the different variable suites assessed in predicting BTV outbreak occurence (Chevan and Sutherland, 1991; Mac Nally, 2000).

3.2.4.2 Approach to Modelling BTV Vector Abundance

After the removal of correlated variables, separately for each species / species group, log-transformed [$\log(x+1)$; x = abundance] maximum abundance was related to the uncorrelated environmental variables [VIFs < 10] (see results Table 3.9 on page 81) using GAMs with a Gaussian error structure. To evaluate the importance of different variable suites, a hierarchical modelling approach was adopted. Firstly the subset of variables was selected - within each of the variable suites - that best explained the variation in abundance of each species. Secondly, these subsets of variables were combined across variable suites into a global model and the selection procedure repeated to produce a final model for each species.

A smoothing spline with four degrees of freedom was used to smooth predictor variables. Variables that contributed significantly to explaining variation in abundance were successively included on the basis of AIC using a forward stepwise selection procedure [R package 'GAM' (Hastie, 2008)]. In this approach continuous predictor variable considered [see Table 6.3 on page 189] may either appear not at all, linearly, linearly in its logarithm, or as a smooth function estimated non-parametrically, while categorical predictors [see Table 6.3 on page 189] were either allowed to appear or not appear. AIC in the stepwise GAM is defined as the $deviance+2*df\phi$, where df is the effective degrees of freedom and ϕ is the dispersion parameter (variance) (Hastie, 1992). The small number of sampled sites precluded partitioning of the species dataset into a calibration and evaluation dataset, therefore the overall fit of models was eval-

uated using the percentage of deviance explained (D^2) (Weisberg, 1980). Significant differences between factor levels of selected soil variables were assessed using multiple Tukeys all-pair comparisons [R package 'multcomp' (Hothorn et al., 2008)].

3.3 Results

3.3.1 BTV Outbreak Occurrence

Moran's I tests were insignificant (P>0.05) for BTV outbreak occurrence at all neighbourhood sizes assessed suggesting that spatial autocorrelation has either no or only a minor influence on patterns of BTV outbreak occurrence at the scale of this study. The examination of the variance inflation factors within and between variable suites, however, did lead to the omission of eight variables from the binary logistic regression of BTV outbreak occurrence which were the minimum and maximum values of the four climatic variables considered [dLST.MIN, dLST.MAX, nLST.MIN, nLST.MAX, EVI.MIN, EVI.MAX, MIR.MIN, MIR.MAX], these variables were highly correlated with the mean value of the respective climate variable. A further 29 variables [16 landscape; 9 climate; 4 terrain/soil] were removed following univariate screening. Additionally non-linear responses were detected [see Section 3.2.4.1 on page 69] for variables ED.25 at the 2 km landscape scale, PLAND.12, COHESION.18 and COHESION.23 at the 5 km landscape scale and dLST.V. These variables were split using two tertiles into low, medium and high values (with the lowest tertile serving as the reference group), to achieve normality in the logit, as required by binary logistic regression. Hence, following the removal of collinear variables and univariate variable screening, a total of 16 variables from the landscape suite were considered and subjected to stepwise selection within the three landscape models. A total of 43 variables from the four variable suites [3 host; 16 landscape; 23 climate; 1 terrain/soil] were considered within the global model and subject to stepwise selection [see Table 3.6 on page 75].

Following stepwise selection of variables within the landscape variable suite, a significant increase in discrimination ability was achieved with increasing buffer scale with model Landscape 5 km having the highest scores for bias-corrected D_{xy} and AUC [see table 3.7 on page 76] [see Appendix A Section A.1 on page 255 for variables selected during the stepwise-selection procedure for landscape-only models]. The

global model, therefore, included landscape variables measured over a 5 km buffer zone.

Variable Suite			
Host	**Landscape**	**Climate**	**Terrain/Soil**
CATTLE	LSI	dLST.MEAN	SLOPE
SHEEP	PRD	dLST.P2	
GOATS	PLAND.12 (non-irrigated arable land)	dLST.P3	
	PD.12 (non-irrigated arable land)	dLST.V	
	ED.12 (non-irrigated arable land)	nLST.MEAN	
	COHESION.12 (non-irrigated arable land)	nLST.A2	
	PLAND.18 (pastures)	nLST.A3	
	PD.18 (pastures)	nLST.P2	
	ED.18 (pastures)	nLST.P3	
	COHESION.18 (pastures)	nLST.V	
	PLAND.23 (broad-leaved forest)	EVI.A1	
	COHESION.23 (broad-leaved forest)	EVI.A2	
	PLAND.25 (mixed forest)	EVI.A3	
	PD.25 (mixed forest)	EVI.P1	
	ED.25 (mixed forest)	EVI.P2	
	COHESION.25 (mixed forest)	EVI.P3	
		EVI.V	
		MIR.A1	
		MIR.A2	
		MIR.A3	
		MIR.P1	
		MIR.P2	
		MIR.P3	

Table 3.6. Variables included in the analysis of BTV outbreak occurrence in Greece and Bulgaria (after the exclusion of variables with VIFs > 10 and univariate variable screening), all variables continuous

The global model achieved a significantly higher level of discriminated between, the observed BTV outbreak presence-absence as measured by the bias-corrected D_{xy} and AUC, compared to the 'best' landscape only model [see Table 3.7 on page 76]. Levels of optimism as a result of the stepwise selection of variables were acceptably low for all models (≤ 0.1). The results of the variance partitioning, of the final global model, showed that selected variables from the climate, landscape and host suites explained 63.06 %, 31.71 % and 5.51 % of the total variance explained by the model respectively, however, no one variable was dominant in determining BTV outbreak risk [see Table 3.8 on page 77].

Model	D_{xy}[a]	Optimism[b]	R^{2}[d]	AUC[e]	Sensitivity[f]	Specificity[g]
$\text{Landscape}_{[Scale:1\,\text{km}]}$	0.420	0.056	0.122	0.710	63.3	77.3
$\text{Landscape}_{[Scale:2\,\text{km}]}$	0.446	0.065	0.132	0.755	60.6	82.6
$\text{Landscape}_{[Scale:5\,\text{km}]}$	0.540	0.061	0.232	0.801	66.8	81.1
Global Model	0.673	0.101	0.131	0.361	84.0	78.0

[a] Bias-corrected Somers Dxy rank correlation [D_{xy} = *2(c 0.5)*]

[b] A measure of model overfitting and the difference between the training and testing values for D_{xy}

[c] Bias-corrected maximum absolute error in between predicted and calibrated probabilities

[d] Nagelkerke generalization of R^2 for maximum likelihood-based models

[e] Receiver Operating Characteristic (ROC) - Area Under Curve (AUC)

[f] Sensitivity of Receiver Operating Characteristic (ROC)
(proportion of true positives which are correctly identified)

[g] Specificity of Receiver Operating Characteristic (ROC) models)
(proportion of true negatives which are correctly identified)

Table 3.7. Fit statistics from internal bootstrap validation and receiver operating characteristic of models of BTV outbreak occurrence in Greece and Bulgaria

Within the final global model, one host, four landscape and 10 climate variables were selected as providing the best level of discrimination between observed BTV presence-absence [see Table 3.8 on page 77]. Only the negative effect of an increasing local cattle population was selected from within the host suite as a significant predictor of BTV risk [see Table 3.8 on page 77].

No metrics at the landscape-level were found to be significant predictors of BTV outbreak risk, however, class-level metrics associated with all four land cover classes considered in the stepwise selection were considered to have a significant effect on the probability of BTV outbreak occurrence. BTV outbreak risk was negatively impacted on by an increasing proportion of non-irrigated arable land within the landscape (PLAND.12) and as the total length of edge of mixed woodland decreased in the landscape (ED.25). BTV outbreak risk increased, however, as areas of both pastures (COHESION.18) and broad-leaved woodland (COHESION.23) became more physically connected, i.e. less fragmented, within the landscape [see Table 3.8 on page 77]. Of the variables considered in the stepwise selection from the climate suite the 10 variables selected described patterns in the seasonality of temperature, moisture

availability and vegetation activity (dLST, nLST, EVI and MIR). Increasing variability in day-time temperature (dLST) and increase stability in night-time temperature (nLST) both increased BTV risk [see Table 3.8 below]. The positive impact of both the annual amplitude and overall variance of the enhanced vegetation index indicate that the annual cycle of vegetation growth, and specifically a major change between summer and winter values, significantly increased BTV risk. No variables from the terrain/soil variable suite were retained as significant predictors of BTV outbreak occurrence during the stepwise selection of variables.

Variable Suite	**Variable**	**OR (95 % CI)**	**% of Variance Explained**
Host	Cattle	0.71 (0.53;0.93)	5.51
Landscape	PLAND.12 (non-irrigated arable land)	0.71 (0.53;0.93)	8.03
	ED.25 (mixed forest)	0.46 (0.33;0.65)	8.09
	COHESION.18 (pastures)		18.44
	Low Values [0.00 - 5.9]	3.80 (2.80;4.80)	
	Medium Values [[5.9 - 84.8]	2.60 (1.75;3.45)	
	High Values [84.8 - 95.8]	2.17 (1.29;3.04)	
	COHESION.23 (broad-leaved forest)		9.04
	Low Values [0.0 - 90.2]	3.80 (2.80;4.80)	
	Medium Values [90.2 - 96.3]	2.51 (1.64;3.37)	
	High Values [96.3 - 100.0]	2.94 (2.12;3.76)	
Climate	dLST.P3	1.33 (1.01;1.75)	3.93
	dLST.V		14.02
	Low Values [0.0000 to 0.0087]	3.80 (2.80;4.80)	
	Medium Values [0.0087 to 0.0107]	2.60 (1.75;3.45)	
	High Values [0.0107 to 0.0158]	2.17 (2.29;3.04)	
	nLST.P2	0.18 (0.06;0.59)	13.70
	EVI.A1	0.14 (0.02;0.83)	3.48
	EVI.P1	0.42 (0.18;0.96)	4.73
	EVI.P3	1.75 (1.12;2.68)	6.13
	EVI.V	6.46 (1.36;30.76)	3.15
	MIR.P3	2.46 (1.20;5.06)	2.11

Table 3.8. Variables selected in the final models, selected by backwards stepwise selection, for the prediction of bluetongue outbreak occurrence in Greece and Bulgaria with Odds Ratios (OR) and their 95 % confidence intervals (OR <1 negative effect OR >1 positive effect) and the percentage of variance explained per variable.

3.3.2 BTV Vector Abundance

Bluetongue virus vectors were recorded at 185 of the sample sites in Greece and Bulgaria. Of these sites *C. obsoletus* s.l. was recorded at 171 and *C. pulicaris* s.l. at 176 of the 185 sample sites. These species groups were found to co-occur at 163 of the sites. *Culicoides imicola* was only present at 17 of the 185 sites, at 13 of these it was found to co-occur with both *C. obsoletus* s.l. and *C. pulicaris* s.l., while at two sites it was found to co-occur with just *C. obsoletus* s.l., at one site with just *C. pulicaris* s.l., and at the remaining site it was the only BTV vector detected. The maximum catch of females was 15,039 for *C. obsoletus* s.l., 2,699 for *C. pulicaris* s.l. and 762 for *C. imicola* [see Figure 3.5, 3.6 and 3.7]. Although *C. obsoletus* s.l. was the most, and *C. imicola* the least abundant, species / species group they displayed more variation in abundance (coefficient of variation = 5.02 and 8.58 respectively), compared to *C. pulicaris* s.l. (coefficient of variation = 2.72).

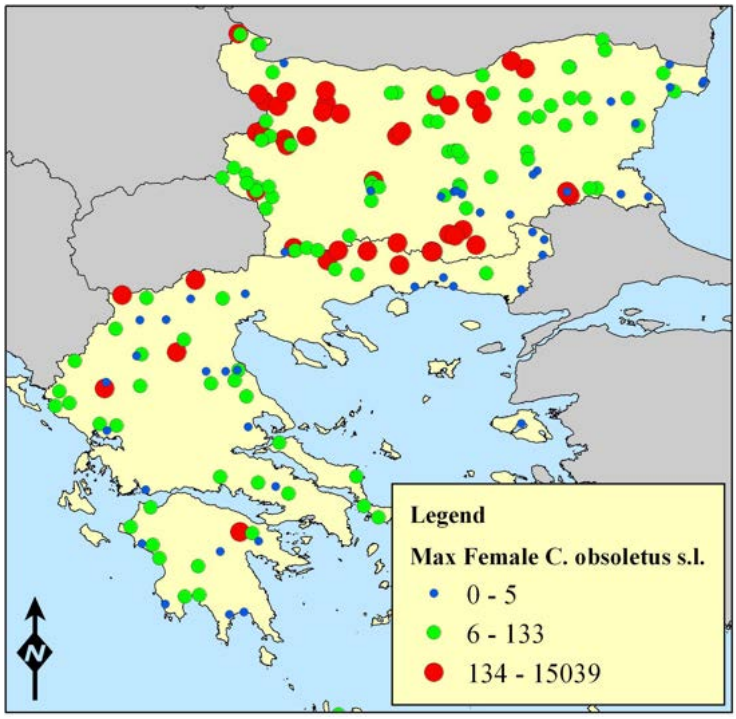


Figure 3.5. Spatial distribution of female *C. obsoletus* s.l. collections displayed with cut points at the 1^{st} and 3^{rd} quartile of the maximum number of females collected at each site for Greece and Bulgaria

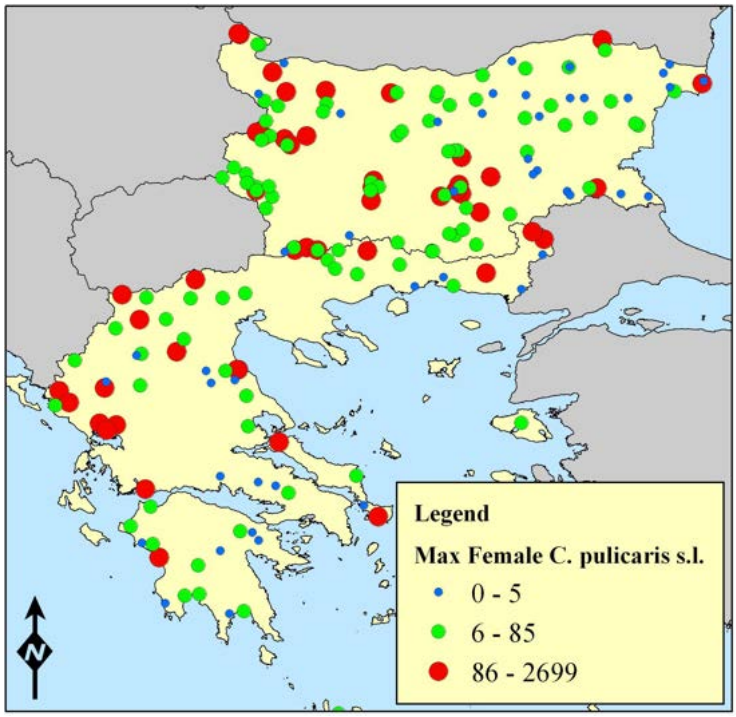


Figure 3.6. Spatial distribution of female *C. pulicaris* s.l. collections displayed with cut points at the 1^{st} and 3^{rd} quartile of the maximum number of females collected at each site for Greece and Bulgaria

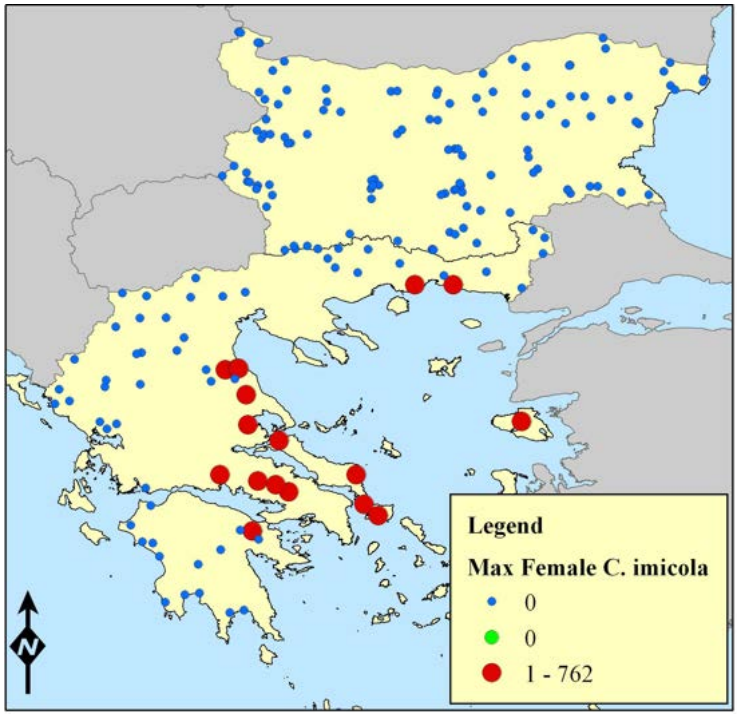


Figure 3.7. Spatial distribution of female *C. imicola* collections displayed with cut points at the 1^{st} and 3^{rd} quartile of the maximum number of females collected at each site for Greece and Bulgaria

Moran's I tests were insignificant for all vector species and groups and neighbourhood sizes suggesting that spatial autocorrelation has either no or only minor influence on patterns of BTV vector abundance at the scale of this study. The examination of the variance inflation factors within and between variable suites, however, did lead to the omission of 10 climatic variables [dLST.MIN, dLST.MAX, dLST.MEAN, nLST.MIN, nLST.MAX, nLST.MEAN, EVI.MIN, EVI.MAX, MIR.MIN, MIR.MAX] and nine landscape variables [LSI, ED.12, ED.16, ED.18, ED.20, ED.21, ED.23, ED.25, ED.26] from the GAMs of species / species group abundance. Hence, following removal of collinear variables, the stepwise selection of variables within the GAMs for each species / species group considered a total of 65 variables from the four variable suites [3 host; 27 landscape; 30 climate; 5 terrain/soil] [see Table 3.9 on page 81].

Forward-stepwise selection of environmental variables found *C. obsoletus* s.l., *C. pulicaris* s.l. and *C. imicola* abundance to be best described by semi-parametric models, which explained 36.88 %, 18.84 % and 31.61 % of the variance in abundance respectively [see Table 3.10 on page 82]. Overall the best performing abundance models, based on a single set of predictors were climatic models, followed by landscape models [see Appendix A Section A.2 on page 256 for variables selected in the within variable suite models]. Interestingly, some impacts of landscape variables were scale dependent between species / species groups. *Culicoides obsoletus* s.l., *C. pulicaris* s.l. and *C. imicola* abundance were best described by landscape variables measured over a 5 km, 2 km and 1 km buffer region respectively [see Appendix A Section A.2 on page 256]. The global abundance models that combined different suites of environmental predictors, however, outperformed those based on single suites of environmental predictors, explaining an additional 7 % to 34 % in *C. obsoletus* s.l. abundance, an additional 11 % to 17 % in *C. pulicaris* s.l. abundance and an additional 4 % to 22 % in *C. imicola* abundance.

Variable Suite			
Host	**Landscape**	**Climate**	**Terrain/Soil**
CATTLE	LSI	dLST.A1	ALTITUDE
SHEEP	MEAN.GYRATE	dLST.A2	SLOPE
GOATS	PRD	dLST.A3	ASPECT
	PLAND.12 (non-irrigated arable land)	nLST.A1	SOIL.TEXTURE* †
	PD.12 (non-irrigated arable land)	nLST.A2	SOIL.PROFILE* $^{\triangle}$
	COHESION.12 (non-irrigated arable land)	nLST.A3	
	PLAND.16 (fruit trees and berry plantations)	EVI.MEAN	
	PD.16 (fruit trees and berry plantations)	EVI.A1	
	COHESION.16 (fruit trees and berry plantations)	EVI.A2	
	PLAND.18 (pastures)	EVI.A3	
	PD.18 (pastures)	MIR.MEAN	
	COHESION.18 (pastures)	MIR.A1	
	PLAND.20 (complex cultivation patterns)	MIR.A2	
	PD.20 (complex cultivation patterns)	MIR.A3	
	COHESION.20 (complex cultivation patterns)	dLST.P1	
	PLAND.21 (agriculture with natural vegetation)	dLST.P2	
	PD.21 (agriculture with natural vegetation)	dLST.P3	
	COHESION.21 (agriculture with natural vegetation)	nLST.P1	
	PLAND.23 (broad-leaved forest)	nLST.P2	
	PD.23 (broad-leaved forest)	nLST.P3	
	COHESION.23 (broad-leaved forest)	EVI.P1	
	PLAND.25 (mixed forest)	EVI.P2	
	PD.25 (mixed forest)	EVI.P3	
	COHESION.25 (mixed forest)	MIR.P1	
	PLAND.26 (natural grassland)	MIR.P2	
	PD.26 (natural grassland)	MIR.P3	
	COHESION.26 (natural grassland)	dLST.V	
		nLST.V	
		EVI.V	
		MIR.V	

Table 3.9. Variables included in the analysis of BTV vector abundance in Greece and Bulgaria (after the exclusion of variables with VIFs > 10**) * indicates a categorical variable, all other variables continuous**
[† **SOIL.TEXTURE factor levels: 1 - No mineral texture (peat soils); 2 - Coarse (**$18\,\%$ **<** $<$ **clay and** $>$ **65% sand); 3 - Medium (**$18\,\%$ $<$ **clay** $<$ $35\,\%$ **and** $\geq$ $15\,\%$ **sand, or** $18\,\%$ $<$ **clay and** $15\,\%$ $<$ **sand** $< 65\,\%$**); 4 - Medium fine (**$< 35\,\%$ **clay and** $< 15\,\%$ **sand); 5 - Fine (**$35\,\%$ **clay** $< 60\,\%$**); 6 Very fine (clay** $> 60\,\%$**)] [**$^{\triangle}$ **SOIL.PROFILE factor levels: 1 - Not wet within** $80\,\mathrm{cm}$ **for over 3 months, nor wet within** $40\,\mathrm{cm}$ **for over 1 month; 2 - Wet within** $80\,\mathrm{cm}$ **for 3 to 6 months, but not wet within** $40\,\mathrm{cm}$ **for over 1 month; 3 - Wet within** $80\,\mathrm{cm}$ **for over 6 months, but not wet within** $40\,\mathrm{cm}$ **for over 11 months; 4 - Wet within** $40\,\mathrm{cm}$ **for over 11 months**]

Species / Species Group	**Variable**	Estimate	± S.E.	Δ AIC	D^2
C. obsoletus s.l.	Intercept	-5.26	(1.29)***	69.13	36.88
	s(LSI, 4)	0.86	(0.25)***		
	COHESION.12	-0.01	(<0.01)**		
	nLST.A1	0.52	(0.12)***		
	nLST.A2	-0.54	(0.43)$^{\triangle}$		
	log(ALTITUDE + 1)	0.44	(0.11)***		
C. pulicaris s.l.	Intercept	1.09	(0.75)	12.61	18.84
	s(COHESION.20, 4)	<0.01	(<0.01)		
	PLAND.26	0.02	(0.02)		
	s(EVI.A3,4)	20.04	(10.82)$^{\triangle}$		
	SOIL.TEXTURE 2	1.38	(0.76)$^{\triangle}$		
	SOIL.TEXTURE 3	2.03	(0.80)**		
	SOIL.TEXTURE 4	2.07	(0.80)**		
	SOIL.TEXTURE 5	1.98	(0.85)*		
C. imicola s.l.	Intercept	1.54	(0.57)**	42.30	31.61
	s(LSI, 4)	-0.19	(0.12)		
	s(MIR.A2, 4)	-43.93	(9.12)**		
	MIR.MEAN	3.72	(2.12)$^{\triangle}$		
	EVI.A2	6.83	(3.24)*		
	SOIL.TEXTURE 2	-0.99	(0.37)**		
	SOIL.TEXTURE 3	-1.06	(0.37)**		
	SOIL.TEXTURE 4	-1.03	(0.37)**		
	SOIL.TEXTURE 5	-1.09	(0.42)**		

Table 3.10. Coefficient estimates with their standard error shown in parenthesis of environmental variables selected by a forward-stepwise procedure for general additive models with a Gaussian error structure for female BTV vector species / species groups [$^{\triangle}$ P≤0.1, *P≤0.05, **P≤0.01, *** P≤<0.001] [non-parametric terms indicated as *s(name of the predictor, numbers of degrees of freedom)*], ‡ see Figure 3.8, 3.9 and 3.10 for fitted smooth terms. [† SOIL.TEXTURE factor levels: 1 - No mineral texture (peat soils); 2 - Coarse (18 % < clay and > 65% sand); 3 - Medium (18 % < clay < 35 % and ≥ 15 % sand, or 18 % < clay and 15 % < sand < 65 %); 4 - Medium fine (< 35 % clay and < 15 % sand); 5 - Fine (35 % clay < 60 %); 6 Very fine (clay > 60 %)]. Land cover classes: 12 - non-irrigated arable land; 20 - complex cultivation patterns; 26 - natural grasslands

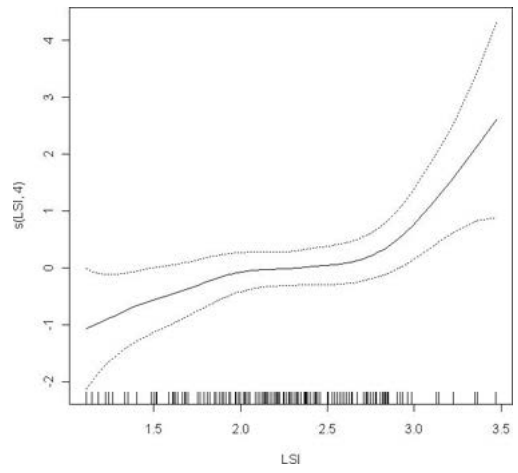
	1	2	3	4	5
1	-	1.38	2.03*	2.06	1.98$^{\triangle}$
2	-0.99*	-	0.65	0.68	0.6
3	-1.06*	-0.07	-	0.03	-0.05
4	-1.03$^{\triangle}$	-0.04	0.03	-	-0.08
5	-1.09$^{\triangle}$	-0.1	-0.03	-0.1	-

Table 3.11. Multiple Tukeys all-pair comparisons of vector abundance between different soil textures, estimates for *C. pulicaris* s.l. shown on upper diagonal, *C. imicola* shown on lower diagonal [P≤0.1 $^{\triangle}$, *P≤0.05, **P≤0.01, ***P≤0.001], SOIL.TEXTURE factor levels: 1 - No mineral texture (peat soils); 2 - Coarse (18 % < clay and > 65% sand); 3 - Medium (18 % < clay < 35 % and ≥ 15 % sand, or 18 % < clay and 15 % < sand < 65 %); 4 - Medium fine (< 35 % clay and < 15 % sand); 5 - Fine (35 % clay < 60 %); 6 Very fine (clay > 60 %)

Seasonal correlates of both temperature and vegetation activity, measures of fragmentation of land cover patches in the landscape, the cohesion of areas of non-irrigated land and natural grasslands in the landscape, altitude and the relative proportion of sand and clay particles in the top horizon of the soil were all found to significantly influence BTV vector abundance. Significant differences in the effects of these predictors on the abundance of the three BTV vector species / species groups considered were, however, observed [see Table 3.10 on page 82].

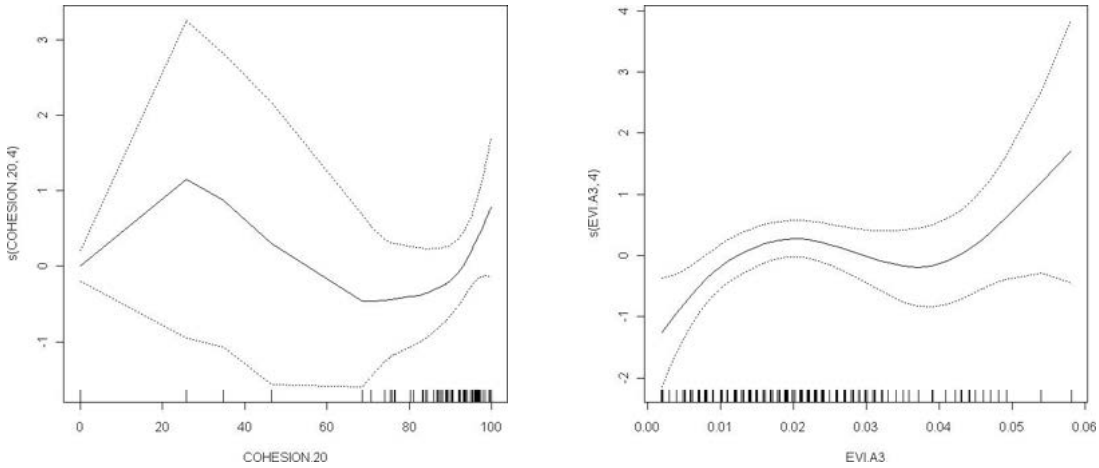
Culicoides obsoletus s.l. abundance was negatively impacted by increasing cohesion of areas of non-irrigated pasture land within the landscape (COHESION.12) and positively impacted by increasing altitude (ALTITUDE). Night-time land surface temperature also had a significant effect on *C. obsoletus* s.l. abundance with a positive effect of increasing annual amplitude (nLST.A1) and a negative effect of increasing biannual amplitude (nLST.A2). In contrast to *C. pulicaris* s.l., the monotonically increasing effect of landscape shape index on *C. obsoletus* s.l. abundance, positively impacted abundance at low and high values, however, medium levels had little effect [see Figure 3.8a on page 84]. *Culicoides pulicaris* s.l. abundance was positively impacted by both an increasing proportion of natural grasslands within the landscape (PLAND.26) and at high levels an increasing cohesion of complex cultivation patterns (COHESION.20). Increasing triannual amplitude in enhanced vegetation index pos-

itively impacted on *C. pulicaris* s.l. abundance at low and high levels, however, had a slight negative effect at medium levels. The underlying soil texture, related to the proportion of clay and sand particles within the top horizon of the soil, was also found to be a significant driver of both *C. imicola* and *C. pulicaris* s.l. abundance, but not *C. obsoletus* s.l. abundance. In contrast to *C. pulicaris* s.l., *C. imicola* abundance was negatively impacted, by peat based soils (SOIL.TYPE 1) in comparison to other soil types [see Table 3.11 on page 83]. *Culicoides imicola* abundance was also positively impacted by increasing mean levels of middle infrared reflectance (MIR.MEAN) and an increasing amplitude of the enhanced vegetation index (EVI.A2). Biannual amplitude of middle infrared reflectance (MIR.A2), however, had a monotonically decreasing effect on *C. pulicaris* s.l. abundance. Very low and very high levels of fragmentation of patches of different land cover types (LSI) had a positive effect on *C. pulicaris* s.l. abundance, i.e. a high abundance of *C. pulicaris* s.l. was associated both with landscapes with large areas of a single land cover type that are dominant in the landscape and with areas that are highly fragmented with long total edge lengths. Medium levels of landscape fragmentation (LSI), however, had a negative impact on abundance [see Figure 3.10a on page 85].



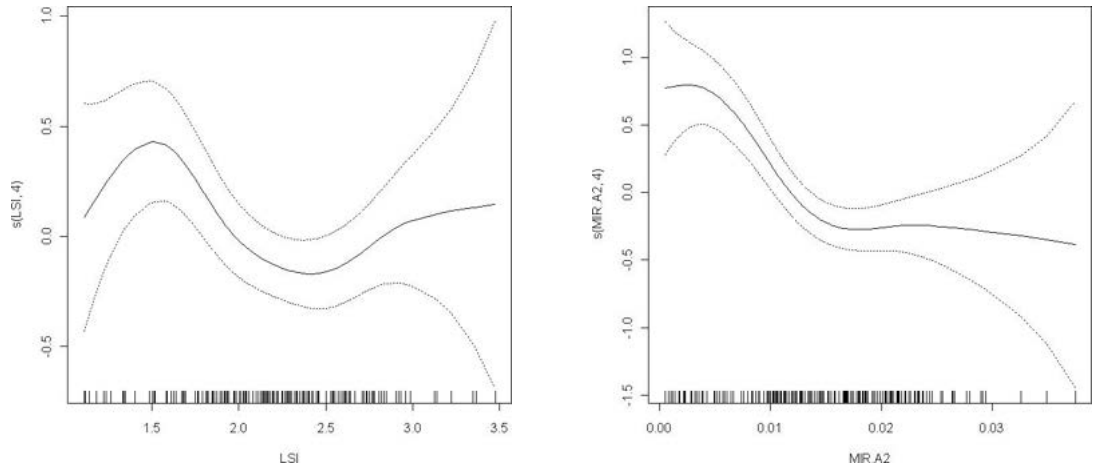
(a). **LSI**

Figure 3.8. Fitted smooth terms (solid lines) [indicated as *s(name of the predictor, numbers of degrees of freedom)*] and 95 % confidence intervals (dashed lines) for landscape shape index for semi-parametric models of *C. obsoletus* s.l. abundance. Ticks in the x-axis represent location of observations along the predictor



(a). **COHESION.20** (b). **EVI.A3**

Figure 3.9. Fitted smooth terms (solid lines) [indicated as *s(name of the predictor, numbers of degrees of freedom)*] and 95 % confidence intervals (dashed lines) for (a) cohesion of complex cultivation patterns (COHESION.20) and (b) triannual amplitude of enhanced vegetation index (EVI.A3) for semi-parametric models of *C. pulicaris* s.l. abundance. Ticks in the x-axis represent location of observations along the predictors



(a). **LSI** (b). **MIR.A2**

Figure 3.10. Fitted smooth terms (solid lines) [indicated as *s(name of the predictor, numbers of degrees of freedom)*] and 95 % confidence intervals (dashed lines) for (a) landscape shape index (LSI) and (b) biannual amplitude of middle infrared reflectance (MIR.A2) for semi-parametric models of *C. imicola* abundance. Ticks in the x-axis represent location of observations along the predictors

3.4 Discussion

Studies undertaken within Europe have demonstrated that climatic variables alone are useful in modelling spatial occurrence and, therefore, the potential distribution area of BTV (Baylis and Mellor, 2001; Calvete et al., 2009; Purse et al., 2004b; Tatem et al., 2003; Wittmann et al., 2001). Guis et al. (2007), however, demonstrated that other non-climatic features could be of use in explaining spatial variation in BTV risk. Conte et al. (2007b) demonstrated that non-climatic factors could improve predictions of vector abundance. The current study, however, is the first to investigate the relative roles of climate, landscape and host factors together in determining BTV outbreak risk and vector abundance. The usefulness of the predictors considered within this study for predicting BTV outbreak presence-absence, suggests that they would act as useful correlates of environmental processes promoting or depressing vector abundance within the same region. Models of vector abundance, however, failed to explain a large degree of the observed variation in both Palaearctic vector and *C. imicola* abundance [*C. obsoletus* s.l. 37 %; *C. pulicaris* s.l. 19 %; *C. imicola* 32 %]. The results of this analysis should be therefore interpreted with caution. The variables selected as significant determinants of vector, however, do indicate that *C. imicola*, *C. obsoletus* s.l. and *C. pulicaris* s.l. have differing optimal ranges of environmental conditions.

Consistent with previous models of BTV outbreak (Calvete et al., 2009; Tatem et al., 2003) and vector occurrence (Baylis et al., 1998; Conte et al., 2007a; Purse et al., 2004a), this study found climatic correlates, related to the measures of both temperature (day-time and night-time LST) and moisture availability/vegetation structure (EVI and MIR) to be useful climatic predictors for determining BTV outbreak occurrence and vector abundance. Key climatic variables factors identified as being useful in discriminating areas of BTV outbreak presence-absence were common with those identified as significant drivers of BTV vector abundance. It is likely that these factors can be related to the thermal limits on virus replication (Mullens et al., 1995; Wittmann and Baylis, 2000) and vector: activity (Carpenter et al., 2008d), develop-

ment (Bishop et al., 1996) and survival (Bishop et al., 1996; Lysyk, 2007). Moisture levels are a significant determinant of the abundance, size and quality of breeding habitats in an area and therefore affect vector bionomics.

Host availability within an area was a significant determinant of BTV outbreak occurrence, with an increasing local populations of cattle negatively impacting on BTV risk. This may reflect host preference within the BTV vector species present in Greece and Bulgaria, however, no host factors were retained as significant predictors of vector abundance. Cattle, sheep and goats are all BTV susceptible, but clinical symptoms are generally restricted to the fine wool and mutton breeds of sheep (Verwoerd and Erasmus, 2004). Sub-clinical presentation of BTV in cattle is likely to have led to under-reporting of BTV in this species, and hence the apparent negative relationship observed between cattle densities and the probability of an outbreak. The lack of extensive sero-surveillance in many European countries, including the UK, mean sub-clinical manifestation of BTV infection may lead to substantial errors in the observed prevalence rate of BTV infection and therefore the assumed absence of disease used to train predictive models of BTV outbreak risk (Guis et al., 2007). The analyses of BTV outbreak occurrence should therefore be interpreted with caution. The dataset of BTV occurrence utilised in this study, however does contain information on true absences of BTV, obtained from serosurveys of sentinel herds, during the same time period as outbreaks were recorded. This is in comparison to other studies which have had to assume farms where BT had not been reported were BT free (Guis et al., 2007) or resort to generating pseudo-absence data (Calvete et al., 2009).

In contrast to climatic variables, the key landscape variables identified as being useful in discriminating areas of BTV outbreak presence-absence were not common with those identified as significant drivers of BTV vector abundance. Guis et al. (2007) hypothesised that high values of lengths of edges (LSI) of woodlands, open prairies and impervious surfaces, indicating increased fragmentation of these land covers within the landscape on sheep farms in Corsica where *C. imicola* is considered the primary vector of BTV, were associated with increased BT risk because of increased vector-

host contact. The present study, however, does not support this suggestion, since increased cohesion of broad-leaved woodland and pasture within the landscape were significant promoters of BTV risk and increasing levels of fragmentation overall in the landscape for the majority of observations in this study also negatively impacted *C. imicola* abundance. In contrast, however, overall levels of fragmentation in the landscape positively impacted on *C. obsoletus* s.l. abundance.

The positive impact on BTV outbreak risk of increased aggregation of pasture land and a decreasing proportion of arable land within the landscape is likely due to these variables describing increasingly suitable areas for sustaining an abundance of BTV susceptible hosts. The negative impact of an increasing proportion of arable land within the landscape on BTV outbreak risk, may also be related to a lack of suitable larval development sites within arable areas [see Chapter 4 on page 91]. The importance of woodland in determining BTV occurrence may be related to the general habitat requirements of the BTV vectors. In particular, in Italy, *C. obsoletus* s.l. is considered to thrive in shaded forest habitats which are likely to contain abundant areas of suitable moist, organically enriched larval development sites (Conte et al., 2007a). At a species / species group level, however, the influence of the underlying soil texture may have better expressed the larval habitat preferences of the individual species. In Cyprus, Mellor and Pitzolis (1979b) noted that the larval development sites favoured by *C. imicola* were generally drier than those preferred by other species, an observation that is supported by this study, with *C. imicola* abundance, in contrast to *C. pulicaris* s.l., negatively impacted by the presence of moisture retentive peat based soils.

In contrast to the global model containing climatic, landscape and host variables, the landscape only models had a higher specificity (accuracy in predicting true negatives) than sensitivity (accuracy in predicting true positives). This may have been as a result of the geographically restricted area over which absence data was collected, resulting in a more tightly constrained range of landscapes being present in the absence data than in the presence data, leading to over-fitting of the model. The

model optimism, i.e. the degree of over fitting to the data, however, was in general low for all models. Calvete et al. (2009) found that locations had a higher probability of being affected with BTV-4 if some of the neighbouring locations within a 200 km radius were also affected. Despite no spatial structure in outbreak occurrence being detected within this study, caution must be exercised in assuming that the natural expansion of BTV within Greece and Bulgaria was complete. BTV transmission may have been limited by restrictions imposed on animal movements or other control measures implemented in the region (Nomikou et al., 2004) and given that transmission was probably vastly under-reported due to circulation in disease resistant animals (Purse et al., 2006), it is likely that many localities in which BTV was not detected might well possess the same degree of environmental suitability for BTV as those in which the virus was found.

3.5 Conclusions

The success of utilising non-climatic environmental correlates in discriminating areas of BTV outbreak presence from absence, indicate their usefulness in describing patterns and relationships in BTV transmission in addition to those already described for climatic correlates (Baylis and Mellor, 2001; Calvete et al., 2009; Purse et al., 2004a,b; Tatem et al., 2003). To date, however, models including the current study, have in describing Palaearctic vector abundance failed to explain a significant proportion of the spatial variation in the observed abundances. This may be due to the fact that all these models were trained on group level, rather than species-specific, vector data and annual population estimates from only a few catches in a season (Baylis et al., 1997). All of these studies, including this one also suffer from limitations as a consequence of utilising light-based vector surveillance, which has been found to provide a biased estimate of species composition or abundance of the Palaearctic vectors (Carpenter et al., 2008d). Significant difference may exist in the species-environment relationships of the constitute species of the Obsoletus and Pulicaris species group confounding the identification of environmental relationships when as-

sessed at the group level. Thus, there is an urgent need to develop species-specific datasets of vector abundance using sampling techniques that can accurately estimate vector populations in a host-equivalent manner. In addition, field-based assessments of the impact of habitat type on both adult and larval bionomics are required in order to uncover the biological basis of the environmental relationships suggested through statistical models. In attempts to provide these biological explanations, the larval development sites utilised by Palaearctic BTV vector populations, and the climatic and non-climatic factors driving the occurrence of these sites are discussed in Chapter 4.

Chapter 4

Distribution and Control of Larval BTV Vector Populations in Southern England

4.1 Introduction

Culicoides larvae are neither genuinely aquatic nor terrestrial, but occupy the ecotone between the two (Kettle, 1962). There is published and anecdotal evidence of *Culicoides* larvae using a wide range of substrates and habitat types for breeding, the relative abundance of species of the Obsoletus and Pulicaris group within and between these different substrates has, however, received little attention. There has only been a single review study of the larval development sites of the UK *Culicoides* fauna; Kettle and Lawson (1952) produced a general classification of UK *Culicoides* larval development sites divided into six broad habitat types based on qualitative assessments of land cover, moisture levels and soil type [bog-land, fresh water marsh, swamp, mud, salt water marsh, and dung]. The classification of Kettle and Lawson (1952) was based on larval collections across the UK, however, there was limited allowance for inter-specific variation with only a relatively small number of larval development sites identified for each species. The classification is therefore likely to severely underestimate the range of larval habitats used by *Culicoides* species.

Only a few records of larval collections of the Palaearctic BTV vector groups have been made in Europe. *Culicoides obsoletus* s.s. larval development sites have been identified in collections made from boggy moorland (Cameron, 1946), marshy soils and ditches (Hill, 1947), areas of acid grassland (Kettle, 1961), muddy puddles with limited vegetation (Schwenkenbecher et al., 2009), watering holes near wet vegetated ditches (Schwenkenbecher et al., 2009), decaying vegetable matter (Edwards et al., 1939; Kettle and Lawson, 1952), maize silage residues (Zimmer et al., 2008), garden waste compost heaps (Kettle and Lawson, 1952) rotting banana stumps (Mellor and

Pitzolis, 1979a) and damp debris within tree holes (Edwards et al., 1939; Hill, 1947). *Culicoides obsoletus* s.s. larvae have also been identified using sheep and horse dung as a larval development substrate (Edwards, 1926; Edwards et al., 1939; Kettle and Lawson, 1952). *Culicoides obsoletus* s.s., however, have not to date been found associated with cattle dung. Conversely *C. dewulfi* (Campbell and Pelham-Clinton, 1960; Schwenkenbecher et al., 2009) and *C. chiopterus* (Campbell and Pelham-Clinton, 1960; Kremer, 1965) larvae have been identified as using cattle dung as a larval development substrate. *Culicoides chiopterus* larvae have even been found in dry cakes of cow dung in livestock pasture (Campbell and Pelham-Clinton, 1960). Sap running from wounds in elm trees (Edwards et al., 1939) and damp trenches (Goetghebuer, 1936) have also been reported to contain small numbers of *C. chiopterus* larvae. *Culicoides scoticus* has been reared from samples of several fungus species [see Table 4.1 on 92]. However, the relatively large numbers of *C. scoticus* which can be recovered in light traps indicates that additional more abundant substrates must also be utilised for larval development. *Culicoides obsoletus* s.s., has also been identified as breeding in decaying fungi but again this is only likely to be of secondary importance (Edwards, 1928). *Culicoides scoticus* larvae have also been reported from mud ruts (Kremer, 1965), maize silage residues (Zimmer et al., 2008) and emergence traps set up in marshy areas (Boorman, 1986).

Fungus Species	**Reference**
Lactarius turpis	(Campbell and Pelham-Clinton, 1960; Buxton, 1960)
Armillaria mellea	(Campbell and Pelham-Clinton, 1960)
Pleurotus cornucopia	(Buxton, 1960)
Russula ochroleuca	(Buxton, 1960)
Daedalia biennis	(Buxton, 1960)
Hypholoma fasciculare	(Buxton, 1960)
Armillaria mellca	(Buxton, 1960)
Boletus bovinus	(Buxton, 1960)

Table 4.1. Fungal species from which *C. scoticus* Downes and Kettle larvae have been reared

Culicoides pulicaris s.s. larvae have been identified in areas of bare mud (Campbell and Pelham-Clinton, 1960), among green algae (Edwards, 1926; Edwards et al., 1939), mud bordering streams and ponds (Kremer, 1965), damp and marshy areas (Goetghebuer, 1919), boggy moorland (Cameron et al., 1946) and even in tree holes (Galli-Valerio, 1925). *Culicoides punctatus* larvae have been identified in areas of organically enriched bare mud (Nielsen, 1964) and in areas of marsh and muddy swamp (Kettle and Lawson, 1952). Small waterlogged areas and meadows in Issyk-Kul, Kyrgyzstan have also been found to contain *C. punctatus* larvae (Konurbayev, 1965). More recently Uslu and Dik (2007) have described *C. pulicaris* s.s. larvae developing in moist soil with organic matter in Konya province, Turkey.

The relative abundances of species of the Obsoletus and Pulicaris group within and between these different substrates has received very little attention. Kettle and Lawson (1952) found that *C. obsoletus* s.s. was equally abundant in both bog (*Sphagnum* sp., *Polytrichium* sp. and *Juncus articulus*) and freshwater habitats, with the larvae from the bog-land found in areas being particularly common where a relatively thin layer of moss was present (<6 inches). It should be noted that the larval habitat associations of Buxton (1960), Campbell and Pelham-Clinton (1960), and Kettle and Lawson (1952) were based on morphological identification of *Culicoides* larvae using descriptions (Kettle and Lawson, 1952) that are now of questionable accuracy [as discussed in Chapter 2 Section 2.3.1 on page 36].

4.1.1 Factors Governing the Occurrence of *Culicoides* Larvae within Substrates

Despite the questions surrounding past identification and the paucity of studies into *Culicoides* larval development sites, it is clear that British *Culicoides* species vary in their larval habitat preferences. The presence or absence of these habitats will vary between different farm types and within individual farms, contributing to spatial variation in species composition and abundance at a local-scale. Evidence from well-studied *Culicoides* species suggests that larval development site suitability is governed

by a range of inter-related soil, microclimatic and vegetation factors. The presence of *C. sonorensis* Wirth and Jones larvae in the United States, where it is the primary vector of both BTV and EHDV, has been correlated with high levels of salt forming ions and indicators of salinity, with high organic content, and high phosphate and nitrate pollution within farmland habitats (Battle and Turner, 1972; Kardatzke and Rowley, 1971; Schmidtmann et al., 1998). High densities of *C. impunctatus* larvae have been correlated with qualitative measures of land cover, including the presence of rushes (*Juncus* species) and bog myrtle (*Myrica gale*) (Blackwell et al., 1994). *Culicoides impunctatus* larval counts, within these habitats, have also been found to be positively correlated with quantitative variables including: a slightly acidic soil pH (pH values of 5 to 6.5) (Blackwell et al., 1999), the presence of high organic content in the soil and a high soil wetness index (Blackwell et al., 1994). Non-climatic environmental factors including farm management may also influence suitability of a specified area for larval development (Jones, 1977; Mullens and Rodriguez, 1988). The identification of ecological correlates of the larval habitats of Palaearctic BTV vector species may improve BTV transmission risk assessment and enable targeting of larval control measures.

4.1.2 Larvicidal Control of *Culicoides*

The larval habitats of *C. imicola* in the Mediterranean Basin are relatively easy to identify (Braverman et al., 1974; Capela et al., 1993; Mellor and Pitzolis, 1979a). The arid summer climate produces dry soil in farmyards so that areas wet enough to support semi-aquatic larvae can usually be readily demarcated. Vector control programmes using insecticides have been used at BTV infected and 'at-risk' premises in several southern European countries (Nomikou et al., 2004; Satta et al., 2004). These programmes were, however, not carried out in a targeted manner, instead they used wholesale spraying of adulticides and larvicides across farmyards, buildings and waterways, without consideration of the habitats used by larval or adult *Culicoides*. Targeted treatment of larval development sites would increase both the success rate

and cost-effectiveness of control programmes, while reducing their environmental impact. In northern Europe, however, moist breeding areas are not so easily demarcated, since levels of moisture are much higher on average across habitats, making it more difficult to distinguish areas containing larvae. In order to develop an ecologically sound pest management scheme to reduce vector population and to help mitigate the risk BTV transmission in the UK, the larval habitats of the Palaearctic BTV vectors require accurate quantification.

Currently, control measures aimed at the reduction or destruction of larval *Culicoides* habitats, may be divided into three main categories, (1) chemical, (2) biological and (3) mechanical. The potential danger to human health of organophosphates (Coggon, 2002; De Silva et al., 2006; Kamanyire and Karalliedde, 2004; Minton and Murray, 1988), and the possible dangers of organochlorines to non-target organisms (Ginsberg, 1945; Sandholzer, 1945) are now well documented and until more species-specific formulations and application techniques are developed, larvicidal chemical control methods are unlikely to be used on a wide scale to reduce transmission of BTV or any other non-zoonotic vector-borne disease in the UK. The development of resistance is also a fundamental problem of relying excessively on a chemical control strategy (Brogdon and McAllister, 1998; Hemingway et al., 2004). Cross-resistance has already developed in larval populations of *C. furens* Poey, *C. melleus* Melander and Brues and *C. mississippiensis* Hoffman, whose salt-marsh breeding habitats had been treated with organochlorine insecticide to control the biting nuisance to humans (Clements and Rogers, 1967; Smith and Davies, 1959).

Biological control refers to methods where entomophagous and entomogenous organisms are used by man, either in manipulated or natural forms, to suppress a pest species (Van Den Bosch and Stern, 1962). The most promising biological vector control agent developed so far is the Gram-positive, soil-dwelling bacterium *Bacillus thuringiensis* serovar *israelensis* (Bti) strain, and a related bacterium *B. sphaericus Neide*, which have been widely used as larvicides against mosquito larvae [for review see Lacey (2007)]. Since, impacts on non-target organisms are few (deBarjac, 1990)

the use of these bacteria represents an appealing alternative to chemical methods of larval control [for review see Carpenter et al. (2008a)]. There are, however, very few listed predators or pathogens of *Culicoides*, although insect-pathogenic fungi have been found to cause high mortality in *C. nubeculosus* larvae in laboratory based exposure assays (Ansari et al., 2010; Unkles et al., 2004). Initial laboratory trials have shown Bti to be ineffective against field collected larvae of *C. impunctatus* (Blackwell and King, 1997), *C. mississippiensis*, *Culicoides guttipennis* Coquillett (Kelson et al., 1980) and colony reared *C. sonorensis* and *Culicoides occidentalis* Wirth and Jones (Kelson et al., 1980). No trials, however, have been conducted to assess the efficacy or practicality of using biological control agents for larval *Culicoides* control in the field.

Mechanical control methods involve either the eradication or biological alteration of larval development sites, in order to render them no longer suitable for larval development, or only capable of supporting small populations of *Culicoides* larvae. Eradication is the most appropriate objective only for very localised potential breeding sites which may develop in small artificial water sources for example, leaking taps and overflowing water troughs. Large-scale habitat modification methods, however, have been successfully used for the control of salt-marsh mosquitoes with only minor adverse impacts on the environment (Dale, 2008). For the Palaearctic BTV vectors, which utilise a diverse range of semi-aquatic larval habitats (see above) there may be limits in practicality in attempting large-scale modifications, both economically and in terms of their environmental impact. With regards to BTV control mechanical modifications of larval development sites, can be achieved through alteration of farm husbandry management practices such as coving muck heaps to target the coprophilic *Culicoides* species (Campbell and Pelham-Clinton, 1960). Such methods are likely to have a reduced impact on the ecology of non-target organisms while still being an effective anti-larval strategy for *Culicoides* and have been recommended as a suitable control measure by DEFRA (DEFRA, 2009b). Little information, however, exists on the effect of mechanical control methods on *Culicoides* populations as a whole.

Waste and water management strategies and their limitations have been investigated for their potential for controlling *C. sonorensis* in the USA (Jones, 1977; Mullens and Rodriguez, 1988). This research focused on the efficacy of draining water trough overflows and dairy waste water evaporation beds. Larvae were found to prefer the gentle slope of polluted habitats, with direct animal access (Schmidtmann et al., 1983) rather than pond edges with slopes of >30° (Mullens and Rodriguez, 1988).

4.1.3 Justification for Research

It is hypothesised that an improved understanding of vector habitat use at a local-scale will improve BTV risk assessment. Further research is therefore required to define suitable habitat types for larval development for the Palaearctic BTV vector species and subsequently, to assess the efficacy of currently recommended methods for livestock-associated larval *Culicoides* control (DEFRA, 2009b). It is hypothesised that the habitat characteristics of larval Obsoletus and Pulicaris group species, defined using ground-based or remotely assessed GIS-based data, can be used to predict the occurrence of these species at the within-farm scale. This hypothesis was investigated through the use the following field-based approach in the south east of England to:

(1) Characterise potential larval development sites for the Palaearctic BTV vector species, through detailed entomological surveys of occurrence and abundance of livestock-associated larval *Culicoides* populations.

(2) Identify which environmental factors, describing variation in temperature, moisture availability, vegetation activity and land cover type and structure are important in driving patterns in the occurrence and abundance of *Culicoides* larvae. To be achieved by matching spatial patterns of larval occurrence and abundance with patterns in these environmental factors.

(3) Determine whether any of the key variables driving habitat suitability can be altered by currently available vector control techniques. If a suitable target for control is identified to carry out a field trial of the selected method and assess its effect on the areas adult *Culicoides* vector population.

4.2 Materials and Methods

4.2.1 Spatial Patterns in Occurrence and Abundance of *Culicoides* Larvae

The spatial pattern in occurrence and abundance of larval *Culicoides* was investigated at six farm sites, A-F [see Section 2.1 on page 28 for further details]. Occurrence of *Culicoides* larvae in each habitat was determined using two methods: (*1*) collection of substrate samples, which were then subject to sugar flotation for the direct extraction of larvae [using the methodology described in Section 2.2.4 on page 34], and (*2*) the use of emergence traps to collect newly emerged adult *Culicoides* directly above their larval development sites [using the methodology described in Chapter 2 Section 2.2.5 on page 35].

Ground surveys for the collection of larvae using substrate samples [method (*1*)] were conducted at 150 sampling points in the period between November and February in 2006 and 2007 [20 sampling points were sampled in winter 2006; 130 sampling points in winter 2007]. During this period the larvae of *C. obsoletus* s.l. and *C. pulicaris* s.l. are considered to over-winter as 4^{th} instar larvae. Within samples *Culicoides* larvae were morphologically identified and separated from other soil organisms using the methodology described in Chapter 2 Section 2.3.1 on page 36. Collected larvae were sent for identification to species level at the University of Aberdeen, as described in Chapter 2 Section 2.4 on page 40.

For method (*2*), emergence traps were left *in situ* at 180 sampling points, each for 14 days, between late April and early May to coincide with the expected peak in *Culicoides* emergence [60 sampling points were sampled in spring 2007; 120 sampling points were sampled in spring 2008]. The mean minimum distance to the nearest neighbouring sampling point within farms for method *1* was 80.2 m and for method *2* was 87.4 m. Within all insect samples, the collected adult *Culicoides* were morphologically identified to species/species group level [see Chapter 2 Section 2.3 on page 36 for methodology], then Obsoletus group females were identified to species level, as

C. obsoletus s.s., *C. scoticus*, *C. dewulfi*, *C. chiopterus*, using a multiplex PCR [see Chapter 2 Section 2.4 on page 40 for methodology].

Geographic coordinates for all sampling points were recorded using a GPS unit [eTrex Legend C (Garmin,US), spatial error 3-5 m] in the British National Grid projection (OSGB 1936). Each sampling point was visually classified into one of 10 habitat categories [see Table 4.2 on page 101]. The habitat categories used represent the broad variations in habitat observed on the six farms, and are representative of common habitat/substrate types present on livestock farms in the south east of England. The number of sample points per habitat class was approximatly proportional to the abundance of that habitat class across the six farms. Habitat classes were evenly distributed across the four farm sites [30 emergence trap sampling points per farm]. A minimum of five sampling points per habitat class were used to account for variation between the sampling points. Some concessions for sample point selection had to be made during the positioning of emergence traps to ensure the traps would remain undamaged by livestock activity and would not impede commercial activities on the farms.

4.2.2 Quantifying Environmental Conditions Surrounding Sampling Points

Variables describing environmental conditions at sampling points were selected based on biological plausibility of a relationship with the occurrence of larval development sites and on their usefulness as predictors of *Culicoides* larval occurrence and abundance within previous studies (Battle and Turner, 1972; Bishop et al., 1994, 1995; Blackwell et al., 1994, 1999; Kardatzke and Rowley, 1971; Schmidtmann et al., 1998; Uslu and Dik, 2010).

In addition to ground-based measures of environmental condition made at the time of sampling, to facilitate future comparisons of drivers of larval livestock-associated *Culicoides* distribution in other areas of the UK and for the potential in the future to generate of risk maps of larval occurrence within farms, land-cover and soil type classifications and remotely-sensed climate data were used to quantify environmental

conditions surrounding each sampling point. These remote, GIS-based, measures of environmental conditions are available as spatially continuous data across the UK.

4.2.2.1 Ground-Based Measures of Environmental Conditions

In addition to the direct measures of habitat/land cover type present at sampling points [see Table 4.2 on page 101], quantitative measures of soil temperature, moisture level and pH were made directly during ground surveys according to the methodology described below. For soil samples collected for larval extraction, measurements were made in the field at the time the sample was collected. For emergence trap sampling point measurements were made at day one (when the traps were set out), at day 14 (when the traps were collected), and summarised as the mean level for each trapping period.

Substrate pH and Temperature

Substrate pH and temperature were measured *in situ* using a HI-99121 soil test pH meter (Hanna Instruments, Leighton Buzzard, UK) [pH +/- 0.01, temperature +/- 0.1 ℃]. This unit automatically corrects the measure of pH for substrate temperature. A small plastic auger was used to create a space for insertion of the probe into the ground to a depth of approximately 5 cm. Following stabilisation of the reading, the pH and soil temperature (in ℃) was recorded.

Substrate Moisture level

The volumetric water content of the soil was measured *in situ* using a ECH20 check meter hand-held reader with EC-5 5 cm probe (Labcell, Alton, UK) as an indicator for soil moisture level. The probe was inserted to a depth of approximately 3 cm to 5 cm, dependent upon substrate depth, and the reading on the check meter allowed to stabilise before being recorded. The substrate moisture level was recorded as a volume percentage in cm·m [accuracy level = 3 %].

Habitat Class	Class Name	Class Description
1	**Open Pasture**	Exposed areas of improved grassland which have been affected by heavy grazing, drainage and/or the application of herbicides, inorganic fertilisers, slurry or high doses of manure. Species composition is limited to those species of grass and common forbs resistant to grazing and often demanding in nutrients.
2	**Wooded Pasture**	Sheltered areas at the margins of improved grassland. Grass and other common pasture species present, however, may be sparsely distributed relative to open pasture. Shelter provided by hedgerows formed of native woody species with a low density of trees greater than 5 m high when mature, with limited leaf litter accumulation.
3	**Arable**	Arable crop land not permanently irrigated
4	**Broadleaved Woodland Leaf Litter**	Areas of vegetation dominated by broadleaved trees more than 5 m high when mature, forming a distinct, although sometimes open canopy, understorey and ground layer with 10 % or less of the canopy made up of coniferous trees. Substrate dominated with dry to moist decaying leaf litter.
5	**Broadleaved Woodland Vegetation**	Areas of vegetation dominated by broadleaved trees more than 5 m high when mature, forming a distinct, although sometimes open canopy, understorey and ground layer with 10 % or less of the canopy made up of coniferous trees. Vegetated substrate dominated by grass species and other low-growing woodland flora.
6	**Coniferous Woodland Ground Substrate**	Vegetation dominated by coniferous trees more than 5m high when mature, forming a distinct closed canopy, understorey and ground layer with 10 % or less of the canopy made up of deciduous trees. Substrate is sparsely vegetated due to closed canopy, some decaying needle drop present.
7	**Marginal Vegetation Surrounding Open Water**	Areas of permanently wet substrate with varying levels of emergent vegetation cover, less than 1 m tall, occurring on the margins of lowland watercourses. The water table is permanently high with an open water source existing beyond the limits of the emergent vegetation, which may contain submerged, free-floating or floating-leaved vegetation. Included areas surrounding lakes, reservoirs, flooded gravel pits, ponds and water filled ditches.
8	**Surrounding Artificial Water Sources**	Localised areas of more or less permanently wet, bare to partially vegetated substrate, created though the periodic inundation of areas surrounding artificial sources of water including leaking taps and overflowing water troughs.
9	**Muck Heaps**	Areas used for the large scale storage of cattle manure and associated bedding.
10	**Organically Enriched Substrate**	Areas immediately surrounding muck heaps/cattle barns, which are subject to high organic pollution related to run off from cattle manure.

Table 4.2. Habitat classes and associated descriptions in which *Culicoides* larvae occurrence and abundance was assessed [modified from the JNCC Phase 1 Habitat Survey classifications (JNCC, 2003)]

4.2.2.2 Remote Measures of Environmental Conditions

Remote, GIS-based, measures of environmental conditions surrounding sampling points were quantified using three suites of ecological correlates: landscape, climate and terrain/soil.

Variables contained within the three variable suites were as follows:

(A) **Landscape Variables** - Landscape structure was characterised based on 250 m circular buffers around sampling points from land cover data derived from the Land Cover Map 2000 (LCM2000) provided by the NERC Centre for Ecology and Hydrology (Fuller et al., 2002a,b). LCM2000 is a thematic classification of spectral data from satellite imagery and incorporates some external data to add context and refine the classification. LCM2000 consists of 16 target classes of land cover types that can be further sub-divided into 27 subclasses to allow broad habitats (i.e. habitats of biodiversity importance under the UK Biodiversity Action Plan) to be mapped across the UK.

Landscape structure within the buffer regions surrounding each sampling point was quantified using the spatial pattern analysis program 'Fragstats' (McGarigal and Marks, 2002). Metrics were calculated at two levels: one for selected land cover classes found in a buffer (class-level metrics) and the other for the whole landscape, i.e. for the entire buffer regardless of the class (landscape-level metrics). Two landscape level metrics: landscape shape index (LSI), Simpson's diversity index (SIDI), were calculate and two class level metrics (per selected land cover class): percentage of landscape covered by patches of class (PLAND.X) and class landscape shape index (LSI.X) (where X is the selected land cover class) [see Table 4.3 on page 103 for metric descriptions]. Class level metrics were calculated for selected agriculture associated land cover types, which were abundant in the areas surrounding samples sites and were expected *a priori* to influence the variation in larval livestock-associated abundance, attributable to differences in the overall availability of suitably moist substrates. Land cover classes selected included: broad-leaved mixed woodland (class 1.1),

arable cereals (class 4.1), arable horticulture (class 4.2), improved grassland (class 5.1), set-aside grassland (class 5.2) and calcareous grassland (class 7.1) and inland bare ground (class 16.1). Selected metrics provide measures of the degree of coverage, fragmentation and diversity within and between different land cover classes.

Variable	Name *(Metric Level; Metric Type)*	Definition
Landscape Level Metrics		
LSI	**Landscape Shape Index** *(Area-Density-Edge Metric)*	Provides a standardized measure of total edge or edge density that adjusts for the size of the landscape. LSI can also be interpreted as a measure of patch aggregation or disaggregation. Specifically, as LSI increases, the patches in the landscape are increasingly disaggregated.
SIDI	**Simpson's Diversity Index** *(Diversity Metric)*	The value of Simpson's index represents the probability that any 2 pixels selected at random would be different land cover types. SIDI = 0 when the landscape contains only 1 patch (i.e. no diversity) and approaches 1 as the number of different land cover class increases and the proportional distribution of area among patch types becomes more equitable
Class Level Metrics		
PLAND.X*	**Percentage of Landscape Covered by Patches of Class X*** *(Area-Density-Edge Metric)*	Quantifies the proportional abundance (in %) of land cover type X* in the landscape.
LSI.X*	**Landscape Shape Index of Class X*** *(Area-Density-Edge Metric)*	A simple measure of the extent of subdivision or fragmentation of land cover type X* within the landscape.

* X represents each of the selected land cover classes

Table 4.3. Selected landscape and class level metrics calculated using the Fragstats landscape pattern analysis software (McGarigal and Marks, 2002)

(B) **Climate Variables** - Variables describing the conditions of temperature and soil moisture at sample sites, that can have a large impact on *Culicoides* life cycles, were extracted from two pansharpened Landsat 7 ETM+ satellite images (15 m resolution), one representing leaf-off (winter) and one leaf-on (spring) conditions [see Chapter 2 Section 2.5.1 on page 41]. From each of these images the following measures of environmental conditions at sampling points were derived: Land Surface Temperature (LST.leaf.on; LST.leaf.off), EVI (EVI.leaf.on; EVI.leaf.off), Tassel Cap Transformation (TC.BRIGHT.leaf.on; TC.BRIGHT.leaf.off; TC.WET.leaf.on; TC.WET.leaf.off; TC.GREEN.leaf.on; TC.GREEN.leaf.off). A complete description of the Landsat satellite data used, and their processing, is provided in Chapter 2 Section 2.5 on page 40.

Land surface temperature (LST) provides a general index of the apparent surface temperature (whether soil or vegetation) (Zhengming, 1999) and has previously been useful in predicting the distributions of *Phlebotomus orientalis* (Thomson et al., 1999) and *C. imicola* (Purse et al., 2004a,b, 2007; Tatem et al., 2003) and the abundance of *C. imicola* (Baylis et al., 1999). EVI is an optical measure of surface vegetation conditions (Tucker et al., 2005) i.e. the vegetation canopy 'greenness', a composite property of leaf chlorophyll, leaf area, canopy cover and canopy architecture (Jiang et al., 2008), that has been correlated with levels of soil moisture (Chen et al., 2006; Waring et al., 2006). Vegetation indices have previously been useful in predicting *C. imicola* distribution (Purse et al., 2004a,b, 2007; Tatem et al., 2003). EVI in particular has previously been useful in predicting Dengue fever cases (Fuller et al., 2009) and the distribution of *Borrelia burgdorferi* infected tick distribution (*Ixodes ricinus* sp.) in relation to Lyme disease risk. Tasselled cap indices have been correlated to the texture and moisture content of soils (Crist and Cicone, 1984; Crist, 1986; Crist et al., 1986) and have previously been useful in predicting tick (*Ixodes scapularis* Say) abundance (Rogers and Mather, 2006) and survival (Ogden et al., 2006) in relation to Lyme disease transmission and in predicting

the risk from *Aedes aegypti* transmitted dengue (Rotela et al., 2007) and for mapping mosquito larval habitats in relation to WNV transmission (Zou et al., 2006).

(C) **Terrain/Soil Variables** - Terrain characteristics at samples sites were derived from the Ordnance Survey (OS) Land-Form Profile 1:10,000 Digital Elevation Model. Land-form profile tiles covering the area of interest were sourced from the Edina Digimap service (EDINA, 2002) as separate tiles in NTF format. ESRI Map Manager 9.1 (ESRI, 2006b) was used to convert these individual files from the NTF format to individual ArcGrid rasters. To allow processing of the elevation data within ArcGIS, the OS DEM tiles, were mosaiced using ArcINFO Workstation (ESRI, 2006a) to form one Arc Grid raster for the area of interest. The vertical units in the resultant ArcGRID represents elevation in metres above mean sea level. Terrain characteristics were summarised from this ArcGrid as the altitude, aspect and slope at sampling points using ESRI Spatial Analyst (ESRI, 2009). Aspect is a circular variable, with the constraint that 0° and 360° represent the same direction. The aspect at each sampling point was therefore calculated using the ArcTangent2 function in Excel (Microsoft Corporation, 2003), according to the mean angle trigonometric approach that transforms the wind direction from cosine (northness) and sine (eastness) variables.

The dominant soil characteristic at sampling points was derived from the NATMAP Soilscapes spatial dataset (1:250,000 scale) (NSRI, 2009). Soilscapes is a simplified rendition of the national soil map (Mackney et al., 1983; Proctor et al., 1998), with soil type, drainage and texture characteristics separated into 30 distinct, ecologically relevant, soil classes. Of these 30 classes five soil classes were found at the sampling points, freely draining slightly acid but base-rich soils (SOIL.TYPE_1), freely draining slightly acid loamy soils (SOIL.TYPE_2), shallow lime-rich soils over chalk or limestone (SOIL.TYPE_3), lightly acid loamy and clayey soils with impeded drainage (SOIL.TYPE_4), slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils (SOIL.TYPE_5).

4.2.3 Approach to Modelling the Spatial Patterns in *Culicoides* Larvae Occurrence

The importance of different predictors amongst the landscape, climate and terrain/soil variable suites in discriminating between areas of immature *Culicoides* presence absence at the local-scale were assessed at the two life stages sampled, larval and emergence. Models of *Culicoides* occurrence at these two stages were fitted using generalized linear modelling with a logistic link function assuming a binomial error distribution [see Chapter 3 Section 3.2.4 on page 68 for further details on logistic regression].

Within spatial data sets, observations that are close in space tend to be more similar than observations that are far apart, i.e. data are spatially correlated. It was expected *a priori* that spatial autocorrelation would influence patterns in *Culicoides* larval occurrence at the local-scale. A Moran's I test was performed on the residuals of the final model of *Culicoides* larval occurrence [R package 'ape' (Paradis et al., 2004)], using neighbourhood sizes of 50 m, 100 m and 500 m. [see Chapter 3 Section 3.2.4 on page 68 for further details of how Moran's I is calculated].

Significant collinearity (correlation between variables) was expected *a priori* within variable suites. Failure to deal with collinearity leads to an increase in type II errors i.e. the failure to reject the null hypothesis when it is untrue. To reduce the initial predictor set to variables that were uncorrelated with each other, correlation both within and between explanatory variable suites was assessed using Variance Inflation Factors (VIF) [R package 'car' (Fox, 2009)]. VIF's of over 10 were used as a rule of thumb to indicate collinearity in the data (Montgomery and Peck, 1992). Where correlation between predictor variables was detected, the covariate with the highest VIF was dropped, the VIFs recalculated. This process repeated until all VIFs were smaller than 10.

Following the removal of correlated variables (see results, Table 4.7 on page 119) two binary logistic regression GLMs were then fitted per life stage assessed, larval and emergence, using maximum likelihood estimation [R package 'RMS' (Harrell,

2009)]. One model containing ground-based variables, the second containing remote, GIS-based, variables from the three variable suites (host, landscape, climate and terrain/soil) [see results Table 4.7 on page 119 for variables used in each model]. The Wald test for significance [R package 'RMS' (Harrell, 2009)] was used to identify variables whose relationship to immature *Culicoides* occurrence showed significant deviations from a linear trend, and therefore invalidated the assumption of linearity in the logit required for logistic regression.

Variable selection within each model was achieved using a backwards-stepwise selection procedure using the method of Harrell (2009) on the basis of Akaike's Information Criterion (AIC) [R package 'RMS' (Harrell, 2009)]. Variables that did not contribute significantly to predicting outbreak occurrence were successively eliminated, until a minimum adequate model was produced (Crawley, 2002). To account for over-optimistic classification measures when validating the models on the training data (Miller et al., 1991), a bootstrap resampling procedure was used for model validation to obtain bias-corrected (i.e. overfitting-corrected) estimates of D_{xy} rank correlation (Somers, 1962) (10,0000 bootstrap samples), Nagelkerke generalization of R^2 for maximum likelihood-based models (Nagelkerke, 1991) and the Receiver Operating Characteristic (ROC) Area Under the Curve (AUC) [R package 'RMS' (Harrell, 2009)] [AIC, Bias-corrected D_{xy}, Nagelkerke R^2 index and AUC are discussed further in Chapter 3 Section 3.2.4 on page 68]. The relative effects of the predictor variables in the final model selected by backwards-stepwise selection were assessed using Odds Ratios (OR) and their 95 % confidence intervals (OR <1 negative effect OR >1 positive effect).

4.2.4 Control of Larval *Culicoides* through Habitat Modification

Species of the Palaearctic BTV vector group *C. obsoletus* s.l. (*Culicoides obsoletus* s.s., *C. dewulfi* and *C. chiopterus*) have previously been identified as breeding in, and substrates contaminated by, livestock faecal matter (Edwards et al., 1939; Campbell and Pelham-Clinton, 1960; Kremer, 1965). It is hypothesised that control techniques aimed at the modification of these organically enriched habitats would have a significant effect on the local adult *C. obsoletus* s.l. population. To test this hypothesis, the effect of one of the DEFRA's recommended, but untested control techniques, of 'covering muck heaps with a plastic, watertight cover' (DEFRA, 2009b) was assessed for its effect on adult *C. obsoletus* s.l. abundance in the treated areas.

Estimates of *C. obsoletus* s.l. abundance were available from weekly trap collections made between Spring 2006 and May 2009 (after the first generational peak in *Culicoides* abundance had occurred) using OVI type, 8W UV down-draught suction traps (Agricultural Research Council, South Africa) at eight farms in the UK [see Figure 4.1 on page 109]. Commercial activities on the farms prevented the muck heaps from remaining covered for a longer time periods. These data were available from a separate BBSRC/DEFRA project 'Epidemiology and control of orbiviral diseases in the UK, with particular reference to bluetongue and African horse sickness' (Contract no. BBSRC: BBS/B/00603, DEFRA: SE-4104). Traps were run from dusk until dawn to coincide with the crepuscular peaks in *Culicoides* activity (Hill, 1947; Kettle, 1957; Parker, 1949; Service, 1969) [see Section 2.2.1 for further details of trapping protocols used].

Muck heaps at four of these farms (farms 1-4) [see Figure 4.1 on page 109 and Figure 4.2 on page 110] were covered with $200\,\mathrm{g{\cdot}m^2}$ (14 by 14 per square inch weave) green tarpaulins (Bradshaws Direct, York UK), which excluded both light and water from the surface of the muck heap. Tarpaulins were weighted and secured with 8 mm polypropylene rope (Wickes, Northampton, UK) and 440 mm by 215 mm by 100 mm medium density blocks (Wickes, Northampton, UK). Muck heaps were covered from early March 2009 (during the seasonal vector-free period), until the end of May 2009

following the spring peak in *Culicoides* emergence. The aim was to prevent the emergence of adults from diapausing 4^{th} instar larvae already present in the muck heap. The remaining four farms were used as controls (farms 5-8) [see Figure 4.1 on page 109 and Figure 4.2 on page 110] with the muck heaps remaining uncovered, to allow an assessment of the overall trend in *C. obsoletus* s.l. populations for the 2009 season compared to previous seasons (2006-2008).

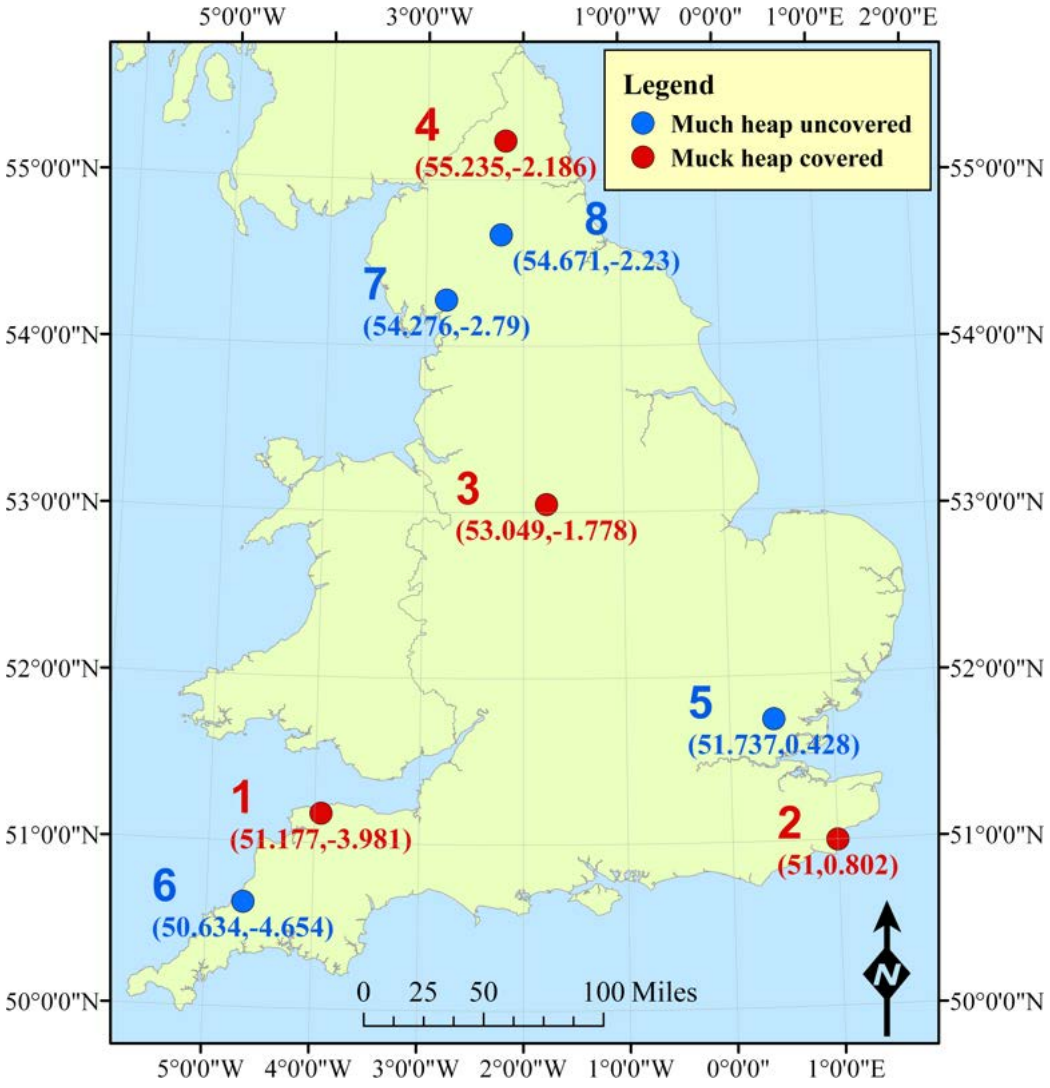


Figure 4.1. Location of treatment (muck heaps covered - farms 1, 2, 3 and 4) and control farms (muck heaps uncovered - farms 5, 6, 7 and 8) with latitude and longitude shown in parentheses

(a). **Farm 1 Uncovered**

(b). **Farm 1 Covered**

(c). **Farm 2 Uncovered**

(d). **Farm 2 Covered**

(e). **Farm 3 Uncovered**

(f). **Farm 3 Covered**

(g). **Farm 3 Uncovered**

(h). **Farm 3 Covered**

(i). **Farm 4 Uncovered**

(j). **Farm 4 Covered**

Figure 4.2. Muck heaps before (uncovered) and after (covered) coverage at farms 1-4

4.2.4.1 Approach to Modelling the Effect of Muck Heap Coverage on Local Adult *C. obsoletus* s.l. Populations

To assess the effect of covering muck heaps on the first generational peak in 'local' adult populations of the BTV vector group *C. obsoletus* s.l., the number of female *C. obsoletus* s.l. caught (y_{jk}) at the j^{th} observation on farm k; with the indicator variable (c_{jk}) indicating whether a muck heap was covered ($c_{jk} = 1$) or not ($c_{jk} = 0$) at the j^{th} observation on farm k; and the time (t_{jk}; in days) of the j^{th} observation on farm k, were modelled using a hierarchical, overdispersed Poisson model with temporal correlation using an AR(2) process modified from Johnson and Hoeting (2003), so that,

$$\begin{aligned} y_{jk} &\sim Poisson(\mu_{jk}), \\ log(\mu_{jk}) &= (1 - c_{jk})b_k^{(U)}(t_{jk}) + c_{jk}b_k^{(C)}(t_{jk}) + \sigma_{jk} + \epsilon_{jk} \end{aligned} \tag{4.1}$$

where,

$$b_k^{(\cdot)}(t) = \alpha_k^{(\cdot)} + \beta_k^{(\cdot)} sin\left(\frac{2\pi}{365}(t - \phi_k)\right) \tag{4.2}$$

is the expected count at time t on farm k, depending on whether the dung heap is covered (C) or uncovered (U), σ_{jk} allows for overdispersion and ϵ_{jk} allows for temporal correlation using an AR(2) process as described by Johnson and Hoeting (2003), so that,

$$\begin{aligned} \epsilon_{jk} &\sim N(\mu_{\epsilon_{jk}}, \sigma_\epsilon^2), \\ \mu_{\epsilon_{jk}} &= \rho_1\epsilon_{j-1,k} + \rho_2\epsilon_{j-2,k}. \end{aligned} \tag{4.3}$$

Between-farm variation in abundance level and temporal autocorrelation was modelled by assuming the parameters for each farm are drawn from higher-level distributions, so that,

$$
\begin{aligned}
\alpha_k^{(\cdot)} &\sim N(\mu_\alpha^{(\cdot)}, \sigma_\alpha^{2(\cdot)}), \\
\beta_k^{(\cdot)} &\sim N(\mu_\beta^{(\cdot)}, \sigma_\beta^{2(\cdot)}), \\
\phi_k &= 182.5 + 182.5\phi_k^{'},\ \phi_k^{'} \sim Beta(a_\phi, b_\phi), \\
\sigma_{jk} &\sim N(0, \sigma_d^2).
\end{aligned}
\tag{4.4}
$$

Only female *C. obsoletus* s.l. were considered in this analysis, as male *Culicoides* do not take blood meals from vertebrates and consequently have no potential to vector BTV between hosts. Insufficient numbers of *C. pulicaris* s.l. were collected for statistical analysis.

Parameters in the model were estimated using Bayesian methods in WinBUGS (Lunn et al., 2000). The R Package 'boa' (Smith, 2007) was used for both convergence assessment and posterior inference of Markov Chain Monte Carlo (MCMC) output produced by WinBugs. Non-informative priors (diffuse normal or diffuse gamma as appropriate) were used for the hierarchical (i.e. farm-level) parameters and for the variance parameter in the temporal correlation models. Priors for the correlation parameters were based on sampling uniformly from the stationary triangle of the AR(2) model. A stationary AR(m) model (where m is the number of time lags) provides either positive or negative correlation between trap catch size that decreases with an increasing separation in time (Johnson and Hoeting, 2003).

MCMC iteratively produces parameter values that are representative samples from the joint posterior. Unlike frequentist analysis where iterative model fitting routines are monitored for convergence to a single point, MCMC output is monitored for convergence to a distribution (Smith, 2007). For the purposes of assessing convergence, two parallel chains were generated, each with different starting values which were overdispersed with respect to the target distribution. The Gelman and Rubin's convergence diagnostic was then used to assess whether approximate convergence had been achieved (Gelman and Rubin, 1992). This statistic is based on the ratio of between-within chain variances (ANOVA) and the upper limit should approach 1.0

on convergence. As a rule of thumb, a 0.975 quantile greater than 1.20 is interpreted as evidence of non-convergence (Smith, 2007). Convergence was also assessed visually through the trace and kernal density plots produced by WinBugs (Lunn et al., 2000).

By sampling from the joint conditional distribution; median values of the posterior, Monte Carlo error, and 95% Bayesian credible intervals for model parameters were obtained. Monte Carlo errors for parameters are an estimate of the difference between the mean of the sampled values (which are used for the estimate of the posterior mean for each parameter) and the true posterior mean. As a rule of thumb the simulation should be run until the Monte Carlo error for each parameter of interest is less than 5% of the sample standard deviation.

4.3 Results

4.3.1 Spatial Patterns *Culicoides* Larvae Occurrence

A total of 767 *Culicoides* larvae were directly collected from 33 of the 150 sampling point assessed directly for *Culicoides* larvae. Only 42 (5.9 %) of these larvae were successfully identified to species level. Of the *Culicoides* larvae which could be indentified species collected included *C. obsoletus* s.s., *C. scoticus*, *C. dewulfi*, *C. chiopterus*, *C. pulicaris* s.l., *C. nubeculosus* and *C. grisescens* Edwards [see Table 4.4 below].

	Habitat Class	Total Number of *Culicoides* Collected	% *Culicoides* larvae not identified to species level*	*C. obsoletus* Meigen	*C. scoticus* Downes and Kettle	*C. dewulfi* Goetghebuer	*C. chiopterus* Meigen	*C. pulicaris* s.l.	*C. nubeculosus* Meigen	*C. grisescens* Edwards
1	Open Pasture [116]	403	95.75	3	1	0	0	1	11	1
2	Wooded Pasture [42]	21	90.48	0	0	0	0	0	2	0
3	Arable [27]	0	-	0	0	0	0	0	0	0
4	Broadleaved Woodland Leaf Litter [28]	31	90.32	0	0	0	0	0	3	0
5	Broadleaved Woodland Vegetation [36]	240	98.33	0	0	0	0	0	4	0
6	Coniferous Woodland Ground Substrate [19]	2	-	0	0	0	0	0	0	2
7	Open Water and Marginal Vegetation [10]	0	-	0	0	0	0	0	0	0
8	Surrounding Artificial Water Sources [17]	2	50.00	1	0	0	0	0	0	0
9	Muck Heaps [18]	38	92.11	0	0	3	0	0	0	0
10	Organically Enriched [17]	47	70.21	0	0	2	7	0	0	0

Table 4.4. Number of *Culicoides* larvae directly collected from substrate per habitat class (total number of sampling points per habitat class shown in parenthesis after class description) *Number of larvae per *Culicoides* species/habitat class successfully identified to species level shown in right hand segment of table

In total 950 adult *Culicoides* were collected using emergence traps from 21 of the 180 sampling points. Species collected included *C. obsoletus* s.l., *C. pulicaris* s.l., *C. nubeculosus*, *C. festivipennis* Kieffer, *C. achrayi* Kettle and Lawson, *C. pictipennis* Staeger, and *C. albicans* Winnertz [see Table 4.5 on page 116]. 360 *C. obsoletus* s.l. individuals were collected from 17 of the sites, 96.67% of these were successfully identified to species level, of which 95.83% were *C. obsoletus* s.s., 8.33% were *C. scoticus*, however, no *C. dewulfi* or *C. chiopterus* were collected in the emergence traps [see Table 4.5 on page 116].

Within the 384 *C. obsoletus* s.l. collected by the emergence traps, 79 were observed to have abdominal pigmentation consistent with that which would be used to describe a parous individual according to the methodology of Dyce (1969). Of these, 78 were identified as *C. obsoletus* s.s. The species-specific PCR failed to identify the remaining individual.

In total, larval development, indicated by the presence of larvae or of emerging adults was detected at only 45 of the 330 sampling points. Of the habitat classes investigated larval development sites were only absent from class 3 (arable) and class 7 (open water and marginal vegetation) [see Table 4.6 on page 117].

Habitat Class	Total *Culicoides* Collected	*C. obsoletus* s.l.**	*C. pulicaris* s.l.	*C. nubeculosus* Meigen	*C. albicans* Winnertz	*C. festivipennis* Kieffer	*C. achrayi* Kettle and Lawson	*C. pictipennis* Staeger	*C. obsoletus* Meigen	*C. scoticus* Downes and Kettle	*C. dewulfi* Goetghebuer	*C. chiopterus* Meigen	Female *C. obsoletus* s.l. PCR Fail
1 Open Pasture [48]	3(1;2)	0(0;0)	2(0;2)	0(0;0)	1(1;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0	0(0;0)	0(0;0)	0(0;0)	0
2 Wooded Pasture [25]	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0	0(0;0)	0(0;0)	0(0;0)	0
3 Arable [14]	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0	0(0;0)	0(0;0)	0(0;0)	0
4 Broadleaved Woodland Leaf Litter [13]	478(343;135)	140(80;60)	0(0;0)	0(0;0)	3(3;0)	335(260;75)	0(0;0)	0(0;0)	114(76;38)	22(0;22)	0(0;0)	0(0;0)	4
5 Broadleaved Woodland Vegetation [25]	41(40;1)	39(38;1)	1(1;0)	1(1;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	37(36;1)	2(2;0)	0(0;0)	0(0;0)	0
6 Coniferous Woodland Ground Substrate [11]	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0
7 Open Water and Marginal Vegetation [8]	107(77;30)	79(49;30)	0(0;0)	27(27;0)	0(0;0)	0(0;0)	1(1;0)	0(0;0)	75(46;29)	1(0;1)	0(0;0)	0(0;0)	3
8 Surrounding Artificial Water Sources [9]	17(17;0)	0(0;0)	0(0;0)	0(0;0)	6(6;0)	0(0;0)	0(0;0)	11(11;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0
9 Muck Heaps [15]	171(117;54)	170(116;54)	0(0;0)	1(1;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	169(115;54)	0(0;0)	0(0;0)	0(0;0)	1
10 Organically Enriched [12]	107(79;28)	104(77;27)	3(2;1)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	0(0;0)	95(72;23)	5(1;4)	0(0;0)	0(0;0)	4

Table 4.5. Number of *Culicoides* collected in emergence traps [total (females;males)] per habitat class (total number of sampling points per habitat class shown in parenthesis after class description) [*C. obsoletus* s.l. group identified to species level as shown in the right hand segment of the table]**

Habitat Class	Larvae Present	
	% of points sampled [No. of points sampled]	
	Direct Larval Sampling	Emergence Trapping
1 Open Pasture [116]	20.6 [68]	4.2 [48]
2 Wooded Pasture [42]	11.8 [17]	0.0 [25]
3 Arable [27]	0.0 [13]	0.0 [14]
4 Broadleaved Woodland Leaf Litter [28]	33.3 [15]	38.5 [13]
5 Broadleaved Woodland Vegetation [36]	63.6 [11]	20.0 [25]
6 Coniferous Woodland Ground Substrate [19]	25.0 [8]	0.0 [11]
7 Open Water and Marginal Vegetation [10]	0.0 [2]	25.0 [8]
8 Surrounding Artificial Water Sources [17]	12.5 [8]	11.1 [9]
9 Muck Heaps [18]	33.3 [3]	33.3 [15]
10 Organically Enriched [17]	20.0 [5]	25.0 [12]

Table 4.6. Percentage of sampling points per habitat class found to be acting as *Culicoides* larval development sites [based on identification of larval presence by either emergence trapping or direct extraction of larvae] (total number of sampling points per habitat class shown in parenthesis after class description)

4.3.2 Modelling Immature Livestock-Associated *Culicoides* Occurrence at the Local-Scale

Moran's I tests were insignificant for both the presence of larval and emerging *Culicoides* at all neighbourhood sizes assessed, suggesting that spatial autocorrelation has no or minor influence on patterns of immature *Culicoides* occurrence at the scale of this study. The examination of the variance inflation factors within and between variable suites, however, did lead to the omission of two climatic variables from both the larval and emergence remote GIS based models of immature *Culicoides* occurrence [TC.BRIGHT leaf on and TC.BRIGHT leaf off]. No significant non-linear responses were observed for predictor variables in either the ground-based or remote models. A total of four variables were therefore included in both larval and emergence ground-based models [see Table 4.7 on 119] and a total of 25 variables were included in both larval and emergence remote GIS-based models (13 landscape, 8 climate and 4 terrain/soil) [see Table 4.7 on page 119].

Following stepwise selection, the variable combinations that best discriminated between areas of *Culicoides* larval development presence-absence were ground-based measures of soil moisture levels, increasing levels which had a positive impact on the probability of larval occurrence [see Table 4.8 and 4.9 on page 120]. No variables were retained as significant predictors of larval *Culicoides* occurrence in the model using remote GIS-based measures of environmental conditions [see Table 4.8 on page 120]. Ground based measure of environmental conditions also provided the best discrimination between the presence and absence of emerging adult *Culicoides* [bias-corrected $D_{xy}{}^{a}$: 0.700; AUC:0.864]. The positive effect of both increasing ground-based measures of soil moisture and soil pH were both found to increase the probability of emerging adult *Culicoides* presence at a sampling site. In contrast to larval *Culicoides* occurrence, the presence-absence of emerging adult *Culicoides* was also described well by remote GIS-based measures of landscape and climatic conditions [see Table 4.8 on page 120]. The positive impact of an increasing proportion of broad-leaved woodland in the landscape (PLAND.1.1), increasing levels of vegetation activity in winter (EVI.leaf off) and increased soil moisture levels in spring all had a positive impact on the presence of emerging *Culicoides*. The presence of emerging adult *Culicoides* was also positively impacted on by increasing levels of fragmentation within the landscape (LSI), however, was negatively impacted on by an increasing diversity of land cover types within the landscape (SIDI) [see Table 4.8 on page 120].

Levels of optimism as a result of the stepwise selection of variables was acceptably low for all models (<0.1). Insufficient identifications to species level were achieved to parameterise species-specific occurrence models.

Ground-Based	Remote GIS-Based (Three Variable Suites)		
	Landscape	**Climate**	**Terrain/Soil**
Observed Habitat Class [10 Levels see Table 4.2]*	LC2000 Land Cover Class*	TC.WET leaf on	Slope
Soil.pH	LSI	TC.WET leaf off	Altitude
Soil.Moisture	SIDI	TC.Green leaf on	Aspect
Soil.Temperature	PLAND.1.1 (Broad-Leaved Mixed Woodland)	TC.Green leaf off	Soil Type [5 levels]* †
	LSI.1.1 (Broad-Leaved Woodland)	EVI leaf on	
	PLAND.2.1 (Coniferous Woodland)	EVI leaf off	
	LSI.2.1 (Coniferous Woodland)	LST leaf on	
	PLAND.5.1 (Improved Grassland)	LST leaf on	
	LSI.5.1 (Improved Grassland)		
	PLAND.5.2 (Set-Aside Grassland)		
	LSI.5.2 (Set-Aside Grassland)		
	PLAND.7.1 (Calcareous Grassland)		
	LSI.7.1 (Calcareous Grassland)		

Table 4.7. Variables included in the analysis of livestock-associated *Culicoides* species abundance (after the exclusion of variables with VIFs > 10), * indicates a categorical variable, all other variables continuous
[† SOIL.TYPE factor levels: 1 - freely draining slightly acid but base-rich soils; 2 - freely draining slightly acid loamy soils; 3 - shallow lime-rich soils over chalk or limestone; 4 - slightly acid loamy and clayey soils with impeded drainage; 5 - slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils]

Model		D_{xy}[a]	Optimism[b]	R^{2d}	AUC[e]
Larval	Ground-Based Measures	0.526	0.034	0.209	0.776
	Remote GIS-Based Measures	*	*	*	*
Emergence	Ground-Based Measures	0.700	0.037	0.311	0.864
	Remote GIS-Based Measures	0.43	0.093	0.146	0.668

[a] Bias-corrected Somers Dxy rank correlation [D_{xy} = 2(c 0.5)]

[b] A measure of model overfitting and the difference between the training and testing values for D_{xy}

[c] Bias-corrected maximum absolute error in between predicted and calibrated probabilities

[d] Nagelkerke generalization of R^2 for maximum likelihood-based models

[e] Receiver Operating Characteristic (ROC) - Area Under Curve (AUC)

Table 4.8. Fit statistics from internal bootstrap validation and receiver operating characteristic of models of immature *Culicoides* larvae occurrence, * indicates no variables retained during the stepwise selection procedure

Variable Suite	Variable	Larval	Emergence	
		Ground-Based	Ground-Based	Remote GIS-Based
	Soil Moisture	8.43 (3.42;20.76)	7.97 (3.00;21.18)	-
	Soil pH	*	4.58 (1.87;11.23)	-
Landscape	LSI	-	-	2.98 (1.06;8.42)
	SIDI	-	-	0.34 (0.12;0.95)
	PLAND.1.1	-	-	1.87 (1.00;3.49)
Climate	EVI leaf off	-	-	0.81 (0.41;1.60)
	TC Wet leaf on	-	-	1.23 (0.62;2.42)

- Indicates variable not considered in stepwise selection

* Indicates variable not selected during stepwise selection

Table 4.9. Variables selected by stepwise selection based on AIC for the prediction of *Culicoides* larval development site occurrence with Odds Ratios (OR) and their 95 % confidence intervals (OR <1 negative effect OR >1 positive effect). Land cover classes: 1.1 broad-leaved mixed woodland

4.3.3 Effects of Habitat Modification - Covering Muck Heaps

No significant effect of covering muck heaps was seen in the estimates of the local female *C. obsoletus* s.l. population. In 6.8 % ($\bar{X}$ 0.068, SD 0.251) of samples the detrended mean trap catches for covered farms was actually higher than for uncovered farms. The MCMC chain appeared to have converged well before the end of the burn-in period and medians values of the posterior and 95 % Bayesian credible interval estimates for the parameters of the model used to describe the influence of muck heap coverage on female *C. obsoletus* s.l. trap catches are given in Table 4.10 on page 122. In Table 4.10 parameters with subscript α relate to functions that do not vary over time, while parameters with subscript β relate to the seasonal dynamics in trap catches, which vary with time. In addition, parameters with supercript C relate to times when muck heaps were covered, parameters with superscript U relate to times when muck heaps were uncovered. Parameter ρ describes the temporal autocorrelation at one time lag [1] and two time lags [2]. The mediun value of the posterior, and 95 % Bayesian credible interval suggest that the majority of the mass for $\rho[1]$ is located over positive values, the posterior density of $\rho[2]$, however, seems to be centred directly over 0, indicating that there is a significant positive influence on the terms at the first but not the second time lag. The model was therefore refit using an AR(1) process, such that $\mu_{\epsilon_{jk}} = \rho\epsilon_{j-1,k}$, this resulted in a reduction in the Deviance Information Criterion (DIC) by 22.9. Parameter σ_d^2 corrects for the overdispersion in the data. The posterior mass of σ_e^2 seems to be located away from zero, indicating that there was a significant amount of overdispersion present. Parameter σ_e^2 relates to the variation in the temporal autocorrelation. The posterior mass of σ_e^2 seems to be located away from zero, indicating that there was also a significant amount of variation in the temporal autocorrelation from week to week [see Table 4.10, and Figure 4.3 on page 123].

Parameter	Parameter	95 % Bayesian Credible Interval
α_ϕ	20.71	(20.71;71.30)
β_ϕ	84.86	(18.87;291.40)
μ_α^C	1.86	(0.11;3.30)
μ_β^C	-8.78	(-11.66; -6.11)
μ_α^U	-5.20	(-5.20;-4.64)
μ_β^U	-5.204	(-5.76;-4.64)
σ_α^C	0.15	(0.01;6.20)
σ_β^C	0.25	(0.01;16.53)
σ_α^U	1.68	(0.57;6.71)
σ_β^U	0.09	(0.01;1.06)
$\rho_{[1]}$	0.62	(0.45;0.76)
σ_d^2	3.13	(2.06;4.08)
σ_ϵ^2	1.68	(0.89;2.95a.phi)

Table 4.10. Median value of the posterior means and 95 % Bayesian credible interval for the AR(1) model parameters ($\rho_{[2]}$ from AR(2) model -0.27(-0.75;0.17))

In addition, no difference was observed in the week number in which *C. obsoletus* s.l. activity began i.e. first collection of the year in the light trap, between 2009 (muck heaps at farms 1-4 covered) and 2007/2008 [see Table 4.11 on page 122].

Year	Farm Number							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**
2007	13	13	14	16	15	16	21	20
2008	14	19	18	17	17	18	15	18
2009	14*	15*	15*	15*	13*	13	13	16

Table 4.11. Week number in which first activity of *Culicoides obsoletus* s.l. was recorded in light trap collections. [* indicates muck heap covered]

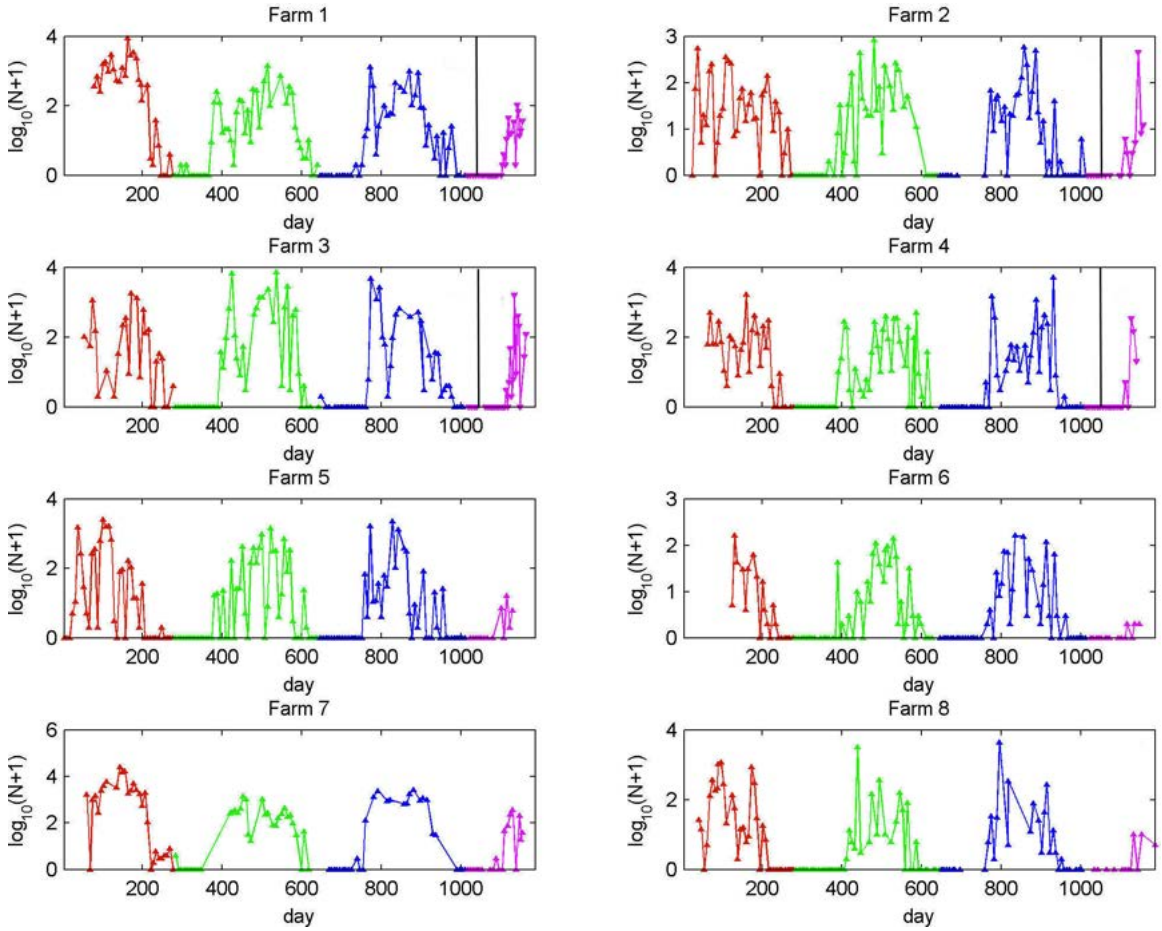


Figure 4.3. Weekly light trap catches of *Culicoides* (total female *C. obsoletus* s.l. caught) between 2006 and 2009 on eight farms. Colours indicate the year: 2006 (red), 2007 (green), 2008 (blue) and 2009 (magenta). Symbols indicate whether the dung heap was uncovered (up-triangles) or covered (down-triangles) at sampling point. Point of coverage of muck heaps indicated by vertical black line, left of which muck heap uncovered, right of which muck heaps were covered [plot provided by S. Gubbins, Institute for Animal Health]

4.4 Discussion

In order to develop an ecologically sound scheme to manage BTV vector populations, the larval development sites of the Palaearctic BTV vectors must be accurately defined. A wide diversity of breeding habitats have previously been observed for the Obsoletus and Pulicaris groups. This project, however, is the first to investigate larval development sites of the Palaearctic BTV vector groups quantitatively in southern England for over 50 years, and is one of only two studies (Schwenkenbecher et al., 2009) to make use of new molecular identification techniques for larval identification to species.

The multiplex PCR assay based on DNA sequence variation at the mitochondrial Cytochrome C oxidase I gene (Schwenkenbecher et al., 2009) has been successfully used for the identification of *Culicoides* larvae in Scotland (Schwenkenbecher et al., 2009). In this study, however, a limited number of identifications to species level were made, despite relatively large numbers of larvae being collected. These difficulties might have arisen if species of *Culicoides* larvae collected are not currently included in the PCR diagnostic assay [species included: *C. obsoletus* s.s., *C. scoticus*, *C. dewulfi*, *C. chiopterus*, *C. pulicaris* s.s., *C. punctatus*, *C. impunctatus*, *C. grisescens*, *C. newsteadi*]. The possibility of additional species within the Obsoletus group in the UK has also been postulated and requires further investigation (J. De Gabriel, personnel communication). The assay of Schwenkenbecher et al. (2009), however, does contain a generic marker for the *Culicoides* genus, which despite morphological identification of *Culicoides* larvae, did not generate a product, likely due to the failure of DNA amplification. The multiplex PCR procedure was initially developed for adult *Culicoides* specimens, hence methodological refinements may be required for it to be as successful in identification of larval specimens.

Following the problems with larval specimen identification the decision was made to focus the investigation on the use of emergence traps, used to collect newly emerged adult *Culicoides* directly above their larval development sites. Emergence traps provided the advantage that adult *Culicoides* are collected which can be identified read-

ily to species, or for the Obsoletus group, to species level using molecular techniques (Nolan et al., 2007). Despite the limited data provided by larval specimens, the combination of these identifications and those made from emergence trap collections allowed *Culicoides* larval development sites to be identified on five of the six livestock farms investigated. The most productive habitats were found to be those associated with broad-leaved woodland, muck heaps and the organically enriched run-off areas surrounding them. An increasing percentage of broad-leaved woodland within the landscape was also found to be a significant predictor of the presence of emerging adult *Culicoides*, within the remote-GIS based model of emergence presence-absence. Neither larvae nor newly emerged adults were collected from arable areas, although this may be due to the relatively small area of habitat sampled compared to their overall abundance within farms. The absence of *Culicoides* of any species, however, indicates that these areas do not form a major source of larval development sites. *Culicoides obsoletus* s.s. was confirmed to breed in vegetated substrate and decaying leaf-litter in broad-leaved woodland, the margins of open water sources, open grassland pasture, muck heaps and the organically enriched run off areas surrounding them. *Culicoides obsoletus* s.s. larval development sites have previously been identified in grassland (Kettle, 1961). This is the first study, however, to associate *C. obsoletus* s.s. with cattle dung, rather than sheep (Edwards, 1926; Edwards et al., 1939) or horse (Kettle and Lawson, 1952) dung. Larval development of *C. scoticus* was recorded in the same habitats as *C. obsoletus* s.s., with the exception of muck heaps. The limited number of *C. scoticus* identified within samples, however, means that their presence within the main muck heap body cannot be discounted, since they were recorded in the run-off areas surrounding them. In support of previous studies both *C. chiopterus* and *C. dewulfi* were again confirmed as dung breeders [*C. dewulfi* (Campbell and Pelham-Clinton, 1960; Schwenkenbecher et al., 2009) and *C. chiopterus* (Campbell and Pelham-Clinton, 1960; Kremer, 1965)]. *Culicoides chiopterus* was also only identified in specimens collected from the organically enriched runoff areas surrounding muck heaps, rather than from the main muck heap body. Very limited collections

of *C. pulicaris* s.l. were made in the emergence traps and only one larvae was successfully identified as *C. pulicaris* s.l. This species group, however, can be confirmed as breeding in areas of open grassland pasture, vegetated substrate in broad-leaved woodland and organically enriched areas surrounding muck heaps.

The models of *Culicoides* larval occurrence at sampling points are interpreted with caution as they are not species-specific. All samples, however, were collected within farms and therefore sampled *Culicoides* are likely to be livestock-associated species. Both *Culicoides* larval presence and the presence of emerging adult *Culicoides* within habitats was found to be strongly associated with increasing substrate moisture levels [soil moisture ranged from 4.5 % to 63 %]. Soil pH conditions examined ranged from pH 4.12 to pH 9.4. The presence of emerging adult *Culicoides* was positively related to alkaline pH levels, this may indicate that pH levels provide a limitation to the *Culicoides* development past the 4^{th} instar stage but not prior to it. Increasing levels of soil moisture have previously been found to be positively correlated with *C. impunctatus* larval counts in Scotland (Blackwell et al., 1994). In contrast to the livestock-associated species studied here, increasing *C. impunctatus* larval counts were found to be correlated with slighty acidic soil (pH 5 to 6.5) (Blackwell et al., 1994).

In this study the presence of emerging adult *Culicoides* within farm were associated with increasing winter-time vegetation activity (EVI leaf on), increasing summer time soil moisture levels and highly fragmented landscape which had a low diversity of different land cover types and an increasing percentage of the landscape covered by broad-leaved woodland. This latter factor is likely related to use leaf litter based larval development sites identified in this study. These remote measures may provide a remote GIS-based ecological correlates for larval habitat that are suitable to sustain *Culicoides* larval through to emergence, that can be used to predict larval habitat occurrence at a wide spatial scale, as although, soil moisture measured directly was very strongly correlated with *Culicoides* larval occurrence, it does not provide a practical measure for wide scale spatial prediction.

Landscape structure is sensitive to the scale at which it is measured [for review see (Klopatek and Gardner, 1999)], however, the scale at which landscape structure influences both adult and/or larval *Culicoides* distribution and abundance at the within-farm scale is undetermined. Examination of a range of buffer sizes may allow the most appropriate scale over which to identify drivers of immature *Culicoides* occurrence and may enable additional associations to be identified. In addition quantification of the relative levels dissolved oxygen content between different habitats and/or sampling points, may allow additional variation in larval occurrence and/or emergence to be explained. *Culicoides* larvae are apneustic (Whitten, 1960), i.e. the tracheal system is closed with no functional spiracles, and oxygen is obtained by diffusion through the body cuticle into the tracheae where oxygen comes out of solution and can be more readily transported as a gas to the areas of need. *Culicoides* are, therefore, adapted to low oxygen concentrations, with minimal dependence on atmospheric oxygen. *Culicoides* larvae are generally found concentrated in the aerobic surface conditions of substrates (Blackwell, 1997, 2001; Fredeen, 1969; Gazeau and Messersmith, 1970; Kitaoka and Morii, 1963; Uslu and Dik, 2006) indicating dissolved oxygen content of the substrates may limit their occurrence and/or emergence.

The larvae collected in this study were primarily 4^{th} instar larvae. This is likely a function of the difficulties observing the much smaller 1^{st}, 2^{nd} and 3^{rd} instar larvae, which are almost translucent and more quiescent in their behaviour, and during the time the specimens were collected, the majority of UK species of *Culicoides* are thought to be over-wintering as 4^{th} instars. Of the techniques that have been utilised for the collection of *Culicoides* larvae from collected substrates [for review see Hribar (1990), Kline and Axtell (1975) and Jones (1978)], the efficiency of larval collection remains low (Blackwell, 1997) and each technique will only recover a proportion of the natural population in a sample. Direct larval extraction will, in addition, only provide a snap-shot measure of *Culicoides* larval presence and abundance within a substrate. This method does not provide a measure of the ability of that substrate to sustain larvae through to emergence. In contrast, emergence traps provide a measure

of the success rate of a habitat to sustain larvae to emergence. The importance of soil pH in the emergence but not the larval models of *Culicoides* occurrence may indicate that long term exposure to unsuitable pH conditions may not be a limiting factor for larval development but may inhibit development to the adult stage. The positive or negative effect on emergence of covering the substrate with the trap, or the proportion of emerging adults collected has not, however, been determined. The relative importance of the different larval habitats for the different *Culicoides* species, therefore, cannot currently be assessed with certainty. Further, long-term assessments of larval presence and abundance within habitats, using a combination of emergence traps and direct extraction of larvae, in conjunction with improved identification techniques, may provide additional information on the suitability of habitats to contain larvae and how, or if, the abundance of over-wintering 4^{th} instar larvae within different habitat types changes over time. In addition habitats found to contain *Culicoides* larvae in late autumn/early winter could be assessed for their ability to sustain larvae through to emergence.

4.4.1 Potential for Vector Control through Habitat Modification

In addition to vector control programs being used alongside vaccination campaigns in Europe, alternative control measures need to be implemented in instances when threats from serotypes not currently covered by inactivated vaccines occur. Inactivated vaccines are only currently available for four serotypes (BTV-1, 2, 4 and 8) and, for example, vaccines for the recent BTV-8 incursion took approximately 18 months to develop. Although vector control alone is unlikely to be successful in entirely preventing the occurrence and transmission of BTV, it may, if used correctly, significantly reduce transmission (Carpenter et al., 2008b).

Widespread application of chemicals for *Culicoides* control is also inappropriate, due the significant associated public health and/or environmental risks. Biological control methods, however, have achieved little success in controlling *Culicoides* larvae (Blackwell and King, 1997; Kelson et al., 1980). Difficulties also arise in employing

an effective control system as the larvae of *C. obsoletus* s.l. appear to have a low density in breeding habitats that are present over a wide geographical area. Large populations of *C. obsoletus* s.l. larvae were mainly associated in this study with areas of decaying organic matter and, in particular, with moist areas where substrate is contaminated with livestock fecal matter. The types of habitats used by *C. obsoletus* s.l. indicate that good water and waste management practices should be key to practical population management of *C. obsoletus* s.l.

The covering of muck heaps is currently recommended by DEFRA for *Culicoides* control on farms, but has not been tested as a means of controlling Palaearctic BTV vector populations. This investigation aimed to examine the hypothesis that the covering of muck heaps would reduce the the first generational peak in adult *Culicoides* abundance on a farm and therefore reduce the risk of disease transmission in the event of an outbreak. The muck heaps at four farms, all created predominately from a mixture of cattle waste and straw bedding, were covered with tarpaulins that excluded both light and moisture from the surface of the heap. The covers were put in place during the winter vector-free period and remained in place till the end of May, after the spring peak in *Culicoides* emergence had occurred, covering at this time aimed to prevent any diapausing *Culicoides* larvae present in the heap emerging. Unfortunately the impact on the first generation of *C. obsoletus* s.l. were disappointing, with no significant effect observed in light trap collections of female *C. obsoletus* s.l. In addition, no apparent change was seen in the onset of recorded female *C. obsoletus* s.l. activity in 2009 compared to 2007 and 2008, which may have been expected if the muck heap was acting as a source of the earliest adult *Culicoides*, due to its higher average temperature as a result of decomposition.

The results of the impact of muck heap coverage on female Obsoletus group abundance must be treated with caution as the analysis was only conducted on group level data and for part of the season. Current financial and labour costs for the molecular identification of all samples from such a large series of entomological collections currently precludes the identification of all *C. obsoletus* s.l. female specimens to species

level. As both molecular and morphological identification techniques develop this data may become available and reanalysis of this experiment would be pertinent, given differences in the ecology of the constituent species.

The long term effect of covering muck-heaps on *Culicoides* populations were not investigated and to be effective as a control measure they may need to be covered year round, preventing oviposition as well as emergence. The areas of seepage around the base of the muck heap were also found to be productive larval development sites. To observe a significant effect from muck heap coverage the run-off areas may additionally need to be kept clean and dry. The practicalities of these measured need to be considered, as well as the effects of long term coverage on the degradation rate of the heap. It is unlikely any one method such as covering muck heaps would have dramatic effect of *Culicoides* numbers. Instead these must be considered as part of an integrated control system, combining not just larvicidal methods but also methods aimed at controlling the adult population, which may included modifications to husbandry and farm management systems but also the use of repellents etc. [for review see Carpenter et al. (2008b)].

4.4.2 The Implications of Pigmented Newly Emerged *Culicoides*

The identification of abdominal pigmentation in newly emerged *C. obsoletus* s.s. provides evidence for a potential source of error in the method for parity determination in the *C. obsoletus* group. Although pigmentation was only observed in the *C. obsoletus* s.s. individuals, the potential for abdominal pigmentation in the other *C. obsoletus* s.l. species can not be excluded due to the relatively small numbers of *C. scoticus* (3 out of 360) and the absence of *C. dewulfi* or *C. chiopterus* from emergence trap collections. There is no record of autogeny in the *C. obsoletus* group and collected *Culicoides* had no opportunity to obtain a blood meal from a vertebrate host, indicating that the observed pigmentation is related to a process other than oogenesis. Braverman and Mumcuoglu (2009) found pigmented newly emerged *C. imicola* in collections from emergence traps, and hypothesised that the develop-

ment of the pigmentation was a function of age, a theory which has also been proposed for *C. variipennis occidentalis* Wirth and Jones (Dyce, 1969). *Culicoides* collected by the emergence traps in this investigation could have been alive for a maximum of 14 days. Braverman and Mumcuoglu (2009) collected pigmented newly emerged *C. imicola* in both samples that were up to 10 days old and up to 24 days old. The large numbers of pigmented individuals collected and the disturbance involved when setting up the traps make it very unlikely that simply resting 'parous' adults were collected. Age, although potentially a contributing factor, is unlikely to be the sole cause of pigmentation development. Limited experiments have monitored a small number of non-bloodfed *C. obsoletus* s.l. individuals over extended periods of time and those which have, have not noted the occurrence of pigmented nulliparous individuals (Goffredo et al., 2004). The determination of the age structure of vector populations plays a key role in the calculation of survivorship, Davidson (1954) proposed that survivorship can be estimated from parity rates (number of parous females / total number of females) if the length of the gonadotrophic cycle is known. Birley and Boorman (1982) showed that for *Culicoides* species from temperate regions estimates of longevity and gonadotrophic cycle length can be made by observing the relative proportions of nulliparous and parous females over eleven successive days, providing that certain conditions are met.

For adult *Culicoides*, age refers to the period since the emergence of the adult insect from the final immature stage. Gonadotrophic age grading methods are used globally for assessing population age structure with females classified on external examination as nulliparous, parous, bloodfed or gravid [for review see Detinova (1962) and Tyndale-Biscoe (1984)]. Nulliparous individuals are classed as those which have not oviposited, and parous individuals those which have oviposited one or more times (Birley and Boorman, 1982). Several methods have been used to determine parity in *Culicoides* including ovarian dilations, ovarial colour differences (Dyce, 1969), ovarial tracheal patterns (Akey and Potter, 1979) and ventral abdominal pigmentation (Dyce, 1969). The later is the most widely used method (Birley and Boorman, 1982; Baldet et al., 2008; Meiswinkel et al., 2008b), and the only one of these methods easily used on a large scale.

The Dyce (1969) method is based on the identification that several species of *Culicoides* develop a permanent red pigmentation in the abdominal fat, increasing in density during the first ovarian cycle (Dyce, 1969; Kay, 1973; Linley and Braverman, 1986) until approximately 72 hours post feeding, coincident with completed ovarian development, after which no further increase in pigmentation is observed (Linley and Braverman, 1986). The development of the same permanent pigmentation following egg maturation has also been observed in autogenous specimens of *C. furens* Poey (Akey, 1987; Linley and Braverman, 1986) and pigment granules have also been recorded in male *C. sonorensis* (Akey, 1987) indicating that the formation of pigment granules is associated with processes other than those involved in blood digestion. The pigment appears to be an ommochrome (Akey, 1987; Linzen, 1974) derived from the amino acid tryptophan (Linzen, 1974) and is confined to the depleted parietal fat-body lining the abdominal wall. The pigment may possibly be a product of excretion, required to regulate tryptophan levels in tissues with inadequate hemolymph circulation, which can occur when the gut is filled with developing eggs (Linzen, 1974). In many species, which have a particularly dark integument this pigment many be difficult to detect but is never completely masked (Dyce, 1969) and the method was found to be 92 % to 94 % accurate for classifying colony reared *C. sonorensis* (Akey and Potter, 1979). Pigmentation changes in the abdominal tergites has also been reported as an accurate method of separating parous and non-parous *C. sonorensis* individuals (Linley and Braverman, 1984; Potter and Akey, 1978). Pigmentation changes, however, do not allow the differentiation of multiparous from uniparous individuals (Walker, 1977).

Currently no other cost effective, accessible technique is available for determining parity status, as follicular relics are rarely identifiable in *Culicoides* and multiple dilations do not necessarily occur in all species of *Culicoides* (Kay, 1973). It has therefore been widely accepted as the generic technique for classifying parity in *Culicoides*. The findings in this investigation, however, adds to a body of evidence that an increase in abdominal pigmentation is not always associated with parity in some species of

Culicoides (Boorman and Goddard, 1970; Braverman and Mumcuoglu, 2009; Dyce, 1969; Walker and Boreham, 1976), potentially leading to errors in the determination the age structure of *C. obsoletus* s.s populations.

4.5 Conclusions

This study aimed to address the paucity of detail regarding the larval habitat use of the Palaearctic BTV vectors, and investigated the possibility of effective larval *Culicoides* control measures that would be cost-effective and practical for British farmers to use. *Culicoides obsoletus* s.l. larvae were found to be highly dependent on substrate moisture levels, but were found in low densities in habitats that are present over a wide geographical area, presenting problems for the development of effective habitat modification schemes. These larvae were mainly associated areas of decaying organic matter, rather than the top layer of the soil horizon, and in particular moist areas where the substrate was formed by, or contaminated with, livestock fecal matter. Despite the lack of success in the habitat modification strategy tested in this study, the types of habitats used by *C. obsoletus* s.l. indicate that good water and waste management practices should be key to practical population management of *C. obsoletus* s.l. Although ground-based measure of environmental condition provided the best level of discrimination between areas of immature *Culicoides* occurrence within farms, remote-GIS based measures provided a good level of discrimination between areas were emerging adult *Culicoides* were present or absent. These GIS-based measures may provide the ability to predict occurrence over a wider spatial scale. Maps of predicted larval development site occurrence may aid in explaining the spatial variation observed in adult *Culicoides* abundance at the local-scale. Tools to quantify this variation in adult abundance within-farms were investigated and are discussed in Chapter 5.

Chapter 5

Evaluation of Carbon Dioxide and the Enantiomers of 1-octen-3-ol as Attractants for Livestock-Associated *Culicoides* in Southern England

5.1 Introduction

Accurate assessment of the abundance and distribution of actively host-seeking arbovirus vectors is an important prerequisite for determining the risk of transmission of pathogens in space and time. In the case of haematophagous flies, a wide variety of trap designs and baits are commonly employed to provide relative estimates of insect activity allowing comparisons in space and time [for review see Silver (2007)]. Absolute measures of haematophagous insect populations expressed as a density or intensity per area or habitat unit are difficult to calculate from trap catches due to variation in trap efficiency both with regards to surrounding environmental conditions and due to varying species responses [for review see Southwood and Henderson (2000)]. The diversity in trap type used reflects both this wide variation in response exhibited by vector species to a specific trapping methodology and that sampling methods are in part a trade-off between accuracy in reflecting populations of the vector of interest and convenience of operation.

For *Culicoides* this trade-off in accuracy verses ease-of-use is particularly apparent. Animal-baited drop traps provide direct assessments of both host-seeking *Culicoides* populations (Jones, 1961; Mullens and Gerry, 1998; Muller and Murray, 1977; Schmidtmann et al., 1980b) and biting intensity on livestock (Campbell and Kettle, 1979; Carpenter et al., 2008d; Gerry et al., 2009; Raich et al., 1997; Schmidtmann et al., 1980a; Turner, 1972), but are too labour intensive to be used for regular surveillance purposes. On the other hand, traps working purely by suction (Fassotte et al., 2008) and vehicle mounted truck traps (Bidlingmayer, 1961; Dyce et al., 1972; Edwards, 1980; Kettle et al., 1998), provide a relatively unbiased approximation of

the total flying population of *Culicoides* (both male and female, host-seeking and non-host-seeking). The use of these latter methods, however, tends to be limited by the small numbers of *Culicoides* caught by the former and by the lack of convenience of the latter. Due to these issues, the use of suction traps with a light source far outstrips any other sampling methodology for *Culicoides*.

A wide range of light traps are available and the efficiency of some of them in collecting *Culicoides* has been compared (Belton and Pucat, 1967; Rowley and Jorgensen, 1967; Venter et al., 2009b). Their accuracy, however, in providing estimates of vector populations representative of the number of *Culicoides* attracted to a host (Carpenter et al., 2008d), or in comparison to the total flying population, is rarely assessed. In Europe, the OVI type 8w UV light-suction trap was recommended as the 'gold-standard' for *Culicoides* surveillance (EFSA, 2007; Mellor et al., 2004) and has been widely used for monitoring across Europe [Belgium (De Deken et al., 2008), Bulgaria (Purse et al., 2006), France (Baldet et al., 2008), Italy (Calistri et al., 2003; De Liberato et al., 2003), The Netherlands (Meiswinkel et al., 2008a), Portugal (Capela et al., 2003), Sicily (Torina et al., 2004), Switzerland (Cagienard et al., 2006)]. This design has been successful in capturing large numbers of adult *Culicoides* across many trap sites, with collections approaching one million individuals in a single night's trapping in the Republic of South Africa (Meiswinkel, 1998). In northern Europe, however, the OVI trap does not provide an unbiased estimate of the host-seeking activity of the Palaearctic BTV vectors (Carpenter et al., 2008d). The distance over which *Culicoides* are attracted to a trap will also significantly affect catches from any habitat, if the range of attraction is greater than the extent of the habitat type. The range over which light acts as an attractant in different landscapes is undetermined for *Culicoides*, and for many other vectors (Killick-Kendrick et al., 1985) and is likely to increase with increasing light intensity. Response of adult *Culicoides* to light also vary dependent on the wavelength emitted from the bulb (Bishop et al., 2004, 2006; Venter and Hermanides, 2006). The heavy weight of OVI traps (>4 kg) also causes practical difficulties for suspending the traps within different habitat types.

Techniques to improve the efficiency of light-baited traps for the collection of haematophagous insects have been based on the addition of semiochemical baits, to improve both the range and/or number of insects collected. Traps baited with semiochemicals, predominantly CO_2, in combination with light, or as the sole attractant, have a long history of use with hematophagous insects, particularly in the USA where they are commonly used as part of wider mosquito-borne arbovirus surveillance campaigns [for example WNV surveillance (Lukacik et al., 2006; Andreadis et al., 2001)]. In general, kairomone-baited traps used without a light source have the advantage of collecting only the host-seeking proportion of the adult population. They also collect species with diurnal activity patterns as well as those that are nocturnal or crepuscular, and have the potential to be more easily standardised against live animal hosts (Gillies and Wilkes, 1969, 1970). Odour-baited traps, however, tend to be highly selective in the species sampled, according to the semiochemical(s) used as bait and how representative these are of the vertebrate host species (Takken and Knols, 1999).

5.1.1 Selection of Semiochemical Bait Combinations for BTV Vector Surveillance

Development of a trap type for vector surveillance, that replicates whole host odour, is unrealistic due to the complexity of host odour profiles. The human body, for example, is known to produce between 300-400 volatile chemicals (Bernier et al., 2002), only a proportion of which are behaviourally active in insect host location and attraction (Logan et al., 2009). Replicating the effects of multicomponent profiles also remains expensive and, in most situations, unavailable for use in the field. Hence, the majority of trap types tend to employ only one or two synergistic components that have unequivocally demonstrated activity and are relatively easy to formulate for release in traps. Some of the fundamental work enabling selection of these components for *Culicoides* trapping has already been carried out, with several kairomones exhaled by hosts identified as activators and attractants for *Culicoides*, including the kairomones CO_2 (Nelson, 1965) and the volatile chemical 1-octen-3-ol (Kline et al.,

1994). An order for the efficiency of the host-derived volatile chemicals for activating host-seeking behaviour has been suggested with, for example, twice as much acetone being required to evoke EAGs (electroantennogram) of a similar amplitude in *C. impunctatus* when compared to 1-octen-3-ol (Bhasin et al., 2000a).

For *Culicoides* dose-dependent responses to CO_2 have been demonstrated in EAG studies (Bhasin et al., 2000a; Grant and Kline, 2003). In the field, abundance in trap catches has been shown to be positively correlated with increasing CO_2 release rates across a range of species (Mullens and Gerry, 1998; Kline et al., 1994) and life stages (Mullens, 1995). Trap responses to host equivalent release rates of CO_2 have been reported for *Culicoides* [500 $ml \cdot min^{-1}$ for a small calf; 1000 $ml \cdot min^{-1}$ for a standard dairy cow (Kinsman et al., 1995; Kirchgessner et al., 1991; Pinares-Patino et al., 2007) and up to 3000 $ml \cdot min^{-1}$ for a large Holstein bull (Mullens, 1995)]. Indicating CO_2 could be incorporated within a trapping system that provides a realistic approximation of the host-seeking population of *Culicoides* in an area. There is evidence, however, that *C. obsoletus* s.l. populations are poorly reflected by CO_2 baited suction traps when compared to standard light trap collections (Mullens et al., 2005; Takken et al., 2008). This indicates that additional semiochemicals may be involved, in addition to CO_2, for host location by this species group. A synergistic combination of CO_2 with another host kairomone identified as being behaviourally active in host location in a trap may overcome the apparent lack of response in *C. obsoletus* s.l. to CO_2 alone, thus improving trap catches in a host equivalent manner. Some trapping systems have also tried to incorporate other aspects such as warming and/or pulsing of the released odour stream [e.g. Mosquito Slayer® (Bantix Worldwide), Mosquito Magnet® (Woodstream Corp.)] to try to replicate release from a host, however, these remain expensive additions to traps for a large scale surveillance system.

In general, unlike CO_2 which has been used at host-equivalent release rates, for many of the other semiochemicals it is only when released at 'supernormal' doses, well above levels naturally found in livestock breath, that their attractiveness to haematophagous insects is apparent in trap catches (Vale and Hall, 1985). Bovine

equivalent concentrations of 1-octen-3-ol (1.3×10^{-5} mg·l^{-1}), for example, failed to elicit an upwind response in *C. impunctatus* when used alone (Bhasin et al., 2000b). For 1-octen-3-ol ranges of 0.06 mg·h^{-1} (Bhasin et al., 2001) to 40.0 mg·h^{-1} (Kline et al., 1994) have previously been used to collect *Culicoides*. Although very high concentrations of 1-octen-3-ol (>100 μg·μ^{-1}) may be repellent (Bhasin et al., 2000b,a; Blackwell et al., 1996). Luckily, 'supernormal' doses are more practical to dispense from traps in the field. Whether these provide a host equivalent response in *Culicoides*, however, is undetermined.

Many of the volatile chemicals eliciting responses in haematophagous insects, that increase the probability of host location, have different isomeric forms. 1-octen-3-ol, for example, is a secondary alcohol derived from 1-octene, which occurs naturally as two optically active enantiomers (S)-(+) and (R)-(-), with carbon-3 as the chiral centre (Kline et al., 2007). The ratio of the enantiomers varies according to the source, but (R)-(-)-1-octen-3-ol has been reported as the predominant enantiomer exhaled from hosts (Hall et al., 1984; Pierce et al., 1989; Dijkstra and Wiken, 1976), with an enantiomeric R:S composition varying from 80:20 to 92:8 in samples of ox breath (Hall et al., 1984). Currently a synthetic 1-octen-3-ol, which is a racemic mixture (50:50, R:S 1-octen-3-ol enantiomers) is available commercially, and utilised as a semiochemical bait in several trap types (Cilek et al., 2003; Cilek and Hallmon, 2005; Lloyd et al., 2008; Mands et al., 2004). The differential attractiveness of the different 1-octen-3-ol enantiomers to *Culicoides* is unknown. For mosquitoes, R-enantiomers acted as better attractants than S-enantiomers, (used at 2.0 mg·h^{-1} in CO_2 (500 ml·s^{-1}) baited traps) (Kline et al., 2007) but, for Tsetse species no such differences in attractiveness was found either in the field or in EAG studies (Hall et al., 1984).

5.1.2 Justification for Research

Semiochemical traps may overcome some of the drawbacks in the technology currently used for *Culicoides* surveillance and research. While the effect of many semiochemicals remains to be assessed, the host kairomones CO_2 and 1-octen-3-ol have been reported as eliciting species-specific electrophysiological and behavioural responses in a wide range of *Culicoides* species, and offer a combination that is cost-effective, easily obtainable and usable in a field situation. Hence these compounds represent a potential bait for traps that will, at least partially, reflect the host-seeking activity of *Culicoides* in the field. In light of the current lack of comparable field based assessments of the efficiency of non-light based traps for *Culicoides* surveillance, this study tests the hypothesis that semiochemical-based trapping techniques can be used to provide representative abundance estimates of the adult livestock-associated *Culicoides* in south east England, through the following three field-based investigations:

(1) Comparison of the relative efficiencies of the use of CO_2 and a range of enantiomers of 1-octen-3-ol [comparing (R)-(-)-1-octen-3-ol and (S)-(+)-1-octen-3-ol, and the commercially available racemic mix (50:50 R:S 1-octen-3-ol)] for the enhancement of trap catches of livestock associated *Culicoides* in south east England.

(2) Investigation of the number and species composition of *Culicoides* collected by semiochemical-baited traps, to that which would be expected, under similar conditions, in standard light-suction surveillance.

(3) Assess whether a representative sample of the host-seeking population was being collected by the semiochemical-baited trap by comparing the species composition and the gender/parity structure of *Culicoides* collected by the most attractive 1-octen-3-ol and/or CO_2 combination to that collected by a sheep-baited drop trap.

To enhance potential applicability for surveillance, the trap type employed [miniature Center for Disease Control traps (Model 512, J.W. Hock, Gainsville, Florida)],

was one that had previously been used unlit and baited with host odours for the collection of mosquitoes [Diptera: Culicidae] (Kline et al., 1990; Mboera et al., 2000; Mullens and Gerry, 1998; Van den Hurk et al., 2006), sandflies [Diptera: Psychodidae] (Pinto et al., 2001) and *Culicoides* (Mullens and Gerry, 1998), and which are portable, inexpensive and easy to operate.

5.2 Materials and Methods

5.2.1 Study Area and Trap Locations

This experiment was conducted on farm A [see Chapter 2 Section 2.1 on page 28 for site details] a mixed dairy/sheep farm with a well studied natural population of *Culicoides* species. Traps were sited in an area of homogeneous grazing pasture flanked by deciduous woodland, and positioned approximately 10 m from the pasture edge, with an inter-trap distance of >50 m to prevent interference between the traps [see Figure 5.1 on page 143].

To allow a comparison between the species composition, gender/parity structure and overall size of the CDC trap catches with that normally expected from traps employed in routine light-suction surveillance, the semiochemical-baited CDC traps were run in parallel with two OVI traps for 14 sampling nights. The OVI traps were situated in the same area of homogeneous pasture at approximately 10 m from the pasture edge, but further than 100 m from any of the other trap locations to avoid interference.

Four semiochemical baited CDC traps were used per sampling night, one trap per semiochemical bait type. The order of semiochemical treatments at CDC trap locations were randomly chosen for the first night, and on each subsequent night the treatments were rotated counter-clockwise to the next location. OVI traps were not rotated, due to restrictions on the availability of homogeneous areas in which to set the traps up, as if both OVI and CDC traps were rotated larger inter-trap distances of >100 m would have been required between all trap locations. This increased distance was unavailable within areas of homogeneous pasture. Following two rotations, trap location 4 (L4) had to be moved to location 5 (L5) due to a parallel animal bait experiment that required installation of a drop trap at L4. As a consequence, during the 20 night experiment, each treatment occupied positions L1, L2, and L3, five times, but L4 only twice and L5 only three times.

5.2.2 Treatments

The experiment was conducted from 10^{th} June 2008 to 27^{th} September 2008 using a four by four Latin square design (20 nights, 80 collections). Traps were operated for three hours at dusk (one hour before sunset to two hours after sunset) to coincide with maximal *Culicoides* activity. Unlit CDC traps [see Chapter 2 Section 2.2.2 on page 30 for trapping protocol] were baited with the one of the following four treatments :

(A) CO_2 with (S)-(+)-1-octen-3-ol

(B) CO_2 with (R)-(-)-1-octen-3-ol

(C) CO_2 with Racemic 1-octen-3-ol [50:50 (R)-(-)-1-octen-3-ol:(S)-(+)-1-octen-3-ol]

(D) CO_2

Five rotations of these treatments were performed, three rotations using a CO_2 release rate of $500\,\mathrm{ml{\cdot}min^{-1}}$ (12 nights, 48 trap catches), followed by two further rotations using a CO_2 release rate of $1000\,\mathrm{ml{\cdot}min^{-1}}$ (8 nights, 32 trap catches). These release rates were chosen to mimic approximate host release rates with, $500\,\mathrm{ml{\cdot}min^{-1}}$ for a small calve and $1000\,\mathrm{ml{\cdot}min^{-1}}$ for a standard dairy cow (Kinsman et al., 1995; Kirchgessner et al., 1991; Pinares-Patino et al., 2007). The average release rate of the (R)-(-)-1-octen-3-ol, (S)-(+)-1-octen-3-ol (supplied by Rothamsted Research) and Racemic 1-octen-3-ol, a 50:50 mix of, R- and S-, 1-octen-3-ol enantiomers (Alfa Aesar, Heysham, UK) for the three hour trapping period was calculated from the difference in the weight of the chromacol vials containing each 1-octen-3-ol sample [see Section 2.2.2 on page 30] between the beginning and end of each trapping period, measured using an Ohaus Scout Pro portable balance [model SPU123, (error 0.003 S.D.)] (Ohaus Corporation, Pine Brook, New Jersey, USA). Each trap and collection bottle was rotated through the trap stations (L1-L5) with its original treatment, to prevent any interference due to contamination of a previous treatment.

The overall species composition, including gender/parity information, collected by traps baited with the most efficient treatment (see results) was then compared

to that attracted to, and collected by, a sheep-baited drop trap adjacent to location four [see Figure 5.1 on page 143], using methodology modified from Carpenter et al. (2008d) [see Chapter 2 Section 2.2.3 on page 32 for trapping protocol]. Collections of *Culicoides* attracted to, and in the immediate proximity of the sheep within the drop trap were made 3-5 times per night for five nights between 18^{th} August and the 24^{th} September 2009. For each of these five nights of sampling, one semiochemical-baited CDC trap (set according to the methodology described in Chapter 2 Section 2.2.2 on page 30) was positioned at location 4 [see Figure 5.1 on page 143], approximately 10 m from the edge of the drop trap cage. The CDC trap was run on the same five nights as the sheep-baited drop trap, and was operated from when the sheep first entered the holding corral until they were released back to the field (approximately two and half hours per night).

Within all insect samples collected *Culicoides* were morphologically identified to species/species group level as *C. nubeculosus*, *C. pulicaris* s.l., *C. obsoletus* s.l. [see Section 2.3 on page 36 for methodology], then Obsoletus group females were identified to species level as *C. obsoletus* s.s., *C. scoticus*, *C. dewulfi*, *C. chiopterus*, using multiplex PCR [see Section 2.4 on page 40 for methodology].

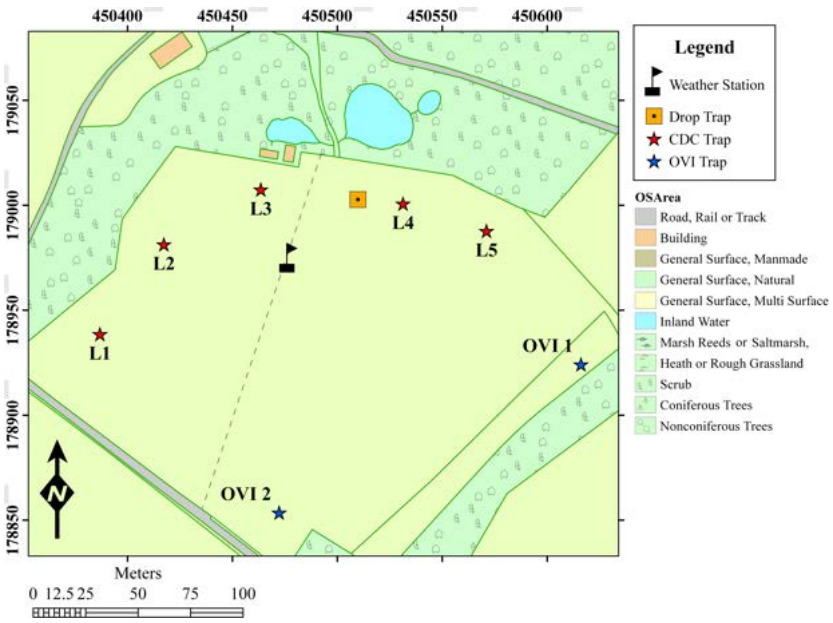


Figure 5.1. Spatial distribution of trap locations at Farm A [with OS Mastermap base map (Ordnance Survey, 2009) and British National Grid graticles]

5.2.3 Meteorological Conditions

Meteorological conditions were recorded using an automatic weather station equipped with a CR800 measurement and control system (Campbell Scientific Inc, Shepshed, UK) [see Figure 5.2 below], located within 100 m of all five CDC trap locations [see Figure 5.1 on page 143]. Air temperature (degrees centigrade (℃), humidity (%), solar intensity (watts per square meter ($W \cdot m^{-2}$)), wind speed (metres per second ($m \cdot s^{-1}$)) and wind direction (degrees (°)) were recorded at 15 minute intervals through the trapping period, and values summarised (mean and maximum values) across each three hour trapping period.

Wind direction is a circular variable, with the constraint that directions of 0° and 360° represent the same direction. A mean transformed wind direction for each trapping period was therefore calculated using the ArcTangent2 function in Excel (Microsoft Corporation, 2003), according to the mean angle trigonometric approach that transforms the wind direction from cosine (northness) and sine (eastness) variables.

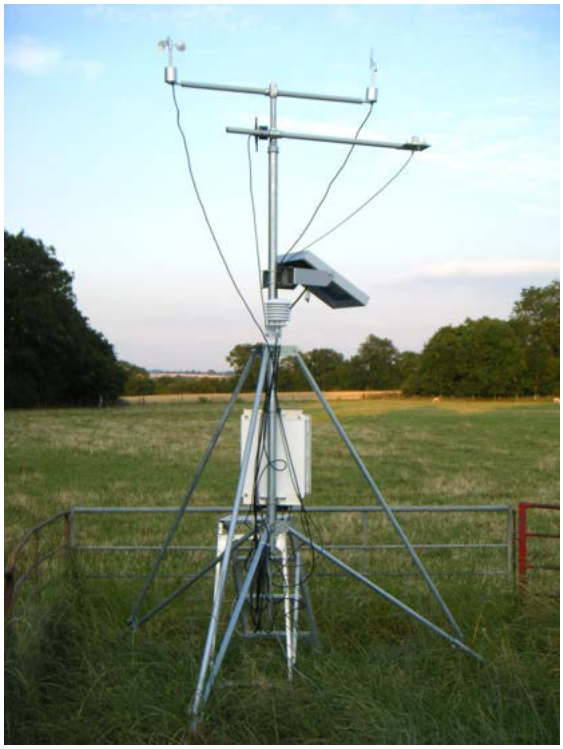


Figure 5.2. Automatic weather station with CR800 Measurement and control system (Campbell Scientific Inc., Shepshed, UK) *in situ* at farm A

5.2.4 Modelling the Impacts of Semiochemical Treatment, Location and Meteorological Conditions on *Culicoides* Trap Catch Size

OVI traps were not rotated as part of the Latin square design used for the CDC traps, hence were analysed separately using paired Wilcoxon rank sum tests [R package 'exactRankTests' (Hothorn and Hornik, 2009)]. Significant differences, and the direction of any differences, in the species composition, and gender and parity structure [nulliparous, parous, blood-fed and gravid] of the semiochemical-baited traps were compared to that which would be expected from standard light-suction surveillance methods under the same conditions [max catch from two OVI traps used per sampling period].

The influence of the semiochemical treatment and CO_2 release rate on *Culicoides* trap catches were presented in two different models. Model A describing the total *Culicoides* catch size, and model B describing female *C. obsoletus* s.l. catch size. The influence of the semiochemical treatment and CO_2 release rate, within both model A and model B, controlling for the effects of trap location and meteorological conditions, were modelled using GLMs. Ten parameters were considered within each GLM, semiochemical treatment, CO_2 release rate, trap location, mean air temperature, mean humidity, mean solar intensity, mean wind speed, maximum wind speed, transformed wind direction and variation in wind direction. Insufficient numbers of *C. pulicaris* s.l. were collected for statistical analysis.

The distribution of model residuals for both model A and model B did not conform to a standard Poisson distribution, with evidence of overdispersion [residual deviance (i.e the unexplained variation, the residual deviance should $\approx$ degrees of freedom (d.f.)) of 2618.4 on 64 d.f. for model A, and 742.7 on 64 d.f. for model B]. In addition, there were a high number of zero catches in the raw catch data, i.e. traps where no *Culicoides* were collected [17 for model A and 38 for model B], rather than the expected 8 and 21 respectively for the Poisson distribution in a 10 parameter model]. To account for both the excessive zeros and overdispersion, a zero-inflated glm [R package 'pscl' (Jackman, 2008; Zeileis et al., 2008)] was used comparing Poisson

(Z.p), negative binomial (Z.nb) and geometric (Z.geo) distributions using pair-wise Vuong closeness tests [R package 'pscl' (Jackman, 2008)]. Vuong closeness tests are a likelihood-ratio-based test for non-nested model selection using the Kullback-Leibler information criterion, these were used to see which distribution best described the data (Vuong, 1989).

Zero-inflated models allow the description of the data to be split into two parts, one explaining the zeros in the data set (zero-inflation model) and the other, given the zero-inflation model describing a proportion of the variation, explaining the data with positive counts (count model) [for further details see Zeileis et al. (2008)]. Explanatory variables can be introduced into either or both parts of the model. For example, if different processes such as meteorological conditions determine that no *Culicoides* occur in catches compared to those that govern the number that will be caught on nights when *Culicoides* are present.

For both model A [Z.p $_{[Voung\ -6.31(P<0.01)]}$ < Z.nb > Z.geo $_{[Voung\ 3.61(P<0.01)]}$] and model B [Z.p $_{[Voung\ -3.63(P<0.01)]}$ < Z.nb > Z.geo $_{[Voung\ 1.09(P<0.01)]}$] the distribution of catch sizes were best described by a zero-inflated negative binomial model.

Initially, both model A and model B included: semiochemical treatment, CO_2 release rate and trap location which were included as categorical explanatory variables; and mean air temperature, mean humidity, mean solar intensity, maximum wind speed, mean wind speed, mean transformed wind direction and variation in wind direction which were included as continuous covariates. The final model was obtained using backward-stepwise selection based on the AIC; non-significant terms were manually discarded, with the best model corresponding to the one with the lowest AIC with all terms significant. Performances of final models were compared based on likelihood ratio tests for nested models [R package 'lmtest' (Hothorm et al., 2009; Zeileis and Hothorn, 2002)]. Differences in catch size between semiochemical treatments, CO_2 release rate, and trap locations were assessed using multiple Tukeys all-pair comparisons [R package 'multcomp' (Hothorn et al., 2008)]. The total variance explained by the final models was assessed using McFadden's Pseudo R^2 (ρ).

This measure is a log-likelihood-based Pseudo R^2 measure [ρ = 1- [L_A - L_O], where L_O is the log-likelihood of the null model and L_A is the log-likelihood of the alternative model]. Values of ρ between 0.20 and 0.40 are considered an excellent model fit (McFadden, 1979).

Variation in the meteorological conditions during trapping at the two different CO_2 release rates were compared firstly using the Fligner-Killeen test of homogeneity of variances. Where no significant difference in the variance was found, mean levels of abundance between the two trapping periods were compared using the Student's t test and where the variances were not equal, using the Wilcoxon rank-sum test [R package 'exactRankTests' (Hothorn and Hornik, 2009)].

Variation in the total trap catch size, species composition and the population gender/parity structure of OVI trap catches (max catch from two OVI trap catches per night) were compared to catches made by the four semiochemical treatments (CDC Traps). OVI and semiochemical-baited CDC traps were operated for the same time period each night, however, significant difference in the variances as shown by the Fligner-Killeen test of homogeneity of variances were present, therefore catches were compared using paired Wilcoxon rank sum tests [R package 'exactRankTests' (Hothorn and Hornik, 2009)].

Paired sheep (total of nights catch, for between $n = 3$ to 5 exposure periods) and semiochemical-baited CDC trap catches were compared using the χ^2 test to assess any significant differences, in the proportion of species/species groups, their gender and parity structure [nulliparous, parous, blood-fed and gravid] which made up the trap catchs collected by the two traps. Due to the differences in sampling time, three to five 10 minute exposures for the sheep-baited drop trap and $\approx$ 2 1/2 hours for the semiochemical-baited drop trap, the total catch of *Culicoides* or *C. obsoletus* s.l. were not comparable.

5.3 Results

5.3.1 1-octen-3-ol Release Rate

An average release rate of approximately 4.1 $mg \cdot h^{-1}$ was recorded for all three 1-octen-3-ol baits trialled [see Table 5.1].

	CO_2 Release Rate	
Semiochemical Treatment	500 $ml \cdot s^{-1}$	1000 $ml \cdot s^{-1}$
A: (S)-(+)-1-octen-3-ol	4.071 (0.544)	4.042 (0.628)
B: (R)-(-)-1-octen-3-ol	4.220 (0.886)	4.083 (0.556)
C: Racemic 1-octen-3-ol	4.056 (0.680)	4.125 (0.853)

Table 5.1. Average 1-octen-3-ol release rate [mean ($\bar{x}$) $\pm$ 1 S.D.] recorded over a three hour period measured over 14 nights per treatment

5.3.2 Species Composition and Gender/Parity Structure of Raw Catch Data

In semiochemical-baited CDC traps 3355 *Culicoides* were collected over 20 nights in 80 samples. Of these individuals 79.67 % were *C. nubeculosus*, 19.91 % *C. obsoletus* s.l. and 0.42 % *C. pulicaris* s.l. The maximum catch for a three hour period reached 1349 for all *Culicoides* species and 137 for *C. obsoletus* s.l. The majority of *Culicoides* collected in the CDC traps were female (98.78 %), and nulliparous (90.55 % of females), with some parous individuals (9.33 %) and, very few gravid (0.06 %) or blood-fed (0.12 %) females [see Figure 5.3 on page 149].

Of the 668 *C. obsoletus* s.l. collected in the CDC traps, the majority were female (99.70 %) and nulliparous (71.92 % of females identified), although a substantial proportion were parous (35.14 % of females identified). For *C. nubeculosus* only 3.68 % females were parous. Amongst female *C. obsoletus* s.l., *C. obsoletus* s.s. dominated (94.89 % of females identified), followed by *C. scoticus* (3.45 % of females identified), *C. dewulfi* (0.15 % of females identified) and *C. chiopterus* (0.45 % of females identified). 1.05 % of female *C. obsoletus* s.l. could not be identified to species level by the multiplex PCR technique [see Figure 5.4 on page 149].

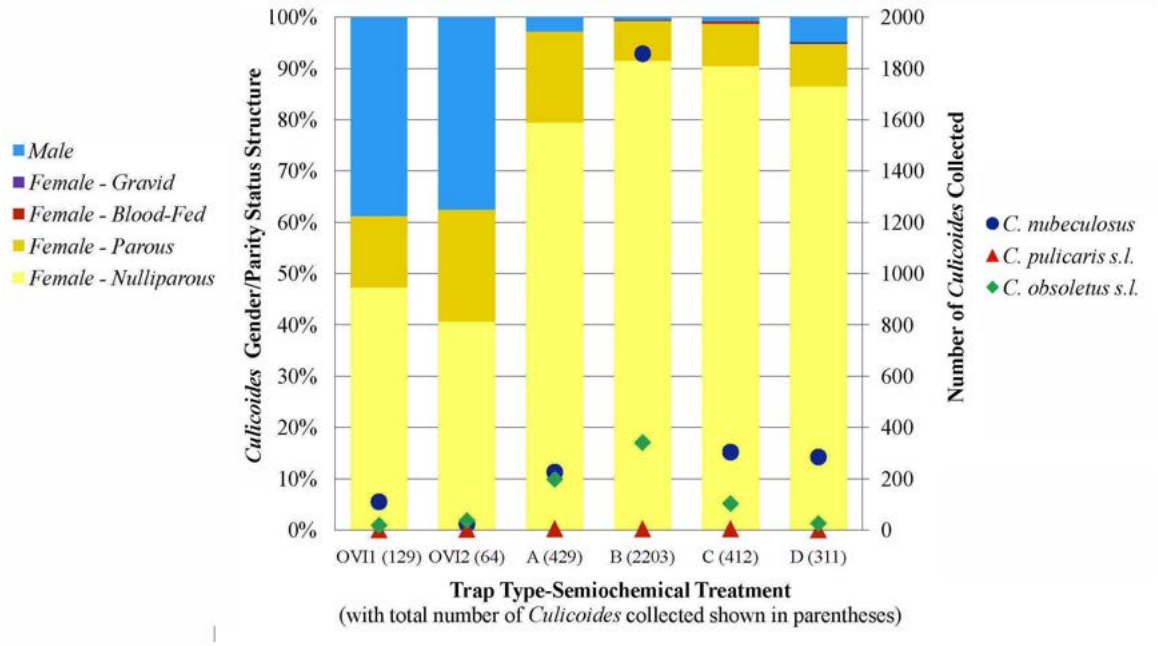


Figure 5.3. Variation in species composition and gender/parity structure of *Culicoides* populations collected in OVI and semiochemical-baited CDC traps (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)

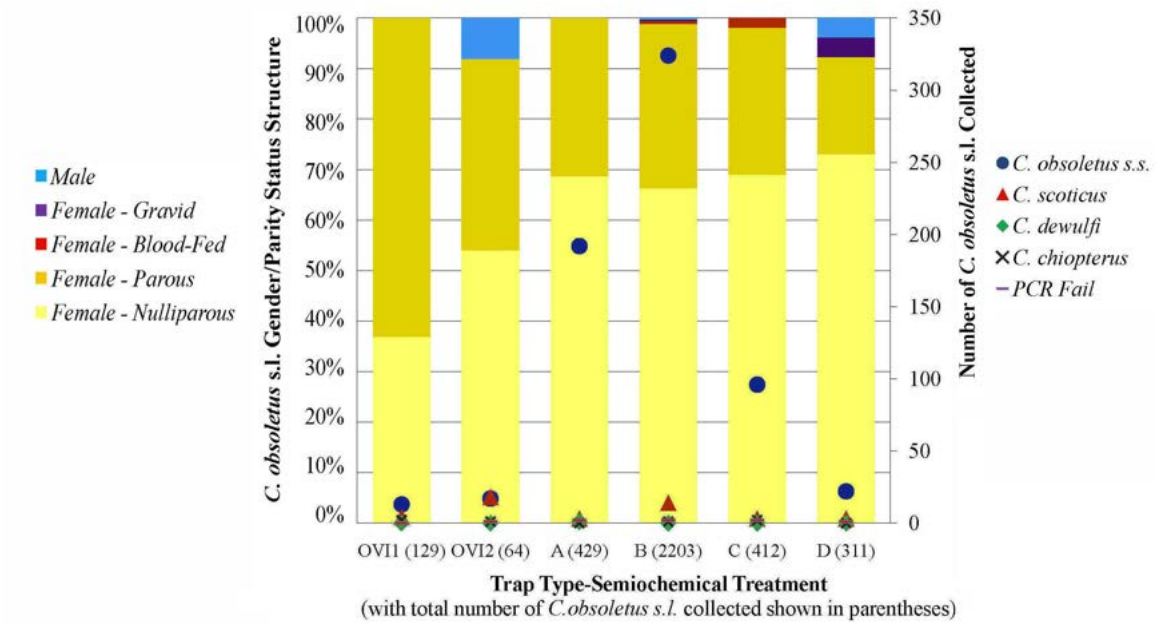


Figure 5.4. Variation in species composition and gender/parity structure of *C. obsoletus* s.l. populations collected in OVI and semiochemical-baited CDC traps (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)

5.3.3 Comparison to Standard Light-Suction Surveillance

The OVI traps collected a total of 193 *Culicoides* over 14 nights (28 samples from two traps). No significant difference in the number *Culicoides* collected per night was detected between the OVI traps and the semiochemical-baited CDC traps [see Table 5.2 on page 151]. The overall species composition showed a similar pattern to that found in semiochemical-baited CDC traps with *C. nubeculosus* again dominant (making up 69.43 % of the total number of individuals collected), followed by *C. obsoletus* s.l. (29.02 %) and *C. pulicaris* s.l. (1.55 %). CDC traps baited with treatment B and C collected significantly ($P \leq 0.05$) more *C. nubeculosus* than the OVI traps and the CDC traps baited with treatment B collected significantly more *C. obsoletus* s.l. ($P \leq 0.01$), than the OVI traps.

61.66 % of the total number of *Culicoides* collected by the OVI traps were female, of which 73.11 % were nulliparous and 26.89 % parous, no blood-fed or gravid individuals were collected [see Figure 5.3 on page 149]. Significantly fewer males were collected by CDC traps baited with treatment C ($P \leq 0.01$) and D ($P \leq 0.05$) than the OVI traps (max catch of two OVI traps made per night) and CDC traps baited with treatments B ($P \leq 0.001$) and C ($P \leq 0.01$) collected significantly more nulliparous females, than the OVI traps did (max catch of two OVI traps made per night).

Of the 53 female *C. obsoletus* s.l. collected at the OVI traps 54.72 % were *C. obsoletus* s.s., 37.74 % *C. scoticus*, and 5.66 % *C. chiopterus*. No *C. dewulfi* were collected in the OVI traps. 1.89 % of female *C. obsoletus* s.l. could not be identified to species level using the multiplex PCR [see Figure 5.4 on page 149]. Treatment B collected significantly more nulliparous *C. obsoletus* s.l. ($P \leq 0.01$) and *C. obsoletus* s.s. ($P \leq 0.01$) than the OVI traps (max catch of two OVI traps made per night). No other significant differences were observed. [see Table 5.4 on page 151].

Treatment	*Culicoides*	*C. nubeculosus*	*C. pulicaris* s.l.	*C. obsoletus* s.l.
A	27.79 (30.38)	14.36 (19.52)	0.36 (0.63)	12.21 (26.00)
B	154.86 (361.91)	131.50 (345.00) *	0.21 (0.58)	22.36 (38.60) **
C	28.29 (49.06)	21.21 (48.04) *	0.21 (0.58)	6.71 (12.37)
D	21.14 (29.80)	18.36 (29.15)	0.00 (0.00)	1.71 (3.54)

Table 5.2. Overall composition of *Culicoides* - Mean Catch ($\bar{x}$) ± S.D. with significant differences of semiochemical-baited CDC trap catches vs that expected in standard light-suction based vector surveillance (max catch of two OVI traps made per night) [*P≤0.001, **P≤0.01, *P≤0.05] (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)**

Treatment	*C. obsoletus* s.s.	*C. scoticus*	*C. dewulfi*	*C. chiopterus*
A	8.35 (20.77)	0.13 (0.34)	0.04 (0.21)	0.00 (0.00)
B	14.13 (30.45) **	0.61 (1.20)	0.00 (0.00)	0.04 (0.21)
C	4.17 (9.54)	0.13 (0.46)	0.00 (0.00)	0.09 (0.29)
D	0.96 (2.40)	0.13 (0.46)	0.00 (0.00)	0.00 (0.00)

Table 5.3. Species composition of the Obsoletus group - Mean Catch ($\bar{x}$) ± standard deviation (SD) with significant differences of semiochemical-baited CDC trap catches vs that expected in standard light-suction based vector surveillance (max catch of two OVI traps made per night) [*P≤0.001, **P≤0.01, *P≤0.05] (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)**

Treatment	*Culicoides*			*C. obsoletus* s.l.		
	Males	Females		Males	Females	
		Nulliparous	*Parous*		*Nulliparous*	*Parous*
A	0.86 (2.93)	21.64 (23.83)	5.29 (9.21)	0.00 (0.00)	7.86 (17.84)	4.36 (8.68)
B	0.79 (2.12)	141.93 (340.69) ***	11.93 (23.35)	0.07 (0.27)	14.57 (26.28) *	7.57 (12.76)
C	0.14 (0.53) **	25.79 (48.46) **	2.21 (3.93)	0.00 (0.00)	4.64 (8.90)	1.93 (3.65)
D	1.07 (1.64)	18.21 (27.97)	1.79 (3.77)	0.07 (0.27)	1.29 (2.37)	0.36 (1.08)

Table 5.4. Gender/parity structure of collected *Culicoides* - mean catch ($\bar{x}$) ± S.D. with significant differences of semiochemical-baited CDC trap catches vs that expected in standard light-suction based vector surveillance (max catch of two OVI traps made per night) [*P≤0.001, **P≤0.01, *P≤0.05] (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)**

5.3.4 Modelling the Impacts of Semiochemical Treatment, Location and Meteorological Conditions on *Culicoides* Trap Catch Size

The final model describing the total number of *Culicoides* (male and female, all species) (model A) selected by backwards-stepwise selection of variables, included six variables within the count section, and one variable within the zero-inflated section of the model [see Table 5.5 below]. Decreasing mean air temperature was found to be a significant predictor on nights of absence of *Culicoides* from trap catches i.e. zero catches. Catch size of *Culicoides* increased with temperature and as wind direction moved clockwise, and decreased with increasing variability in the wind direction and increasing wind speed. Mean humidity, mean solar incidence and maximum wind speed were found to have no significant effect on either the presence or absence of *Culicoides* in trap catches, within the range of conditions experienced during trapping [see Table 5.13 on page 160].

Parameter	**Estimate**	95 % **Confidence Intervals**	ΔAIC
Count Model Coefficients			
Intercept	0.81	-1.56 ; 3.18	
Treatment B	0.79	-0.12 ; 1.70	9.73
Treatment C	-0.76	-1.70 ; 0.17	-
Treatment D	-1.18	-2.13 ; -0.23	-
Location *ii*	2.60	1.78 ; 3.43	24.07
Mean Air Temperature	0.15	0.01 ; 0.28	1.70
Mean Wind speed	-0.12	-0.18 ; -0.06	8.70
Mean Transformed Wind Direction	0.02	0.01 ; 0.03	12.10
Variation in Wind Direction	-0.02	-0.03 ; -0.01	13.48
Zero-Inflation Model Coefficients			
Mean Air Temperature	-0.52	-0.38 ; -0.05	13.48

McFadden Pseudo R^2: 0.10

Table 5.5. Regression coefficients for the zero-inflated negative binomial model resulting from backwards-stepwise selection of variables in model A (describing the total number of *Culicoides* (male and female, all species) collected during a three hour trapping period) [variables included within the count model have a significant relationship to nights when *Culicoides* were present within trap catches. Variables included within the zero-inflation model have a significant relationship to nights when *Culicoides* were absent from trap catches] (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)

The final model describing the total number of collected female *C. obsoletus* s.l. (model B), selected by backwards-stepwise selection of variables, included five variables within the count section, and four variables within the zero-inflated section of the model [see Table 5.6 below]. Catch sizes of female *C. obsoletus* s.l. increased with increasing mean humidity and decreased with temperature and high wind speed. Increasing mean humidity and mean wind speed, however, were also was found to be a significant predictor on nights of absence of *C. obsoletus* s.l. from trap catches i.e. zero catches in addition to the positive effect on the likelihood of zero catches of increasing maximum windspeed. Mean wind direction and mean solar incidence were found to have no significant effect on the either the presence or absence of female *C. obsoletus* s.l. in trap catches, within the range of conditions experienced during trapping [see Table 5.13 on page 160]

Parameter	**Estimate**	95 % **Confidence Intervals**	ΔAIC
Count Model Coefficients			
Intercept	-0.65	-5.03 ; 3.74	
Semiochemical B	0.95	-0.05 ; 1.95	15.77
Semiochemical C	-0.71	-1.69 ; 0.28	-
Semiochemical D	-1.85	-2.94 ; -0.77	-
Location *ii*	2.31	1.34 ; 3.27	14.48
Mean Air Temperature	-0.16	-0.29 ; -0.03	3.02
Mean Humidity	0.05	0.00 ; 0.09	1.33
Mean Wind Speed	-0.12	-0.18 ; -0.05	14.09
Zero-Inflation Model Coefficients			
Intercept	-19.49	-38.03 ; -0.96	
Mean Humidity	0.19	-0.02 ; 0.40	2.21
Mean wind speed	-4.31	-8.30 ; -0.33	4.56
Maximum wind speed	3.87	0.19 ; 7.55	4.34
Mean Transformed Wind Direction	-0.19	-0.32 ; -0.06	15.03

McFadden Pseudo R^2: 0.14

Table 5.6. Regression coefficients for the zero-inflated negative binomial model resulting from backwards-stepwise selection of variables in Model B (describing the total number of female *C. obsoletus* s.l.) collected during a three hour trapping period) [variables included within the count model have a significant relationship to nights when female *C. obsoletus* s.l. were present within trap catches. Variables included within the zero-inflation model have a significant relationship to nights when female *C. obsoletus* s.l. were absent from trap catches], (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)

5.3.4.1 Effect of Location

Initial models indicated that for both model A (total *Culicoides*) and model B (female *C. obsoletus* s.l.), trap location 1 collected significantly fewer (P≤0.001) female *Culicoides* and female *C. obsoletus* s.l. than locations 2, 3, 4, 5. No significant differences in catch sizes, during a three hour trapping period, for both model A (total *Culicoides*) and model B (female *C. obsoletus* s.l.) were found between trap locations 2, 3, 4 and 5 [see Table 5.7].

	L1	**L2**	**L3**	**L4**	**L5**
L1	-	2.57***	2.81***	3.53***	2.94***
L2	2.39***	-	0.25	0.96	0.37
L3	2.00***	-0.39	-	0.72	0.13
L4	3.42***	1.03	1.42	-	-0.59
L5	2.85***	0.46	0.85	-0.57	-

Table 5.7. Multiple Tukeys all-pair comparisons of catch size between trap locations collected during a three hours trapping period (estimate for model A (total *Culicoides* catch size) shown on upper diagonal, model B (female *C. obsoletus* s.l. catch size) shown on lower diagonal) [*P≤0.001, **P≤0.01, *P≤0.05]**

5.3.4.2 Effect of CO_2 Release Rate

Initial models and multiple Tukey's all-pair comparisons of trap catch size [see Table 5.8], indicated that no significant difference in the total *Culicoides* catch size [see Figure 5.5 on page 156] or the female *C. obsoletus* s.l. catch size [see Figure 5.6 on page 156] was observed within semiochemical treatments between the two different CO_2 releases rates (500 ml·min^{-1}; 1000 ml·min^{-1}). Hence for the final models *Culicoides* collected using the two CO_2 release rates were amalgamated, and variation in trap catch size compared solely on the 1-octen-3-ol bait used [A, B, C and D] where treatment D (CO_2 alone) was used as a control.

	Semiochemical Treatment			
	A1:A2	**B1:B2**	**C1:C2**	**D1:D2**
Total Number of *Culicoides* Collected	0.966	0.982	1.000	0.271
Total Number of Female *C. obsoletus* s.l. Collected	1.000	0.984	0.070	0.126

Table 5.8. Multiple Tukeys all-pair comparisons of model A (total *Culicoides* catch size) and model B (female *C. obsoletus* s.l. catch size), collected during a three hours trapping period, compared within treatments between two CO_2 release rates (1: $500\,ml{\cdot}min^{-1}$; 2: $1000\,ml{\cdot}min^{-1}$) [*P≤0.001, **P≤0.01, *P≤0.05] (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)**

CO_2	*Culicoides*				Female *C. obsoletus* s.l.			
Release Rate	***i* (L1)**	**Index**	***ii* (L2, L3, L4, L5)**	**Index**	***i* (L1)**	**Index**	***ii* (L2, L3, L4, L5)**	**Index**
$500\,ml{\cdot}s^{-1}$	$3.8(2.7)^A$	1.0	$69.9\ (38.7)^A$	1.0	$0.7\ (0.5)^A$	1.0	$6.9\ (2.4)^A$	1.0
$1000\,ml{\cdot}s^{-1}$	$5.0\ (2.9)^A$	1.3	$31.3\ (7.8)^A$	0.4	$1.9\ (1.3)^A$	1.2	$16.4\ (6.8)^A$	2.4

Table 5.9. Mean Catch [$\bar{x}$ (±SD)] per trap day ($500\,ml{\cdot}s^{-1}$: $n = 12$; $500\,ml{\cdot}s^{-1}$: $n = 8$) for model A (total *Culicoides* catch size) and model B (female *C. obsoletus* s.l. catch size), for different CO_2 release rates of semiochemical-baited CDC traps. Means in the same column with the same letter were not significantly different (P>0.05) when the effects of variation due to changing meteorological conditions were taken account of [index is the ratio of the CO_2 release rate mean catch with the mean catch obtained by the base release rate $500\,ml{\cdot}s^{-1}$]

CO_2 release rates were assessed sequentially [$500\,ml{\cdot}min^{-1}$: 10^{th} June to 27^{th} July; $1000\,ml{\cdot}min^{-1}$: 30^{th} July to 27^{th} September]. The effects of seasonality on the *Culicoides* population could not be accounted for, though variation in meteorological conditions between the two sampling periods was explicitly included within the model covariates. Wind direction was predominantly northeasterly at an intensity of $1\,m{\cdot}s^{-1}$ to $2\,m{\cdot}s^{-1}$ (range $0.004\,m{\cdot}s^{-1}$ to $6.279\,m{\cdot}s^{-1}$) [see Figure 5.7 on page 157]. No significant difference in the temperature, humidity, solar intensity or wind direction experienced during trapping was detected between the two periods [see Table 5.10 on page 157]. Wind speeds were on average significantly higher (P≤0.001) during the period the traps were operated with a CO_2 release rate of $500\,ml{\cdot}min^{-1}$ compared to when they were operated with a release rate of $1000\,ml{\cdot}min^{-1}$ [see Table 5.10 on page 157].

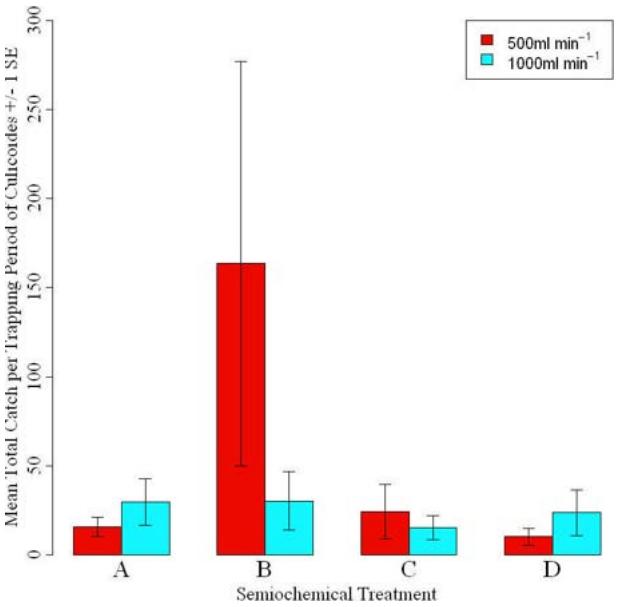


Figure 5.5. The effect of CO_2 release rate and semiochemical treatment on the mean number *Culicoides* (male and female, all species) collected per three hour trapping period ±1 S.E. (effect of varying meteorological conditions not accounted for) (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)

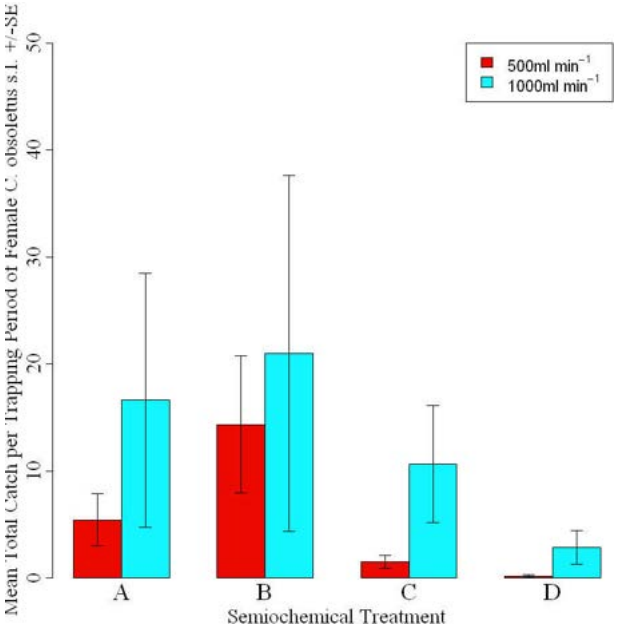
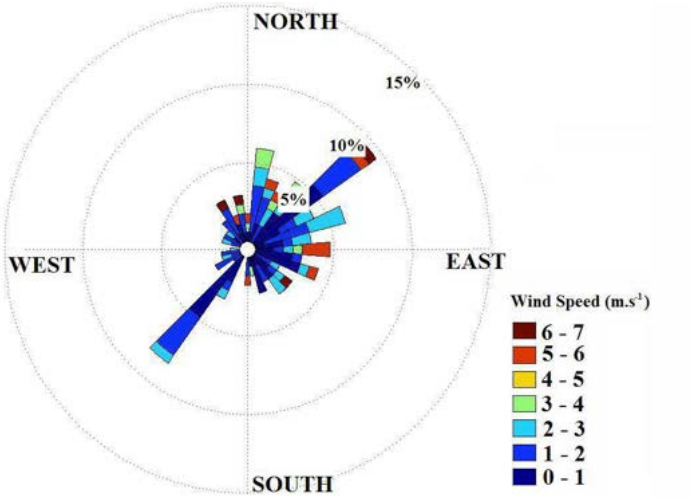


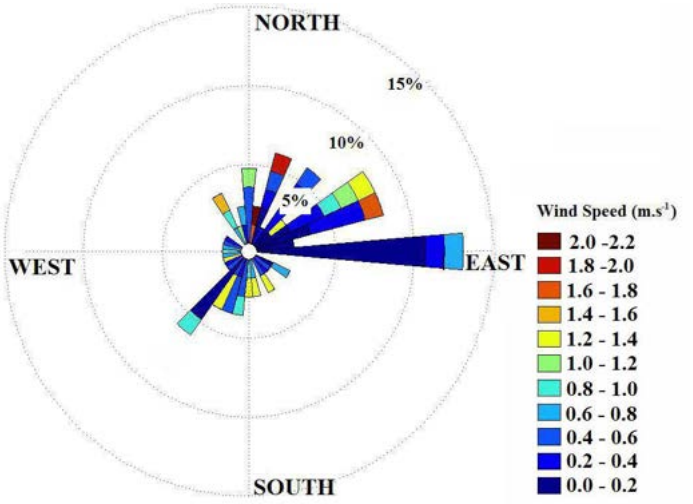
Figure 5.6. The effect of CO_2 release rate and semiochemical treatment on the mean number of female *C. obsoletus* s.l. collected per three hour trapping period ±1 S.E. (effect of varying meteorological conditions not accounted for) (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)

CO_2 Release Rate ($ml \cdot min^{-1}$)	Mean Air Temperature (°C)	Mean Humidity (%)	Mean Solar Intensity ($W \cdot m^{-2}$)	Mean Wind Speed ($m \cdot s^{-1}$)
500	15.56 (9.65-22.69)^A	77.77 (52.83-93.60)^A	11.17 (0.00-107.97)^A	1.67 (0.11-6.28)^A
1000	15.36 (10.72-22.76)^A	75.86 (49.65-92.00)^A	14.33 (0.00-124.94)^A	0.66 (0.00-2.20)^B

Table 5.10. The mean (with range shown in parenthesis) of meteorological variables experienced during trapping [means in the same column with the same letter were not significantly different (P>0.05)]



(a). CO_2 500 $ml \cdot min^{-1}$



(b). CO_2 1000 $ml \cdot min^{-1}$

Figure 5.7. Wind rose diagram representing frequency of different wind speed and wind directions during sampling [generated using Matlab® 7.1 (Ma, 2008; The MathWorks, 2006)]

5.3.4.3 Effect of Semiochemical Treatment

Semiochemical treatment significantly influenced both the total number of *Culicoides* (model A), and female *C. obsoletus* s.l. (model B) collected, when at least one *Culicoides* was collected [see count models in Table 5.5 on page 152 and Table 5.6 on page 153]. For the total number of *Culicoides* collected (model A) there was no significant difference in the number of *Culicoides* collected between traps baited with semiochemical treatments A and B, or between traps baited with treatments A and D, or treatments C and D. Traps baited with treatment B collected significantly more *Culicoides* than traps baited with treatment C ($P \leq 0.01$) and traps baited with treatment D ($P \leq 0.001$). For the total number of female *C. obsoletus* s.l. collected (model B) there was no significant difference in the number collected between traps baited with semiochemical treatments A and B, A and C, and C and D. Traps baited with treatment B collected significantly more female *C. obsoletus* s.l. than traps baited with treatment C ($P \leq 0.01$) and traps baited with treatment D ($P \leq 0.001$). Traps baited with treatment A collected significantly more female *C. obsoletus* s.l. than traps baited with treatment D ($P \leq 0.01$).

	A	**B**	**C**	**D**
A	-	0.79	-0.76	-1.18
B	0.95	-	-1.55**	-1.97***
C	-0.71	-1.66**	-	-0.42
D	-1.85**	-2.80***	-1.15	-

Table 5.11. Multiple Tukeys all-pair comparisons of catch size between semiochemical treatments taking into account variation due to changing meteorological conditions (estimate for model A (total *Culicoides* catch size) shown on upper diagonal, model B (female *C. obsoletus* s.l. catch size) shown on lower diagonal) [*P≤0.001, **P≤0.01, *P≤0.05] (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)**

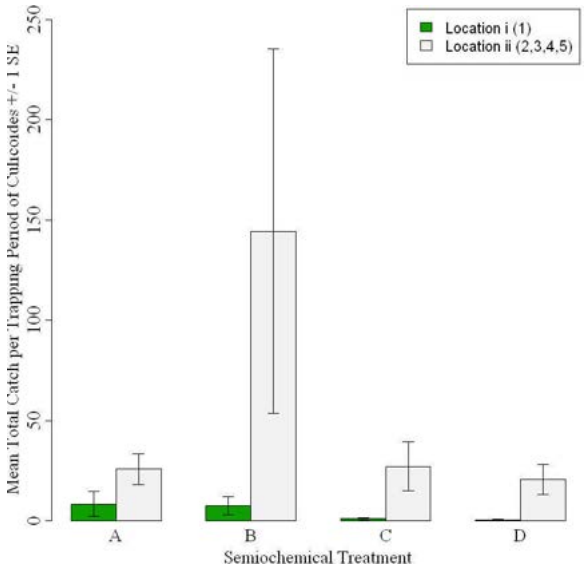


Figure 5.8. The effect of semiochemical treatment and location (as used in final model) on the mean total number of *Culicoides* collected (model A) per three hour trapping period ±1 S.E. (effect of varying meteorological conditions not accounted for) (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)

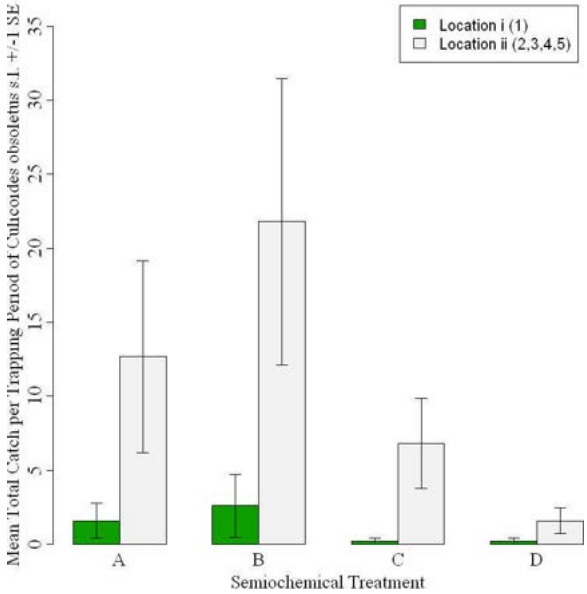


Figure 5.9. The effect of semiochemical treatment and location (as used in final model) on the mean number of female *C. obsoletus* s.l. collected (model B) per three hour trapping period ±1 S.E. (effect of varying meteorological conditions not accounted for) (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)

Treatment	*Culicoides* *i* (L1)	Index	*Culicoides* *ii* (L2, L3, L4, L5)	Index	Female *C. obsoletus* s.l. *i* (L1)	Index	Female *C. obsoletus* s.l. *ii* (L2, L3, L4, L5)	Index
A	1.6 (6.3)	8.0	12.7 (7.7)	7.9	8.4 (1.2)	21.0	25.8 (6.5)	1.3
B	2.6 (4.5)	13.0	21.8 (90.7)	13.6	7.4 (2.1)	18.5	144.4 (9.7)	7.0
C	0.2 (0.5)	1.0	6.8 (12.2)	4.3	1 (0.2)	2.5	27.13 (3.0)	1.3
D	0.2 (0.2)	1.0	1.6 (7.4)	1.0	0.4 (0.2)	1.0	20.6 (0.9)	1.0

Table 5.12. Mean catch (with standard error shown in parenthesis) per trap day ($n = 20$) for model A (total *Culicoides*) and model B (female *C. obsoletus* s.l.), for different treatments of semiochemical-baited CDC traps [Index is the ratio of the treatment mean catch with the mean catch obtained by the control treatment D] (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)

5.3.4.4 Meteorological Limits on Recorded *Culicoides* Activity

Of the meteorological covariates assessed in this study, the absence of *Culicoides* in catches were best described by a model that included only the negative effect of mean air temperature. While, in descending order of influence, when at least one *Culicoides* was collected: the negative effect of variation in wind direction, the positive effect of mean transformed wind direction, the negative effect of mean wind speed and the positive effect of mean air temperature, all significantly influenced the number of *Culicoides* collected [see Table 5.5 on page 152 and Table 5.13 below].

Treatment	*Culicoides*	Mean Air Temperature (℃)	Mean Wind Speed ($m{\cdot}s^{-1}$)
A	**0**	14.49 (9.65;20.73)	11.76 (0.05;3.74)
	> 1	15.73 (9.84;22.76)	1.26 (0.00;6.28)
B	**0**	15.77 (9.84;22.76)	3.76 (0.00;6.28)
	> 1	13.50 (9.84;18.20)	9.00 (0.05;2.40)
C	**0**	16.14 (9.65;22.76)	1.48 (0.00;6.28)
	> 1	15.48 (9.65;22.76)	2.74 (0.00;6.28)
D	**0**	13.30 (9.65;17.29)	10.61 (0.05;6.28)
	> 1	16.21 (9.84;22.76)	0.94 (0.00;3.48)

Table 5.13. The mean (with range shown in parenthesis) of meteorological conditions experienced during trapping in which (*i*) zero and (*ii*) >1 *Culicoides* (model A) were collected (Treatment - A: CO_2 with (S)-(+)-1-octen-3-ol, B: CO_2 with (R)-(-)-1-octen-3-ol, C: CO_2 with Racemic 1-octen-3-ol, D: CO_2)

Of the meteorological covariates assessed in this study, the absence of female *C. obsoletus* s.l. in catches was described best by a model that included, in descending order of importance: the negative effect of mean transformed wind direction, the negative effect of mean wind speed, the positive effect of maximum wind speed and the positive effect of mean humidity. While, in descending order of importance: the negative effect of mean wind speed, the negative effect of mean air temperature and positive effect of mean humidity, all significantly influenced the number of female *C. obsoletus* s.l. collected, when at least one *C. obsoletus* s.l. was collected [see Table 5.6 on page 153 and Table 5.14 below].

Treatment	Female *C. obsoletus* s.l.	Mean Air Temperature (℃)	Mean Humidity (%)	Mean Wind Speed ($m \cdot s^{-1}$)
A	**0**	16.03 (9.65;22.69)	76.65 (52.83;93.60)	6.06 (0.05;3.74)
	> 1	15.04 (9.84;22.76)	77.29 (49.65;92.30)	1.15 (0.00;6.28)
B	**0**	15.88 (9.65;22.69)	76.95 (52.90;93.60)	1.45 (0.18;3.74)
	> 1	15.27 (9.84;22.76)	77.43 (49.65;92.30)	4.39 (0.00;6.28)
C	**0**	15.81 (9.84;22.76)	74.29 (52.83;88.30)	5.83 (0.05;3.74)
	> 1	15.21 (9.65;20.73)	79.23 (49.65;93.60)	1.33 (0.00;6.28)
D	**0**	15.53 (9.65;22.69)	76.39 (52.83;93.60)	4.78 (0.05;6.28)
	> 1	15.39 (9.84;22.76)	78.15 (49.65;92.00)	0.71 (0.00;3.33)

Table 5.14. The mean (with range shown in parenthesis) of meteorological conditions experienced during trapping in which (*i*) zero and (*ii*) >1 female *C. obsoletus* s.l. (*2*) were collected

5.3.5 Sheep-Baited Drop Trap Comparison

The sheep-baited drop trap collected a total of 424 *Culicoides*, of which 99.5 % were *C. obsoletus* s.l. (422 individuals), in addition one *C. pulicaris* s.l. and one *C. nubeculosus*, both nulliparous, were collected. Of the 422 *C. obsoletus* s.l. collected in the sheep-baited drop trap, nulliparous females dominated (79.9 % of females) followed by parous (14.7% of females collected) and blood-fed (3.3 % of females collected). No gravid individuals were collected. The semiochemical baited CDC trap [(R)-(-)-1-octen-3-ol + CO_2 500 $ml \cdot s^{-1}$] collected a total of 128 *Culicoides* all of which were *C. obsoletus* s.l., of these 83.33 % were *C. obsoletus* s.s., 11.11 % *C. scoticus*, 2.22 % *C. dewulfi* and 1.11 % *C. chiopterus*. The multiplex PCR failed to identify 2.22 %

of Obsoletus group females collected by the semiochemical baited CDC trap [species specific identifications of individuals collected by the sheep-baited drop trap are currently unavailable]. Of the 128 *C. obsoletus* s.l. collected in the semiochemical baited CDC traps nulliparous individuals dominated (78.1 % of females collected) followed by parous individuals (21.9 % of females collected). No blood-fed or gravid individuals were collected [see Figure 5.10 on page 162]. There was no significant difference in gender/parity structure of the population of *C. obsoletus* s.l. collected using the sheep-baited trap compared to the semiochemical-baited CDC trap [see Table 5.15 on page 162].

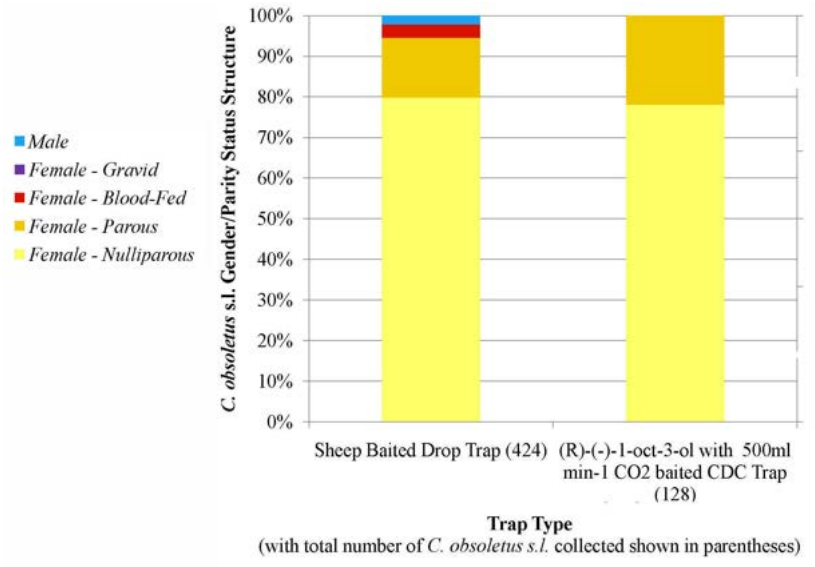


Figure 5.10. Variation in gender/parity structure of *C. obsoletus* s.l. population collected in (R)-(-)-1-octen-3-ol with 500 ml·s^{-1} CO^2 baited CDC trap compared to sheep-baited drop trap, over five nights of sampling

Trap Type	Males	Females		
		Nulliparous	Parous	Blood-Fed
(*i*) Sheep-Baited Drop Trap	2.1^A	3.3^A	80.0^A	14.6^A
(*ii*) Semiochemical-Baited CDC Trap [(R)-(-)-1-octen-3-ol with 500 ml·s^{-1} CO_2]	0.0^A	0.0^A	78.1^A	21.9^A

Table 5.15. Gender/Parity structure of collected *C. obsoletus* s.l.; shown as the percentage of total catch over five nights of trapping [*P≤0.001, **P≤0.01, *P≤0.05, proportions in the same column with the same letter were not significantly different (P>0.05)]**

5.4 Discussion

Current light-based surveillance techniques do not provide an unbiased estimate of the species composition, or abundance, of host-seeking Palaearctic BTV vectors (Carpenter et al., 2008d). Representative host-equivalent estimates of vector abundance are required for accurate local-scale prediction of BTV vector abundance, and the associated risk of BTV transmission. This investigation reflected the need to develop a trapping system for the estimation of host-seeking populations *Culicoides* within different farm-land habitats, to enable an assessment of the local within-farm drivers of the spatial variation of *Culicoides* abundance. It represents the first field trial to study the attractiveness of 1-octen-3-ol, and its different isomers, with CO_2 for the collection of livestock-associated *Culicoides* species in England, including *C. obsoletus* s.l. The choice of these semiochemicals was governed by their demonstrated efficacy and synergistic effect on trap catches for the collection of *C. impunctatus* (Bhasin et al., 2001) and other *Culicoides* species (Bishop et al., 2008; Kline et al., 1994).

5.4.1 Species Composition and Gender/Parity Structure

All four members of the *C. obsoletus* group were collected by each of the semiochemical-baited CDC traps but, *C. obsoletus* s.s predominated in all treatments. *Culicoides dewulfi* was not collected by the OVI traps, but was found in a very small proportion of CDC trap catches and may occur in very small populations locally. OVI traps generally performed poorly in comparison with semiochemical-baited CDC traps, although detailed quantitative comparisons cannot be made as the OVI traps were not rotated through the trap locations in the same way as the CDC traps. Preliminary comparisons between (R)-(-)-1-octen-3-ol and CO_2 ($500\,ml{\cdot}s^{-1}$) baited CDC trap and sheep-baited drop trap catches indicate that host equivalent estimates of host-seeking population structure were being made by the semiochemical baited CDC trap catches. A surprisingly large proportion of the *C. nubeculosus* were collected by the semiochemical-baited traps, far more than in the OVI traps or in historical collections from the same site during the same seasonal period made using OVI and sheep-baited drop

traps (Carpenter et al., 2008d). This indicates that, like *C. chiopterus*, *C. nubeculosus* may be underestimated in light trap catches. *Culicoides nubeculosus* is a known vector of *Onchocerca* species (Balin, 1979; Dohnal et al., 1990; Mellor, 1974, 1975; Steward, 1933) and is a potential vector of several arboviruses including BTV (Jennings and Mellor, 1988). The low attractiveness of sheep to *C. nubeculosus*, which made up only 2 out of 424 *Culicoides* collected in the sheep-baited drop trap, mirrors the results of Carpenter et al. (2008d) at the same site in late summer. If this low biting intensity on sheep is maintained throughout the *Culicoides* season, in spite of the presence of large background populations, *C. nubeculosus* may have a low vector potential for ovines. The host preferences of *C. nubeculosus*, however, will have a large influence on its potential vector role for transmission of BTV since this species is primarily considered to be cattle-associated (Campbell and Pelham-Clinton, 1960) and the biting rates on cattle, and therefore the overall potential for transmission of BTV, has not been determined in the present study.

5.4.2 Meteorological Limitations on Recorded *Culicoides* Activity

The inclusion of meteorological variables within the models allowed any variation in trap catches due to changing conditions affecting both trap efficiency and the background population of *Culicoides*, to be accounted for between replicates. This is a factor that has been overlooked in many previous trials of semiochemical attractiveness to both *Culicoides* (Bhasin et al., 2001; Cilek and Kline, 2002; Kline et al., 1994) and other vectors (Foil and Hribar, 1995; Holloway and Phelps, 1991; Kline et al., 2007; Van Essen et al., 1994). Meteorological covariates with a significant relationship to the number of *Culicoides* collected within the semiochemical-baited trap catches were associated with temperature, humidity, wind speed and variability in wind direction. These factors have previously been associated with biting rates on sheep (Carpenter et al., 2008d). The significance of wind associated variables may have arisen from the wind's effect on *Culicoides* flight activity, the ability to navigate close enough a trap's entrance to be collected and its effect on semiochemical plume dis-

persion (Murlis et al., 1992), or more likely a combination of these effects. Mean wind speeds during trapping ranged from $0.11\,\mathrm{m{\cdot}s^{-1}}$ to $6.28\,\mathrm{m{\cdot}s^{-1}}$ although the majority of wind during the experiment was under $2\,\mathrm{m{\cdot}s^{-1}}$, with *Culicoides* and *C. obsoletus* s.l. activity both recorded between $0.00\,\mathrm{m{\cdot}s^{-1}}$ to $6.28\,\mathrm{m{\cdot}s^{-1}}$, a slightly wider range than when *Culicoides* were collected from sheep by Carpenter et al. (2008d) who recorded activity between $0\,\mathrm{m{\cdot}s^{-1}}$ to $4.1\,\mathrm{m{\cdot}s^{-1}}$.

Wind direction had a significant negative effect on the number of *Culicoides* collected when *Culicoides* were present in a trap catch, however, this was only a significant predictor for absence of female *C. obsoletus* s.l. from trap catches. Although influential in this study, these effects may be more site-specific, due to the impacts of surrounding land cover types and terrain, than for example mean air temperature. Semiochemical plume dispersion within different habitat types has been shown to vary (Murlis et al., 2000; Zollner et al., 2004) and the abundance of *Culicoides* within different habitats activated to host-seek following exposure to the plume may vary (Bishop et al., 1995) both influencing the variation in the number of individuals trapped between habitats.

Culicoides obsoletus s.l. activity was recorded at mean air temperatures between 9.84 ℃ and 22.76 ℃, this minimum temperature is lower than the range over which *C. obsoletus* s.l. activity was recorded by Carpenter et al. (2008d) at the same site, although similar to a sheep-baited experiment in north-eastern Spain by Gerry et al. (2009), who recorded *C. obsoletus* s.l. activity down to 8 ℃. Mean air temperature had a positive effect on the total number of *Culicoides* that you would expect to catch per night but surprisingly a negative effect on the number of female *C. obsoletus* s.l. This may be associated with the fact that due to its Palaearctic distribution *C. obsoletus* s.l., which is considered to be a cold-adapted species group, the need to avoid desiccation overrides the impulse to host seek. This may also explain the positive effect rising humidity levels had on the number of female *C. obsoletus* s.l. collected. With *C. obsoletus* s.l. activity recorded at humidity's ranging from 29.65 % to 92.30 % similar levels to that observed on sheep by Carpenter et al. (2008d), who

recorded activity between 26.6 % to 94.0 % humidity, at the same site.

Further investigations into the trap responses under a greater variety of meteorological conditions, paired with an unbiased estimate of active *Culicoides* population density, such as via suction for which trap efficiency have already been defined (Taylor, 1962), could be made. This may enable the range of meteorological conditions under which the trap may be operated effectively to be determined, and provide information for the development of correction factors for correcting trap catches under conditions which affect the trap's efficiency but not the active population of *Culicoides*, improving the accuracy with which the *Culicoides* population can be reflected under changing conditions.

5.4.3 Effects of CO_2 Release Rate

The lack of significant differences observed in both the total number of *Culicoides* and the number of female *C. obsoletus* s.l. attracted to the traps between the 500 $ml \cdot min^{-1}$ and 1000 $ml \cdot min^{-1}$ release rates for the 'CO_2 only' bait and any of the synergist bait combinations of 1-octen-3-ol and CO_2 was surprising. Dose-dependent responses to CO_2 have been identified in other *Culicoides* species (Kline et al., 1994; Mullens, 1995; Mullens and Gerry, 1998) but these results were usually observed over a much wider range of release rates, up to 2000 $ml \cdot min^{-1}$. The threshold to trigger an increase in the rate of attraction of a trap to *Culicoides*, if dose-dependent responses are present in *C. obsoletus* s.l., may not have been reached or it may only occur in the presence of other host odours and neither of these possibilities were tested in this study. Seasonality in the background vector population may also have influenced this comparison, since the 1-octen-3-ol baits were trialled with the higher CO_2 rate (August-September) after the lower rate (June-July), although meteorological conditions were explicitly accounted for in the model. Simultaneous comparison of catch sizes over a wider range of CO_2 release rates would provide a more accurate assessment of the degree of dose-dependence in trap response to CO_2 of *C. obsoletus* s.l. and other livestock-associated species in southern England.

5.4.4 Effects of Semiochemical Treatment

Despite CO_2 being shown to be an effective attractant for *C. impunctatus* (Bhasin et al., 2001; Carpenter, 2001) and other species of *Culicoides* (Bishop et al., 2008; Cilek and Kline, 2002; Gerry and Mullens, 1998; Kline et al., 1994; Mullens, 1995), this investigation supports previous evidence that *C. obsoletus* s.l. populations respond poorly to CO_2 baited suction traps when compared to standard light trap collection (Mullens et al., 2005; Takken et al., 2008). CO_2 alone was found to be a poor attractant of female *C. obsoletus* s.l. with significantly fewer *Culicoides* being collected at the CO_2 only baited traps compared to traps when either (R)-(-)-1-octen-3-ol or (S)-(+)-1-octen-3-ol was added to the CO_2 bait.

The differential attractiveness of CO_2 between *C. impunctatus* and the *Culicoides* species / species groups observed in this study may reflect difference in host preference, since *C. obsoletus* s.l., *C. pulicaris* s.l. and *C. nubeculosus* are considered to be livestock associated, mammophilic species (Campbell and Pelham-Clinton, 1960) and *C. impunctatus* an opportunistic (Blackwell et al., 1994, 1995) and often anthropophilic feeders (Cameron et al., 1946; Hill, 1947; Parker, 1949). For mosquitoes, Dekker and Takken (1998) found that CO_2 was used to a greater degree in host location in opportunistic species compared to more specialised feeders.

Both (R)-(-)-1-octen-3-ol and (S)-(+)-1-octen-3-ol when added to CO_2 attracted significantly more *Culicoides* and female *C. obsoletus* s.l. than when CO_2 was used alone. Further comparisons of mixed CO_2/1-octen-3-ol baits to 1-octen-3-ol only baited traps will allow any synergistic effects of 1-octen-3-ol and CO_2, similar to that identified in other haematophagous insects to be examined (Bhasin et al., 2001; Kline et al., 1990, 1994; Ritchie et al., 1994; Takken and Kline, 1989).

The commercially available racemic 1-octen-3-ol was found not to attract significantly more *Culicoides* than CO_2 alone, indicating that mixing the two isomers has, to some degree, a neutralising effect on their attractiveness. Similarly, the low attractiveness of racemic 1-octen-3-ol to *C. obsoletus* s.l has previously been detected using Mosquito Magnet® traps by Carpenter et al. (2008d) at the same site when compared to a sheep-baited drop trap. This may have been as a result of the ratio at which they are mixed i.e. 50:50 rather than closer to the ratio observed in oxen breath, of 80:20 to 92:8 (Hall et al., 1984). (S)-(+)-1-octen-3-ol has previously been identified as having a neutral or even negative effect on trap catches for the collection of mosquitoes (Kline et al., 2007). The poor performance of the racemic 1-octen-3-ol as an attractant is unlikely to be a direct neutralising or repellent effect of (S)-(+)-1-octen-3-ol, as once variation due to changing meteorological conditions was taken into account, this enantiomer did not collect significantly less *Culicoides* or less *C. obsoletus* s.l. than (R)-(-)-1-octen-3-ol. Of the two, however, only (R)-(-)-1-octen-3-ol collected significantly more *C. obsoletus* s.l. ($P \leq 0.01$) than would be expected in standard light-suction trap surveillance. More extensive testing may show further divergence between the two 1-octen-3-ol isomers in attractiveness. Based on the limited evidence available for other haematophagous insects and the data presented in this investigation it is likely that (R)-(-)-1-octen-3-ol, the major isomer present ox odour, will be the more effective bait when used with CO_2 for the collection of *Culicoides*. In light of this the semiochemical combination (R)-(-)-1-octen-3-ol with CO_2 at a release rate of $500\,ml{\cdot}s^{-1}$ was compared to a sheep-baited drop trap, the results of this short trial indicate that this semiochemical bait combination was accurately reflecting the *Culicoides* population structure attracted to a host.

Insufficient numbers of *C. pulicaris* s.l. were collected to make statistical interpretations on the effect of semiochemical treatment and CO_2 release rate on their collection. Historical collections from the same site as this study during the same seasonal period made using OVI and sheep-baited drop traps indicate that *C. pulicaris* s.l. may occur in very small populations locally (Carpenter et al., 2008d). Repetition of

the current experiment at a site with a large known population of *C. pulicaris* s.l. will allow comparison of the level of attraction of semiochemical-baited traps between the two species groups.

With regards to future testing of different semiochemical baits, electrophysiological experiments and behavioural responses using bioassay systems and wind tunnels in the laboratory are useful for screening a wide range of candidate compounds for behavioural activity/responses before field testing (Bhasin et al., 2000a), further testing using these techniques may allow additional useful host location cues in *C. obsoletus* s.l. to be identified and would provide further insight into the most effective combination of host kairomones, that could be taken forward to be compared for their efficacy and ease of use in the field.

5.5 Conclusions

The greater total numbers of *Culicoides* and the number of *C. obsoletus* s.l. collected in CDC traps baited with (R)-(-)-1-octen-3-ol and CO_2, indicates these semiochemicals would be suitable for use as an effective surveillance tool for livestock associated *Culicoides* species at the within-farm scale in southern England. The use of a CO_2 release rate higher than $500\,ml{\cdot}s^{-1}$ is unnecessary for attraction of *Culicoides* when CO_2 is combined with 1-octen-3-ol. Based on the results of this study, unlit CDC traps baited with (R)-(-)-1-octen-3-ol ($4\,mg{\cdot}h^{-1}$) and CO_2 ($500\,ml{\cdot}s^{-1}$) were used to estimate *Culicoides* populations at a within-farm scale in the study discussed in Chapter 6.

Chapter 6

Habitat Associations and Spatial Patterns in Abundance of Adult BTV Vector Populations in Southern England at the Local-Scale

6.1 Introduction

The distribution and abundance of adult *Culicoides* across landscapes is influenced to varying degrees by the abundance and proximity of a range of resources, namely hosts, oviposition and resting sites. Following emergence, adult *Culicoides* disperse from their larval development sites in search of these resources (Kettle, 1951). The prevalence of these resources and by implication the dispersal of *Culicoides* within and between farms will be influenced in turn by both biotic and abiotic conditions including anthropogenic factors such as farm husbandry. Kettle (1951) estimated, for example, that the density of adult female *C. impunctatus* in birch woodland fell by a factor of 10 for every 59 m travelled from the larval development site, an area of boggy ground covered by *Sphagnum* sp. and *Juncus articulatus*. The effect of such factors on spatial patterns in the abundance of adult Palaearctic BTV vector species have, however, not been quantified at this local-scale.

The majority of recorded observations of the Palaearctic vectors species have been made at the group level, due to the cryptic morphology of these species. For many of the postulated adult *Culicoides* habitat associations, collections have been made at single locations, with the physical and topographical descriptions of the areas surrounding these locations often vague or missing. Surveillance of the presence and abundance of *Culicoides* species in Europe has also been focused on the use of light traps to collect adults. In addition to the limitations of light trapping discussed in the previous chapter, light trap location is often dependent on the proximity to a power source and in turn, collections are generally made close to farm buildings.

As a consequence of the limitations of light trap surveillance [see Chapter 5], the historical 'species-habitat associations' discussed below are restricted to *Culicoides* collections made by suction-only traps. Suction-only traps collect individuals passively. The *Culicoides* collected are therefore more likely to originate from adjacent habitat rather than being drawn in by light from a radius of several hundred metres or more. These passive methods, however, do not specifically target the host-seeking population. Investigations of species-habitat associations of the Palaearctic BTV vectors, *C. obsoletus* s.l. and *C. pulicaris* s.l., in northern Europe using suction-only traps are, however, limited. Service (1971, 1974) collected *C. obsoletus* s.s., *C. scoticus C. dewulfi*, *C. chiopterus*, *C. pulicaris* s.s. and *C. punctatus* in suction traps at two locations within deciduous woodland in south east England, this woodland was dominated by Oak (*Quercus robur* L.) and Ash (*Fraxinus excelsior* L.) with ground vegetation consisting of mainly Dog's Mercury (*Mercurialis perennis* L.), Ground Ivy (*Glechoma hederacea* L.) and various grasses up to 20-25cm in height. Since the work of Service (1971, 1974) pre-date the availability of molecular tools for species identification, species-specific results within the Obsoletus group were based only on the male specimens that were collected, in which *C. obsoletus* s.s. predominated (101 out of 174 male *C. obsoletus* s.l. collected (Service, 1971)). Within this woodland habitat, however, at the group level Service (1971) found catches of *C. obsoletus* s.l., *C. punctatus* and *C. pulicaris* s.s. to increase with increasing trap height (23 cm to 550 cm, maximum canopy height 10 m). Quantitative data regarding the vertical activity profile of *C. obsoletus* s.l. within different habitats has not been collected. *Culicoides chiopterus*, however, has been suggested to have a lower flight profile than the other Obsoletus group species in open grassland pasture (S. Carpenter, personal communication). Kettle (1951) found the vertical distribution of *C. impunctatus* to be significantly different between areas of deciduous woodland and open moorland. Areas of moorland bracken cover limited low level (2 ft) *C. impunctatus* activity in comparison to areas of moorland covered by *Juncus* sp, while the leaf canopy within deciduous woodland appeared to provide a vertical limit to *C. impunctatus* activity

(Kettle, 1951). The presence or absence of a herb layer in comparison to bare leaf litter within deciduous woodland, however, had no significant limiting effect on low level *C. impunctatus* activity (Kettle, 1951). It has also been noted that the number of resting adult *C. impunctatus* collected on trees appears to be related to both the height and availability of cover (Boorman and Goddard, 1970; Carpenter et al., 2008c) and the tree examined (Blackwell et al., 1992). These findings indicate that the level of vegetation cover and its structure will affect the dispersal of *Culicoides* within and between habitat types as they move from their emergence sites to areas suitable for the location of hosts, mates and sites for oviposition and resting.

Holmes and Boorman (1987) collected both *C. obsoletus* s.l. and *C. pulicaris* s.l. from suction traps at the edge of open grassland bordered by deciduous woodland, and a freshwater marsh/stream. Campbell and Pelham-Clinton (1960) suggested that *C. obsoletus* s.s. breeding in media-enriched habitats (i.e. dung) were bivoltine, while spring and autumn generations breeding in freshwater marshes were univoltine. Holmes and Boorman (1987) indicated, based on observations in the peaks of adult numbers collected by both light traps and suction traps, that the Obsoletus group may be trivoltine, with the first emergence being primarily from marshy areas and the second and third emergence being primarily from more widely distributed habitats such as leaf litter. This suggests that there may be significant inter-annual variation in habitat use by *C. obsoletus* s.l. The finding of Holmes and Boorman (1987), however, pre-date the availability of molecular tools for species identification, therefore their conclusions on the seasonality of the Obsoletus group must be interpreted with caution as the timing of the three emergence periods were defined based on peaks and troughs in the abundance of parous Obsoletus group females not differentiated to species level. The timing of the emergence of the constitute species of the Obsoletus group were then considered within these 'emergence periods' based on the number of males of each species collected. Holmes and Boorman (1987) defined the start of a new emergence period as the point at which the parous rate starts to fall, the observation of pigmented newly emerged nulliparous female *C. obsoletus* s.s. in Chapter 4 may

have yet undetermined implications for this definition. Errors in the initial definition of the emergence periods may have obscured any difference between the seasonality in the Obsoletus group species, and by implication the temporal variation in habitat use.

In the wider Palaearctic area, Dzhafarov (1962) found *C. pulicaris* s.l. on the lower parts of shrubs, tamarisk (wild pomegranate), rushes, the edge of wheat fields and the burrows of rodents, jackals, foxes and in the nests of birds in Azerbaijan. Small numbers of both *C. scoticus* and *C. chiopterus* have been associated with dense tugai (flood-land) forest, shrub thickets and open forest (Dzhafarov, 1960). Large numbers of *C. pulicaris* s.s. and *C. punctatus* have also been found in shrub thickets, open forest and the boundary between meadows and caragan semi-desert (Dzhafarov, 1960). Small numbers of *C. pulicaris* s.s. and *C. chiopterus* have also been associated with mulberry trees on flood-land (Dzhafarov, 1960).

6.1.1 Factors Governing the Occurrence of Adult Palaearctic BTV Vectors within Farm Land Habitats

A range of climate (Calvete et al., 2008; Conte et al., 2007a; Purse et al., 2004a, 2007) terrain and soil (Conte et al., 2007b) factors have been found to be associated, through multivariate modelling of farm based surveillance data, to spatial patterns in *C. obsoletus* s.l. and *C. pulicaris* s.l. distribution at a regional level [see Chapter 3 on page 49]. Limited information is, however, available on Palaearctic BTV vector abundance and distribution at the local (<1 km) scale and studies on the entry of both *C. imicola* (Calvete et al., 2009) and the Palaearctic BTV vectors (Meiswinkel et al., 2008b) into livestock accommodation have been conducted within farms. Takken et al. (2008) used Liberty Plus CO_2 baited traps (American Biophysics, USA) to sample *Culicoides* in four broad habitats (or 'ecosystems'), wetland, peat bogs and moors, flood plains and livestock farms. Both *C. pulicaris* s.l. and *C. obsoletus* s.l. abundance was found to be strongly associated with farm land habitats. Although *Culicoides* numbers are widely acknowledged to vary spatially

within farms (Bishop et al., 1994, 1995), no quantitative assessments of the relative abundance of the Palaearctic BTV vectors within different farm land habitats have been made. Historical studies that pre-dated the availability of molecular tools for species identification, have also only considered *C. obsoletus* s.l. and *C. pulicaris* s.l. at the group level and have additionally primarily focused only on the southern margins of these species' distributions within Europe (Calvete et al., 2008; Conte et al., 2007a,b; Purse et al., 2004a, 2007).

The livestock-associated Australian BTV vector, *C. brevitarsis* Kieffer, is the only BTV vector whose spatial patterns in habitat preference have been investigated at a local (within a 300 hectare area) scale in farm land (Bishop et al., 1994, 1995). Using a non-light based surveillance method, variation in habitat type was found to significantly affect the spatial pattern in abundance of both 'resting' (Bishop et al., 1994, 1995) and 'active' *C. brevitarsis* adults (Bishop et al., 1994). Higher abundances of *C. brevitarsis* estimated using backpack-vacuum sampling, were associated with dam margins and open pasture in comparison to areas of irrigated or wooded pasture and creek margins (Bishop et al., 1994). Bishop et al. (1994) also found that within a farm increased proximity of cattle to light traps increased trap catches. Bishop et al. (1995) found *C. brevitarsis* to be 21 times more abundant in grass tussocks (*Poa* species) than in pasture grass, with abundance positively correlated to tussock size, indicating a strong influence of vegetation structure and type, in addition to the influence of host presence on *C. brevitarsis* abundance.

6.1.2 Justification for Research

A greater understanding of the relative importance of various climatic and non-climatic drivers of local vector abundance will enable us to relate the demographic rates and carrying capacities permitted by different habitats to the risk for BTV transmission within those habitats. The identification of productive habitat types have previously enabled more targeted, and therefore cost-effective, control strategies to be developed for *Anopheline* mosquitoes in relation to malaria transmission

(Protopopoff et al., 2007, 2008). If suitable habitat-associations are identified for the Palaearctic BTV vectors similar spatially targeted control programmes can be developed.

Grubb's theory of species co-existence (Grubb, 1977) suggests that different species will be favoured in different combinations of environmental conditions. To date, however, habitat association for the adult Palaearctic BTV vectors has been considered at the group level [see Chapter 3]. It is hypothesised that significant differences in the sensitivity of the Obsoletus group species to environmental conditions may occur resulting in spatial variation in the occurrence and abundance of these species at the local level. In the current work this hypothesis was investigated firstly using the semiochemical-baited trapping system developed in Chapter 5, in a field-based investigation into the spatial patterns in abundance and occurrence of livestock-associated *Culicoides* species, with Obsoletus group specimens identified to species level. Secondly these species-specific estimates of *Culicoides* abundance within and between farms were used to identify which environmental factors, ground-based and remotely-sensed, are important in driving patterns in the abundance of adult *Culicoides*, by matching spatial patterns of adult *Culicoides* prevalence with patterns in these environmental factors.

6.2 Materials and Methods

6.2.1 Study Sites and Habitat Classes Sampled

The spatial pattern in abundance of adult *Culicoides* was investigated during the summer of 2009 at four farms sites, A, D, E and F [see Section 2.1 on page 28 for further details]. These farms had all been identified as having large areas of potential and confirmed *Culicoides* breeding habitats and a wide diversity of surrounding landscape types [see Chapter 4 on page 91].

Sampling was carried out twice during the 2009 vector season [Sampling round A: June-July, B: August-September] at 100 sampling points across the four farm sites [see Appendix B Figure B.2 on page 260, Figure B.5 on page 263, B.6 on page 264 and Figure B.7 on page 265]. The mean minimum distance to the nearest neighbouring sampling point within farms was 183.9 m. Each sampling point was visually classified into one of 11 habitat categories [see Table 6.1 on page 177]. The habitat categories used represent the broad variations observed on the four farms, and are representative of common types present on livestock farms in the south east of England. The number of sample points per habitat class were, where possible, proportional to the abundance of that class across the four farms. A minimum of five sampling points per habitat class were used, however, to allow variation between the points to be taken into account. Specific sample point locations were additionally selected to achieve a distribution across the four farm sites and so that traps would remain undamaged by livestock activity and would not impede commercial production on study farms.

Geographic coordinates for all sampling points, were recorded using a GPS unit [eTrex Legend C (Garmin, USA), spatial error 3-5 m] in the British National Grid projection (OSGB 1936).

Habitat Class	Class Name	Class Description
1	**Improved Grassland (Pasture)**	Pastures which have been affected by heavy grazing, drainage and/or the application of herbicides, inorganic fertilisers, slurry or high doses of manure. Vegetation composition is limited to those species of grass and common forbs resistant to grazing and often demanding in nutrients, with many of the species which would be expected in an unimproved pasture absent.
2	**Unimproved Grassland (Meadow)**	Unimproved neutral grassland across an enclosed lowland landscapes, may include grasslands cut for hay, but not those where the primary use is for livestock grazing.
3	**Arable**	Arable crop land not permanently irrigated
4	**Broadleaved Woodland**	Vegetation dominated by broadleaved trees more than 5 m high when mature, forming a distinct, although sometimes open canopy, understorey and ground layer with 10 % or less of the canopy made up of coniferous trees.
5	**Coniferous Woodland**	Vegetation dominated by coniferous trees more than 5m high when mature, forming a distinct closed canopy, understorey and ground layer with 10 % or less of the canopy made up of deciduous trees.
6	**Transitional - Pasture* to Broadleaved Woodland**	Boundary area between broadleaved woodland (see class 4 for full description) and improved grassland (see class 1 for full description), *trap placed in class 1 within 10 m of the boundary line between the classes.
7	**Transitional - Broadleaved Woodland* to Pasture**	Boundary area between broadleaved woodland (see class 4 for full description) and improved grassland (see class 1 for full description), *trap placed in class 4 within 10 m of the boundary line between the classes.
8	**Hedgerow**	Intact, stock-proof, hedges with a diversity of native woody species and a good hedgerow bottom flora with a low density of trees greater than 5 m high,
9	**Marginal Vegetation Surrounding Open Water**	Emergent vegetation, less than 1 m tall, occurring on the margins of lowland watercourses where the water table is permanently high with an open water source existing beyond the limits of the emergent vegetation, which may contain submerged, free-floating or floating-leaved vegetation. Includes areas surrounding lakes, reservoirs, flooded gravel pits, ponds and water filled ditches.
10	**Surrounding Artificial Water Sources**	Localised habitats created though the periodic inundation of vegetation surrounding artificial sources of water such as leaking taps, overflowing water troughs etc.
11	**Muck Heaps**	Areas used for the large scale storage of cattle manure, associated bedding and the areas immediately surrounding them which are subject to high organic pollution, related to run off from the muck heap.

Table 6.1. Habitat classes and associated descriptions in which adult *Culicoides* abundance was assessed [modified from the JNCC Phase 1 Habitat Survey classifications (JNCC, 2003)]

6.2.1.1 *Culicoides* Sampling Methodology

Estimates of the abundance of host-seeking *Culicoides* at each sampling point were made using the semiochemical-baited trapping system with treatment B developed in Chapter 5, i.e. unlit new standard miniature CDC traps (Model 512, J.W. Hock, Gainsville, Florida) were baited with CO_2 (at 500 ml min^{-1}) and (R)-(-)-1-octen-3-ol (at approximately 4mg $hour^{-1}$). Full details of the trapping protocol and equipment used are given in Chapter 2 Section 2.2.2 on page 30.

Traps were operated overnight, with a trapping period spanning from at least one hour before sunset to two hours after sunset, to coincide with maximal *Culicoides* activity (Hill, 1947; Kettle, 1957; Parker, 1949; Service, 1969). All samples were collected within one hour of the end of the nightly trapping period. Sampled *Culicoides* were sorted and identified to group, and where possible species level, according to the methodology described in Chapter 2 Section 2.3.2 on page 38. Sampled female *C. obsoletus* s.l. were identified to species level (*C. obsoletus* s.s., *C. scoticus*, *C. dewulfi*, *C. chiopterus*) using multiplex PCR according to the methodology described in Chapter 2 Section 2.4 on page 40.

6.2.2 Quantifying Environmental Conditions Surrounding Sampling Points

Variables quantifying environmental conditions surrounding sampling points were selected from four variable suites (host, landscape, climate and terrain/soil) based on their hypothesised influence on the abundance of livestock-associated *Culicoides* abundance.

Variables contained within the four suites were as follows:

(A) **Host Variables** - To capture details of the availability of BTV susceptible livestock within the area surrounding sampling points, the presence-absence of cattle (C) and sheep (S) with access within 50 m of the sampling point were recorded at the time of trap collection. The livestock type present in an area has previously been found to be useful for predicting BTV outbreak occurrence

in Corsica (Guis et al., 2007) and Spain (Calvete et al., 2009). Where sheep or cattle were present, the number of cattle and the number of sheep with access within 50 m of the trap site were recorded ($C_{abundance}$; $S_{abundance}$), to give an estimate of host availability at each sampling point. Areas where either sheep or cattle were present at the time of sampling were used for grazing livestock for at least 50 % of the period in which adult *Culicoides* activity was observed [approximately May to October (Birley and Boorman, 1982; Edwards et al., 1939; Hill, 1947)]. Wild ruminants were not considered in this analysis as these hosts are expected to make up a low proportion of hosts for *Culicoides* in agricultural areas in southern England and geographical datasets are currently not available for these species in this area.

(B) **Landscape Variables** - In addition to the observed habitat class at the sampling points [11 Levels see Table 6.1 on page 177] landscape structure was characterised based on 250 m circular buffers around sampling points from land cover data derived from the Land Cover Map 2000 (LCM2000) provided by the NERC Centre for Ecology and Hydrology (Fuller et al., 2002a,b). [see Chapter 4 Section 3.2.3 on page 60 for further details of LC2000]

Landscape structure within the buffer regions surrounding each sampling point was quantified using the spatial pattern analysis program 'Fragstats' (McGarigal and Marks, 2002). Metrics were calculated at two levels: one for selected land cover classes found in a buffer (class-level metrics) and the other for the whole landscape, i.e. for the entire buffer regardless of the class (landscape-level metrics). Two landscape level metrics: landscape shape index (LSI), Simpson's diversity index (SIDI), were calculate and two class level metrics (per selected land cover class): percentage of landscape covered by patches of class (PLAND.X) and class landscape shape index (LSI.X) (where X is the selected land cover class) [see Chapter 4 Table 4.3 on page 103 for further description of these metrics]. Class level metrics were calculated for selected agriculture associated land cover types, which were abundant in the areas surrounding

samples sites and were expected *a priori* to influence the variation in *Culicoides* abundance, attributable to differences in the overall availability of larval development sites, host abundance and areas suitable for resting for the avoidance of desiccation. Land cover classes selected included: broad-leaved mixed woodland (class 1.1), coniferous woodland (class 2.1), improved grassland (class 5.1), set-aside grassland (class 5.2) and calcareous grassland (class 7.1). Selected metrics provide measures of the degree of coverage, fragmentation and diversity within and between different land cover classes, and have previously been useful in explaining patterns in the occurrence of bluetongue (Guis et al., 2007) and other vector-borne diseases (Brownstein et al., 2005; Danson et al., 2004; Gleiser et al., 2002; Graham et al., 2004; Jackson et al., 2006).

(C) **Climate Variables** - Variables describing the conditions of temperature and soil moisture at sample sites, that can have a large impact on *Culicoides* life cycles, were extracted from two pansharpened Landsat 7 ETM+ satellite images (15 m resolution), one representing leaf-off (winter) and one leaf-on (spring) conditions [see Chapter 2 Section 2.5.1 on page 41]. From each of these images the following measures of environmental conditions at sampling points were derived: Land Surface Temperature (LST.leaf.on; LST.leaf.off), Enhanced Vegetation Index (EVI.leaf.on; EVI.leaf.off), Tassel Cap Transformation (TC.BRIGHT.leaf.on; TC.BRIGHT.leaf.off; TC.WET.leaf.on; TC.WET.leaf.off; TC.GREEN.leaf.on; TC.GREEN.leaf.off). A complete description of the Landsat satellite data used, and their processing, is provided in Chapter 2 Section 2.5 on page 40 [see Chapter 4 Section 3.2.3 on page 60 for further details of LST, EVI and Tasselcap transformations and their usefulness for describing variation in temperature, moisture availability and vegetation activity with regard to predicting the distribution of both adult and larval vectors]

(D) **Terrain/Soil Variables** - Terrain characteristics at samples sites were derived from the Ordnance Survey (OS) Land-Form Profile 1:10,000 Digital Elevation Model sourced from the Edina Digimap service (EDINA, 2002) and summarised

as the altitude, aspect and slope at sampling points. A complete description of the data used, and its processing, is provided in Chapter 4 Section 3.2.3 on page 60. Variables describing the slope and altitude of areas have previously been found to be useful in predicting adult *C. imicola* and *C. obsoletus* s.l. abundance in Italy (Conte et al., 2007a,b).

The dominant soil characteristic at sampling points was derived from the NATMAP Soilscapes spatial dataset (1:250,000 scale) (NSRI, 2009). Soil type has previously been found to be useful in predicting adult *C. imicola* and *C. obsoletus* s.l. abundance in Italy (Conte et al., 2007a). Soilscapes is a simplified rendition of the national soil map (Mackney et al., 1983; Proctor et al., 1998), with soil type, drainage and texture characteristics separated into 30 distinct, ecologically relevant, soil classes. Of these 30 classes, five soil classes were found at the sampling points, freely draining slightly acid but base-rich soils (SOIL.TYPE_1), freely draining slightly acid loamy soils (SOIL.TYPE_2), shallow lime-rich soils over chalk or limestone (SOIL.TYPE_3), lightly acid loamy and clayey soils with impeded drainage (SOIL.TYPE_4), slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils (SOIL.TYPE_5).

6.2.3 Approach to Modelling Vector Abundance at the Local-Scale

Generalized additive models (GAMs) and constrained ordination were used to investigate the relationship of livestock-associated *Culicoides* species abundance to environmental predictor variables at the local-scale [GAMs are discussed further in Chapter 3 Section 3.2.4 on page 68]. Multivariate constrained ordination methods were used in addition to GAMs for individual species/species group to describe continuous variation in community composition and relate that variation in species composition to environmental gradients. Selection of variables within constrained ordination is not species specific, rather selection is based on predictor variables explaining successively the highest proportion of the variance in the species data as a whole. This approach allows the identification of important environmental correlates for overall commu-

nity composition to be identified (in addition to those identified by GAMs for single species) and can help identify environmental predictors for the rarer species in the community, which might otherwise be impossible to select in single species models, due to the small number of observations.

Within spatial data sets, observations that are close in space tend to be more similar than observations that are far apart, i.e. data are spatially correlated. It was expected *a priori* that spatial autocorrelation would influence patterns in *Culicoides* abundance at the local-scale. The global test of the positive eigenvalues (positive principal coordinates of neighbour matrices (PCNMs)) using the methodology of Borcard and Legendre (2002), however, showed no significant spatial structure within the *Culicoides* species community composition ($P \leq 0.05$). This indicates that significant spatial autocorrelation was not present, hence PCNM's were not included as explanatory variables as no conditioning by spatial predictors was required. A Moran's I test was performed on the residuals of the final model GAMs of adult *Culicoides* abundance [R package 'ape' (Paradis et al., 2004)], using neighbourhood sizes of 50 m, 100 m and 500 m. [see Chapter 3 Section 3.2.4 on page 68 for further details of how Moran's I is calculated].

Prior to selection of variables, collinearity (correlation between variables) both within and between explanatory variable groups was assessed using Variance Inflation Factors (VIF) [R package 'car' (Fox, 2009)]. Failure to deal with collinearity leads to an increase in type II errors i.e. the failure to reject the null hypothesis when it is untrue. VIF's of over 10 were used as a rule of thumb to indicate collinearity in the data (Montgomery and Peck, 1992). Where correlation between predictor variables was detected, the covariate with the highest VIF was dropped, the VIFs recalculated and this process repeated until all VIFs were smaller than 10.

Species abundance at each sampling point was defined as the maximum catch of females of that species / species group from the two semiochemical-baited trap catches made at each sampling point. Only female *Culicoides* were considered in this analysis, as male *Culicoides* do not take blood meals from vertebrates and consequently have no

potential to vector BTV between hosts. *Culicoides* species / species group abundance data was log transformed [log(x+1); x = abundance] to achieve normality (P>0.05), as assessed by the Shapiro-Wilkes normality test (Shapiro and Wilk, 1965) with regard to its use within the GAMs and for constrained ordination as species abundance was expected to increase multiplicatively as the number of environmental factors increased.

6.2.3.1 Generalized Additive Models

After the removal of correlated variables, separately for each species / species group, log-transformed [log(x+1); x = abundance] max abundance was related to uncorrelated environmental variables [VIFs < 10] using GAMs with a Gaussian error structure. A smoothing spline with four degrees of freedom was used to smooth predictor variables. Variables that did not contribute significantly to explaining variation in abundance were successively eliminated on the basis of AIC using a forward stepwise selection procedure [R package 'GAM' (Hastie, 2008)]. Such that each continuous predictor variable considered [see Table 6.3 on page 189] may either appear not at all, linearly, linearly in its logarithm, or as a smooth function estimated non-parametrically, while categorical predictors [see Table 6.3 on page 189] were either allowed to appear or not appear. AIC in the stepwise GAM is defined as the *deviance+2*df*ϕ, where *df* is the effective degrees of freedom and ϕ is the dispersion parameter (variance) (Hastie, 1992). The small number of sampled sites precluded partitioning of the species dataset into a calibration and evaluation dataset, therefore the overall fit of models was evaluated using the percentage of deviance explained (D^2) (Weisberg, 1980).

6.2.3.2 Constrained Ordination

The gradient length of the transformed species data was tested using Detrended Correspondence Analysis (DCA) (Hill and Gauch, 1980). According to DCA the community was homogeneous enough to justify linear ordination methods. The length

of the gradient in species abundance for DCA axes I to IV was 2.59, 2.03, 2.52, 1.96 S.D. units (mean standard deviation of species turnover) respectively.

Environmental predictors and their relative importance for determining abundance within livestock associated *Culicoides* communities were assessed using RDA, according to the methodology of Legendre (1998) [R package 'Vegan' (Oksanen et al., 2010)]. Redundancy Analysis (RDA) is the multivariate extension of multiple regression analysis i.e. from a single response and multiple predictors to multiple response variables, e.g. several species, and a common matrix of predictors [for further details see (Legendre, 1998)]. RDA first models each species distribution with respect to the explanatory variables and then performs the ordination for all species in the community with respect to the modelled relationships within the environment. Testing the null hypothesis that there is no significant relationship between variation in the species data and some linear combination of the environmental variables (i.e., that the environmental variables do not explain a significant proportion of the species data).

A Hellinger distance transformation (Rao et al., 1995) of the log-transformed species abundance data were also used to avoid distortion of the ordination diagrams (Legendre and Gallagher, 2001) and allow accurate assessment of the adjusted redundancy statistic (R_a^2) (Peres-Neto et al., 2006). The Hellinger transformation also permits the use of RDA models, which are Euclidean-based, with community composition data containing many zeros (long gradients) as the result of rare species, which otherwise might excessively influence the ordination (Legendre and Gallagher, 2001). Long gradients may also arise due to wide species tolerances on average.

After the removal of correlated variables, the variables listed in Table 6.3 on page 189 were incorporated into the RDA using a forward stepwise procedure by permutation of residuals under the reduced model [R package 'Vegan' (Oksanen et al., 2010)]. Two stopping criteria were used to obtain a parsimonious model, the first with $\alpha = 0.05$ as a stopping rule, the second an α equal to the value of the R_a^2 of the maximal model (the model in which all variables initially considered are included). The use of the adjusted redundancy statistic (R_a^2) as an additional stopping criterion minimises

the inclusion of superfluous variables (Blanchet et al., 2008). The significance of included variables was tested by comparing them to Monte Carlo permutations (5,000 times) of the null model. Variance partitioning was also used to partition the variation in species composition between sample points into non-overlapping and overlapping components of the variation explained by each suite of predictor variables (Borcard et al., 1992).

6.3 Results

A total of 100 sample points across four farm sites were each sampled twice during the 2009 vector season, 22 samples points were on farm A, 23 sample points on farm D, 27 sample points on farm E and 28 sample points were on farm F [see Appendix B on page 258]. A total of 1997 *Culicoides* were collected from 68 of the 100 sample points. Of these individuals 63.45 % were *C. obsoletus* s.l., 34.50 % *C. nubeculosus* and 1.35 % *C. pulicaris* s.l., the remaining 0.70 % of collected *Culicoides* consisted of *C. albicans* Winnertz (3 individuals), *C. stigma* Meigen (10 individuals) and *C. puncticollis* Becker (1 individual). The maximum catch for one overnight trapping period reached 449 for all *Culicoides* species and 448 for *C. obsoletus* s.l. The majority of *Culicoides* collected in the traps were female (99.15 %, present in 67 out of 68 sites), and nulliparous (84.10 % of females) the remaining individuals were parous (15.90 %) with no blood-fed or gravid females collected.

Of the 1267 *C. obsoletus* s.l. collected, the majority were female (99.45 %) and nulliparous (76.14 % of females identified). A substantial proportion were parous (23.86 % of females identified) in contrast to *C. nubeculosus* for which only 1.33 % females were parous. 14.81 % of collected female *C. pulicaris* s.l. were parous, all other species collected were nulliparous. Amongst those female *C. obsoletus* s.l. that could be identified to species level, *C. obsoletus* s.s. dominated (71.3 % of female *C. obsoletus* s.l.), followed by *C. scoticus* (5.63 % of female *C. obsoletus* s.l.), *C. dewulfi* (0.40 % of female *C. obsoletus* s.l.). No *C. chiopterus* were identified within the female *C. obsoletus* s.l. identified to species level [see Table 6.2 on page 188]. The multiplex PCR, however, failed to identify 22.70 % of collected female *C. obsoletus* s.l. to species level. These unidentified individuals came mostly (95.45 %) from one sample point and accounted for 61.90 % of the *C. obsoletus* s.l. collected at that point. This sample point was therefore excluded from further analysis.

Of the 11 habitat classes sampled only in class 3 (arable) were no *Culicoides* collected [see Table 6.2 on page 188]. The largest number of *Culicoides* and in particular *C. obsoletus* s.l. were collected from areas of improved grassland (class 1) and tran-

sitional areas of pasture to broad-leaved woodland (class 2). Greater numbers of *C. obsoletus* s.s. were collected within transitional habitats when traps were placed on the pasture side of the habitat transition compared to the woodland habitat. In contrast, greater numbers of *C. scoticus* were collected when traps were placed on the woodland side of the habitat transition compared to the pasture side. Small numbers of *C. pulicaris* s.l. were collected from areas of grassland both improved (class 1) and unimproved (class 2), coniferous woodland (class 5), transitional broad-leaved woodland to pasture (class 7), surrounding artificial water sources (class 6) and muck heaps (class 10) [see Table 6.2 on page 188]. *Culicoides nubeculosus* was primarily collected from areas of improved pasture (class 1) and areas surrounding artificial water sources. *Culicoides nubeculosus* was not collected from wooded habitats, however, large numbers were collected from broad-leaved woodland to pasture transitional areas where traps were placed on the pasture side (class 6) [see Table 6.2 on page 188].

Habitat Class	*Culicoides*	*C. obsoletus* s.l.**	*C. pulicaris* s.l.	*C. nubeculosus* Meigen	*C. albicans* Winnertz	*C. stigma* Meigen	*C. punctcollis* Becker	*C. obsoletus* Meigen	*C. scoticus* Downes and Kettle	*C. dewulfi* Goetghebuer	*C. chiopterus* Meigen	PCR Fail
1 Improved Grassland (Pasture) [23]	46.6 (1081)	16 (657)	0.3 (8)	17.8 (410)	0 (1)	0.2 (4)	0.1 (1)	15.4 (367)	0.6 (13)	0.1 (2)	0 (0)	12.1 (275)
2 Unimproved Grassland (Meadow) [5]	17.8 (89)	14.2 (71)	0.8 (4)	2.6 (13)	0 (0)	0.2 (1)	0 (0)	13.4 (67)	0.8 (4)	0 (0)	0 (0)	0 (0)
3 Arable [12]	0.1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.1 (0)
4 Broad-Leaved Woodland [13]	5.8 (89)	5.9 (89)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4.1 (62)	1.8 (24)	0 (0)	0 (0)	0 (3)
5 Coniferous Woodland [6]	6.2 (45)	5.3 (44)	0.2 (1)	0 (0)	0 (0)	0 (0)	0 (0)	4.7 (39)	0.7 (5)	0 (0)	0 (0)	0.7 (0)
6 Transitional - Pasture* To Woodland [7]	52.4 (374)	31.9 (229)	0.4 (3)	20 (141)	0.1 (1)	0 (0)	0 (0)	31.6 (222)	0.3 (2)	0 (0)	0 (0)	0 (5)
7 Transitional - Broad-Leaved* Woodland to Pasture [7]	9.6 (77)	9.6 (77)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	8.1 (66)	1.3 (10)	0.1 (1)	0 (0)	0 (0)
8 Hedgerow [11]	2.8 (30)	2.3 (25)	0 (0)	0.3 (3)	0 (0)	0.2 (2)	0 (0)	1.8 (20)	0.5 (5)	0 (0)	0 (0)	0.1 (0)
9 Marginal Vegetation Surrounding Open Water [5]	1.6 (8)	1.4 (7)	0.2 (1)	0 (0)	0 (0)	0 (0)	0 (0)	1.2 (6)	0 (0)	0.2 (1)	0 (0)	0 (0)
10 Surrounding Artificial Water Sources [6]	20.7 (131)	2.7 (23)	1.5 (9)	16 (96)	0 (0)	0.3 (3)	0 (0)	2.3 (19)	0.3 (3)	0 (0)	0 (0)	0.2 (1)
11 Muck Heaps [5]	9.2 (56)	6.4 (38)	0.2 (1)	2.4 (16)	0.2 (1)	0 (0)	0 (0)	5.2 (30)	1 (5)	0.2 (1)	0 (0)	0 (2)

Table 6.2. Mean number of female *Culicoides* collected (maximum collected per species from two sampling periods) per habitat (with total collected over two sampling periods shown in parenthesis), for transitional classes * indicates habitat class trap placed in (total number of sampling points per habitat class shown in parenthesis after class description) [*C. obsoletus* s.l. group identified to species level as shown in the right hand segment of the table]**

Based on the examination of the variance inflation factors two variables were excluded from both the GAMs and the RDA, TC-BRIGHT leaf on and TC-BRIGHT leaf off. A total of 30 variables [4 host; 14 landscape; 8 climate; 4 terrain/soil] (out of an original 32) were therefore considered within each GAM and the RDA [see Table 6.3 below]. *Culicoides albicans*, *C. stigma* and *C. puncticollis* were all insufficiently abundant in the study region for models of their abundance to be parameterised, with 3, 10 and 1 individual respectively found in all catches across all sampling points. RDA and GAM models of species abundance therefore included only the following species / species groups *C. obsoletus* s.s., *C. scoticus*, *C. dewulfi*, *C. pulicaris* s.l. and *C. nubeculosus*.

Variable Suite			
Host	**Landscape**	**Climate**	**Terrain/Soil**
Sheep Presence-Absence*	Observed Habitat Class [11 Levels see Table 6.1]*	TC.WET leaf on	Slope
Cattle Presence-Absence*	LSI	TC.WET leaf off	Altitude
Sheep Abundance	SIDI	TC.Green leaf on	Aspect
Cattle Abundance	PLAND.1.1 (Broad-Leaved Mixed Woodland)	TC.Green leaf off	Soil Type [5 levels]* †
	LSI.1.1 (Broad-Leaved Woodland)	EVI leaf on	
	PLAND.2.1 (Coniferous Woodland)	EVI leaf off	
	LSI.2.1 (Coniferous Woodland)	LST leaf on	
	PLAND.5.1 (Improved Grassland)	LST leaf on	
	LSI.5.1 (Improved Grassland)		
	PLAND.5.2 (Set-Aside Grassland)		
	LSI.5.2 (Set-Aside Grassland)		
	PLAND.7.1 (Calcareous Grassland)		
	LSI.7.1 (Calcareous Grassland)		

Table 6.3. Variables included in the analysis of livestock-associated *Culicoides* species abundance (after the exclusion of variables with VIFs > 10), * indicates a categorical variable, all other variables continuous
[† SOIL.TYPE factor levels: 1 - freely draining slightly acid but base-rich soils; 2 - freely draining slightly acid loamy soils; 3 - shallow lime-rich soils over chalk or limestone; 4 - slightly acid loamy and clayey soils with impeded drainage; 5 - slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils]

Forward-stepwise selection of environmental variables found both *C. obsoletus* s.s. and *C. scoticus* abundance at the local-scale to be best described by parametric models, explaining 21.65 % and 30.78 % of the variance in abundance, respectively [see Table 6.9 on page 196]. *Culicoides pulicaris* s.l. and *C. nubeculosus* abundance were best described by semi-parametric models, which explained 28.68 % and 48.64 % of the variance in abundance respectively [see Table 6.9 on page 6.9 and Figure 6.1 on page 191]. None of the predictor variables from any of the four variable suites considered in this study explained a significant amount of the variation in abundance of *C. dewulfi*, with no variables being retained during the stepwise selection procedure.

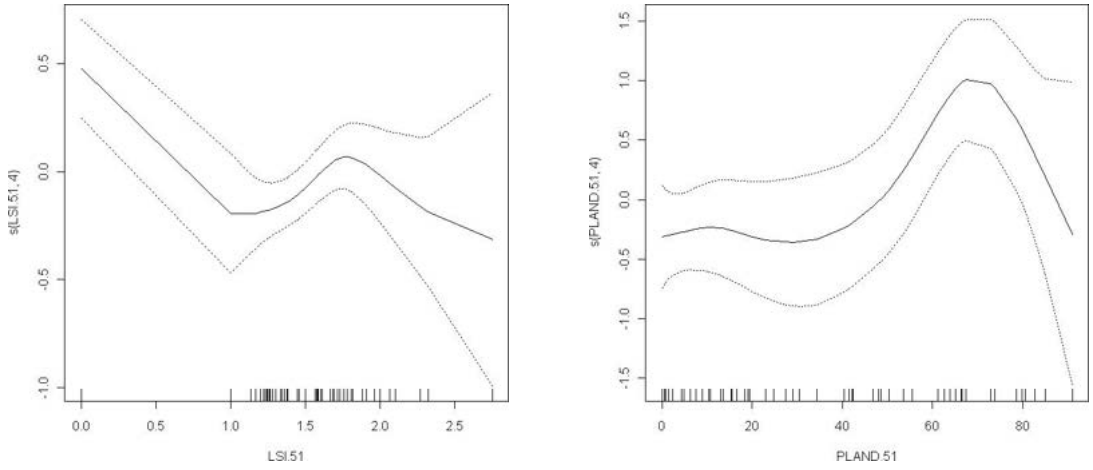
Culicoides obsoletus s.s. abundance at the local-scale was best described by the positive effect of the presence of sheep within 50 m for the sampling point, however was negatively impacted by an increasing percentage of calcareous grassland within 250 m of the sampling point (PLAND.7.1) and an increase in winter-time land surface temperature (LST.leaf.off) [see Table 6.4 on page 192].

An increasing percentage of broad-leaved woodland within 250 m of the sampling point (PLAND.5.1) positively impacted *C. scoticus* abundance at the local-scale. *Culicoides scoticus* abundance, however, was negatively impacted by an increasing percentage of calcareous grassland within 250 m of the sampling point (PLAND.7.1) and an increase in the level of winter-time vegetation activity (EVI.leaf.off) [see Table 6.4 on page 192]. The dominant soil characteristic at a sampling point also significantly influenced *C. scoticus* abundance within farms. Higher abundances of *C. scoticus* were associated with areas of slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils (SOIL.TYPE 5) over both areas of freely draining slightly acid loamy soils (SOIL.TYPE 2) and areas of shallow lime-rich soils over chalk or limestone (SOIL.TYPE 3) [see Table 6.5 on page 193].

An increasing percentage of calcareous grassland within 250 m of the sampling point (PLAND.7.1) negitively impacted *C. pulicaris* s.l. abundance at the local-scale [see Table 6.4 on page 192]. In addition increased level of fragmentation of areas of improved grassland within the landscape (LSI.5.1) was found to have a negative effect

on *C. pulicaris* s.l. abundance at low and high values but a positive effect at medium levels [see Figure 6.1a on page 191].

Culicoides nubeculosus abundance was best described by the negative impact of both the presence of cattle within 50 m of the sampling point and an increasing percentage of calcareous grassland within 250 m of the sampling point (PLAND.7.1). Higher abundance of *C. nubeculosus* were associated with an increasing percentage (PLAND.5.2) and reduced fragmentation (LSI.5.2) of set-aside grassland within 250 m of the sampling point, in addition to increased winter-time vegetation activity (EVI leaf off and TC.GREEN leaf off) and summer-time soil moisture levels (TC.WET leaf.on) [see Table 6.4 on page 192]. An increasing percentage of improved grassland within 250 m of the sampling point (PLAND.5.1) was also found to significantly influence *C. nubeculosus* abundance, with little impact on abundance observed at low values, a positive effect at medium to high values and a negative effect at very high values [see Figure 6.1b on page 191].



(a). ***C. pulicaris*** **s.l.** (b). ***C. nubeculosus***

Figure 6.1. Fitted smooth terms (solid lines) [indicated as *s(name of the predictor, numbers of degrees of freedom)*] and 95 % confidence intervals (dashed lines) for (a) landscape shape index of improved grassland (LSI.5.1.) for semi-parametric models of *C. pulicaris* abundance and (b) the percentage of landscape covered by improved grassland (PLAND.5.1.) for semi-parametric models of *C. nubeculosus* abundance. Ticks in the x-axis represent location of observations along the predictors

Variable Suite	Variable	*Culicoides* Species / Species Group			
		***C. obsoletus* s.s.**	***C. scoticus***	***C. pulicaris* s.l.**	***C. nubeculosus***
	Intercept	1.48 (0.21)***	5.97 (3.27)$^{\triangle}$	0.59 (0.12)***	-59.70 (18.27)***
Host	Sheep Presence	0.76 (0.33)*	-	-	-
	Cattle Presence	-	-	-	-0.95 (0.38)*
Landscape	PLAND.1.1 (Broad-Leaved Woodland)	-	0.01 (0.00)'$^{\triangle}$	-	-
	s(PLAND.5.1, 4) (Improved Grassland)	-	-	-	0.01 (0.00) *** ‡
	PLAND.7.1 (Calcareous Grassland)	-0.02 (0.01)*	-0.01 (0.01)*	-0.01 (0.01)*	-
	PLAND.5.2 (Set-Aside Grassland)	-	-	-	0.08 (0.06)***
	s(LSI.5.1, 4) (Improved Grassland)			-0.25 (0.08)***‡	
	LSI.7.1 (Calcareous Grassland)		-	-	-0.54 (0.18)***
Climate	LST.leaf.off	-0.57 (0.19)**	-	-	-
	EVI leaf off	-	-8.27 (4.77)$^{\triangle}$	-	-16.23 (10.67)$^{\triangle}$
	TC.Green leaf off	-	-	-	106.79 (32.73)***
	TC.WET leaf on	-	-	-	10.49 (3.75)***
Terrain/Soil	Soil Type 2	-	-0.26 (0.32)	-	
	Soil Type 3	-	-0.12 (0.28)	-	
	Soil Type 4	-	-0.17 (0.34)	-	
	Soil Type 5	-	0.50 (0.30)$^{\triangle}$	-	

- indicates variable not included in final model

Table 6.4. Coefficient estimates with their standard error shown in parenthesis of environmental variables selected by a forward-stepwise procedure for general additive models with a Gaussian error structure for female livestock-associated *Culicoides* species [$^{\triangle}$ P≤0.1, *P≤0.05, **P≤0.01, *** P≤<0.001] [non-parametric terms indicated as *s(name of the predictor, numbers of degrees of freedom)*], ‡ see Figure 6.1 for fitted smooth terms. [† SOIL.TYPE factor levels: 1 - freely draining slightly acid but base-rich soils (reference level); 2 - freely draining slightly acid loamy soils; 3 - shallow lime-rich soils over chalk or limestone; 4 - slightly acid loamy and clayey soils with impeded drainage; 5 - slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils]

	1	2	3	4	5
1	-	-0.256	-0.122	-0.172	0.504
2		-	0.134	0.084	0.760*
3			-	-0.050	0.626**
4				-	$0.676^{\triangle}$
5					-

Table 6.5. Multiple Tukeys all-pair comparisons of *C. scoticus* abundance between different soil types shown on upper diagonal [$^{\triangle}$ P≤0.1, *P≤0.05, **P≤0.01, *** P≤<0.001], SOIL.TYPE factor levels: 1 - freely draining slightly acid but base-rich soils (reference level); 2 - freely draining slightly acid loamy soils; 3 - shallow lime-rich soils over chalk or limestone; 4 - slightly acid loamy and clayey soils with impeded drainage; 5 - slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils

Forward-stepwise selection of variables using RDA (Hellinger distance-transformed) identified three variables as significant in determining livestock-associated *Culicoides* species assemblages, one climatic variable [EVI leaf on], one landscape variable [LSI.51 - (improved grassland)], and one terrain/soil variable [SOIL.TYPE] [see Table 6.6 on page 194]. All variables were significant at the 0.05 level as calculated by the Monte Carlo permutation test (5,000 times). No variables from the host variable suite were selected. Within linear ordinations inertia represents the variance in transformed species abundance. Overall inertia, for the transformed livestock-associated *Culicoides* species abundance data was 0.280 of which 28.31 % was explained by the selected environmental variables (sum of the five canonical axes and five PCA axes), R_a^2 0.22 [see Table 6.6 on page 194]. Variance explained by ordination analyses decreases with the dimensionality of the problem, i.e. as the number of samples and species increase. Three environemal variables were considered above, the cumulative variance explained by the 1^{st}, 2^{nd} and 3^{rd} PCA axes in unconstrained RDA explained 91.23 % of the total variance in species composition. The selected constrained axes explained 16.38 % of the variation in *C. obsoletus* s.s. abundance, 28.02 % of variation in *C. scoticus* abundance, 12.40 % of variation in *C. dewulfi* abundance, 27.09 % in *C. pulicaris* s.l. abundance and 40.10 % of variation in *C. nubeculosus* abundance [see Table 6.9 on page 196]. The partial or independent effects of climate (spring-time

EVI) was only 2 % and that of landcover (LSI.51 - improved grassland) was only 4 %. Terrain/soil factors (SOIL.TYPE) accounted for 16 % of the total variance in community composition. No further variance was explained by the shared effects of climate, landscape variables and terrain/soil variables [See Figure 6.2 below].

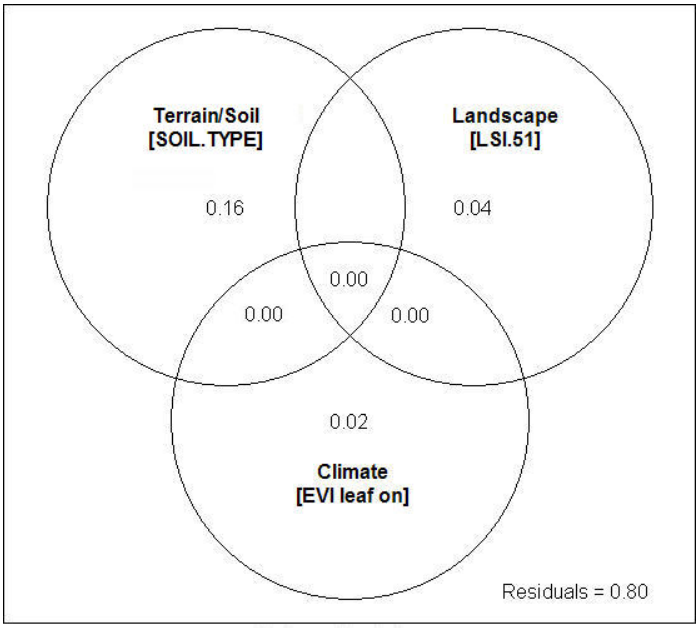


Figure 6.2. Variance decomposition by linear redundancy analysis (RDA) of female livestock-associated *Culicoides* species assemblages at the local-scale (Hellinger distance-transformed) into its purely climate, purely landscape, purely terrain/soil, and mixed components, adjusted R-squared reported [R_a^2] (EVI - enhanced vegetation Index, LSI - landscape shape index, SOIL.TYPE - characteristics of dominant soil type)

Variable Suite	Variable	F	*P* value	R_a^2
Climate	EVI leaf on	2.59	0.05	0.02
Landscape	LSI.5.1 (Improved Grassland)	3.81	0.01	0.04
Terrain and Soil	SOIL.TYPE	4.15	<0.01	0.16

Table 6.6. Significance of environmental variables selected by a forward-stepwise procedure in linear redundancy analysis (RDA) of female livestock-associated *Culicoides* species assemblages at the local-scale (Hellinger distance-transformed), as tested by comparing them to Monte Carlo permutations (5,000 times) of the null model (EVI - enhanced vegetation Index, LSI - landscape shape index, SOIL.TYPE - characteristics of dominant soil type)

RDA ordination diagrams (correlation triplots) are shown in Figure 6.3 on page 197, in order to show the correspondence between environmental predictors and species composition. Correlation between selected environmental predictors and *Culicoides* species/species groups and the five RDA Canonical axes are shown below in Table 6.7 and Table 6.8 respectively.

Variable	Canonical Axes				
	I	**II**	**III**	**IV**	**V**
LSI.51 (Improved Grassland)	-0.244	-0.365	-0.649	-0.475	0.205
EVI.leaf.on	0.136	0.497	-0.246	-0.143	-0.772
SOIL.TYPE 2†	0.528	-0.039	-0.353	0.707	0.103
SOIL.TYPE 3†	-0.328	0.325	0.542	-0.270	0.145
SOIL.TYPE 4†	-0.219	0.501	-0.496	-0.074	0.357
SOIL.TYPE 5†	-0.326	-0.535	-0.007	0.177	-0.625

Table 6.7. Correlations between canonical RDA axes and environmental variables selected by linear redundancy analysis (RDA) (using a forwards stepwise procedure) of livestock-associated *Culicoides* species abundance (Hellinger distance-transformed) (EVI - enhanced vegetation index, LSI - landscape shape index, SOIL.TYPE - characteristics of dominant soil type) [† SOIL.TYPE factor levels: 1 - freely draining slightly acid but base-rich soils (reference level); 2 - freely draining slightly acid loamy soils; 3 - shallow lime-rich soils over chalk or limestone; 4 - slightly acid loamy and clayey soils with impeded drainage; 5 - slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils]

Variable	Canonical Axes				
	I	**II**	**III**	**IV**	**V**
C. obsoletus s.s.	-0.200	0.264	-0.145	-0.063	-0.029
C. dewulfi	0.056	-0.082	0.015	-0.115	0.022
C. scoticus	-0.356	-0.454	0.100	-0.017	-0.018
C. pulicaris s.l.	0.204	0.170	0.366	-0.019	-0.012
C. nubeculosus	0.653	-0.212	-0.106	-0.0126	-0.017

Table 6.8. Correlations between canonical RDA axes and *Culicoides* species/species group from linear redundancy analysis (RDA) (using a forwards stepwise procedure) of livestock-associated *Culicoides* species abundance (Hellinger distance-transformed)

The RDA triplot indicates that the five livestock associated *Culicoides* species assessed have differing responses to environmental conditions. *Culicoides obsoletus* s.s. abundance increased in areas of slightly acid loamy and clayey soils with impeded drainage (SOIL.TYPE 4) and was correlated with increasing spring-time EVI (leaf on). Patterns in *C. scoticus* abundance, in contrast, were strongly correlated with decreasing spring-time EVI (leaf on) and the presence, and increasing fragmentation, of areas of improved grassland within the landscape (LSI.5.1) and were also associated with areas of slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils (SOIL.TYPE 5). *Culicoides dewulfi* showed little correlation with any selected variable. *Culicoides nubeculosus* was correlated with areas of freely draining slightly acid but base-rich soils (SOIL.TYPE 1). Increasing *C. pulicaris* s.l. abundance was correlated with increasing spring-time EVI (leaf on) and areas of freely draining slightly acid loamy soils (SOIL.TYPE 2)

Model Type	***Culicoides* Species / Species Group**				
	***C. obsoletus* s.s.**	***C. scoticus***	***C. dewulfi***	***C. pulicaris* s.l.**	***C. nubeculosus***
GAM	21.65	30.78	*	28.68	48.64
RDA	16.38	28.02	12.40	27.09	40.10

Table 6.9. Percentage of variance explained per species / species group by Gaussian general additive models [GAM] and by constrained linear redundancy analysis [RDA] (Hellinger distance transformed) of livestock associated *Culicoides* species abundance (log-transformed) at the local-scale (*no variables selected in stepwise procedure)

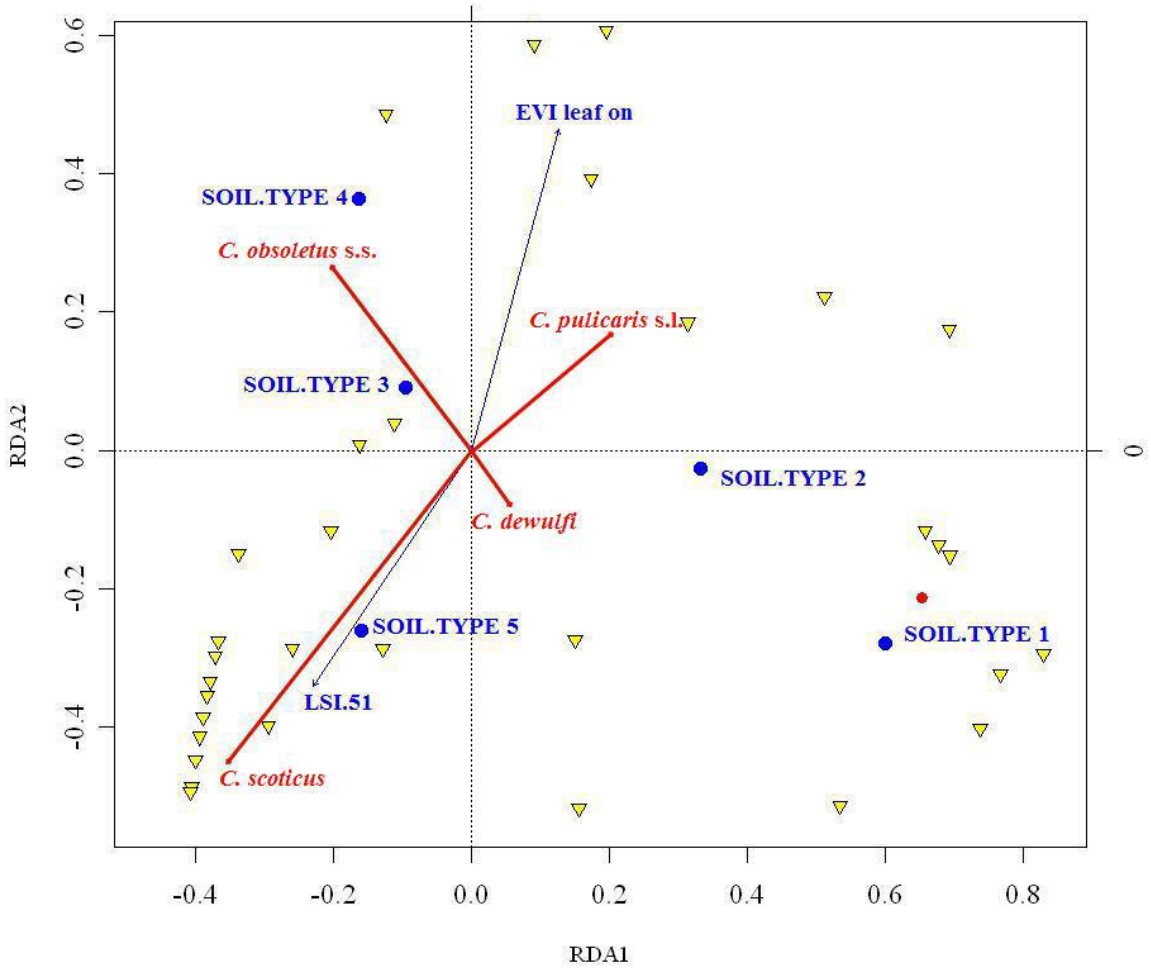


Figure 6.3. RDA ordination correlation triplot diagrams (showing: fitted site scores) for the four explanatory variables selected by forward-stepwise selection using RDA, all RDAs were calculated using Hellinger distance-transformed species data. Blue Arrows: continuous environmental vectors, whose length = importance of the variable to ordination and direction: arrow points towards increase in value. Blue Labels (no arrow): categorical environmental factor levels centroids labelled, whose distance from the origin = importance of the variable to ordination, and position: factor levels association with the canonical axes. Red arrows: *Culicoides* species / species group whose locations on the plot indicate the species distributional similarity with each other. Yellow triangles = sites. Land cover classes: improved grassland (class 5.1) [† SOIL.TYPE factor levels: 1 - freely draining slightly acid but base-rich soils; 2 - freely draining slightly acid loamy soils; 3 - shallow lime-rich soils over chalk or limestone; 4 - slightly acid loamy and clayey soils with impeded drainage; 5 - slowly permeable seasonally wet slightly acid but base-rich loamy and clayey soils]

6.4 Discussion

The distribution and abundance of *Culicoides* both within and between farms exhibits a large degree of spatial variation and variations of up to two orders of magnitude, with regard to both adult abundance (see above) and larval numbers (see Chapter 4) were observed in this study. To date, however, all papers reporting on the distribution and abundance of *Culicoides* in Europe have focused on their spatial distribution and abundance at a regional scale ($\geq$1 km). This study is the first to investigate Palaearctic livestock-associated *Culicoides* species in relation to their environmental requirements at the local within-farm scale. All previous investigations have also been based on *Culicoides* occurrence and abundance sampled using light-suction traps, which have since been found not to provide an accurate estimate of Palaearctic BTV vector species composition (Carpenter et al., 2008d). This study utilised a semiochemical-based trapping system specifically targeting the host-seeking population of *Culicoides*, allowing host-equivalent estimates of *Culicoides* populations within discrete areas of habitat to be obtained.

A large degree of variation in both the number and species composition of *Culicoides* collected between the different habitat types examined was observed, however, the habitat type was found not to be a significant predictor of *Culicoides* species abundance in either the GAMs or RDA. Of the 11 habitat classes sampled only in class 3 (arable) were no *Culicoides* collected, this is likely related to the absence of suitable hosts from areas of arable land and also a lack of suitable larval development sites [see Chapter 4]. The underlying soil type, its composition and drainage was found to be a significant determinant for *Culicoides* species community composition, and specifically for determining *C. scoticus* abundance. All five of the soil compositions encountered in this study had a loamy texture, with medium to high fertility, but ranged from freely draining to having impeded drainage. Although *Culicoides obsoletus* s.s., *C. scoticus* and *C. nubeculosus* were all found to be abundant in improved grassland (pasture) (class 1) areas, all three were associated with different underlying soil characteristics at a community level. These associations may be attributable to variations

in the larval development sites utilised by the species, however, both *C. obsoletus* s.s. and *C. scoticus* were collected from similar habitat types in Chapter 4. The dominant soil characteristics are also likely to be correlated with other environmental conditions at a site, in particular the structure and type of vegetation present. EVI is an optical measure of surface vegetation conditions (Tucker et al., 2005) i.e. the vegetation canopy 'greenness', a composite property of leaf chlorophyll, leaf area, canopy cover and canopy architecture (Jiang et al., 2008), that has been correlated with levels of soil moisture (Chen et al., 2006; Waring et al., 2006). Spring-time vegetation activity was found to be a significant determinant of *Culicoides* species composition with both *C. obsoletus* s.s. and *C. pulicaris* s.l. abundance correlated with increasing levels of vegetation activity. The positive impact of increasing vegetation activity in an area is potentially related to these species requirement for shaded, moist leaf-litter associated larval development sites [see Chapter 4 on page 91], which are likely to become more abundant within highly vegetated areas. In contrast, however, *C. scoticus* abundance was found to be negatively correlated with spring-time vegetation activity when assessed at a community level and negatively correlated to winter-time vegetation activity when assessed as an individual species. NDVI, a vegetation index closely related to EVI has previously been a useful predictor for *C. imicola* presence-absence (Purse et al., 2004a,b; Tatem et al., 2003). *Culicoides obsoletus* s.l. occurrence has been positively correlated with mean NDVI in Spain (Calvete et al., 2008) and in Italy *C. obsoletus* s.l. abundance has been associated with a high percentage of forest cover surrounding sites and higher than average levels of vegetation activity (Conte et al., 2007a). This study supports these findings showing that measures of vegetation activity in an area are important for determining *C. obsoletus* s.l. occurrence and abundance, however, the data suggests that within the Obsoletus group, *C. obsoletus* s.s. and *C. scoticus* may have an opposing relationship with measures of an area's vegetation activity. These findings should be interpreted with caution, however, due to the small number of *C. scoticus* collected. The larval development sites of *C. scoticus* remain poorly defined, however, the negative association to in-

creasing vegetation activity in an area indicates it may favour less vegetated areas, than *C. obsoletus* s.s. Increased abundance *C. scoticus* within farms were, however, found to be associated with substrates with increasingly impeded drainage, this is likely a reflection of the importance of soil moisture levels in determining *Culicoides* larval occurrence identified in Chapter 4, indicating that although *C. scoticus* may prefer less vegetated areas it does still have a preference for moist conditions.

An increased level of fragmentation, and therefore greater total edge lengths, of areas of improved grassland within the landscape was found to negatively impact *C. pulicaris* s.l. abundance when considered both as an individual species and in terms of this species groups relationship community composition. In contrast, increasing *C. scoticus* abundances were found to be negatively impacted by increasing fragmentation of grassland within the landscape, improved grassland areas when considered as an individual species and calcareous grassland in terms of community species composition. Guis et al. (2007) suggested that the importance of woodland edge length in determining BTV outbreak risk in Corsica was the result of increased vector-host interaction as *C. imicola* moved between areas with abundant hosts i.e. pastures to woodland areas in which breeding and resting sites may be found. A similar movement of *C. pulicaris* s.l. between areas of improved grassland which are predominantly used for grazing livestock and other areas of land cover interspersed between the patches of grassland in search of resting and oviposition sites may explain the importance of landscape fragmentation in determining *C. pulicaris* s.l. abundance. If increasing fragmentation results in an an increase in the success of this species group by increasing the potential to find suitable hosts and larval development sites in close proximity. The negative impact of fragmentation of grassland on *C. scoticus* abundance may again be related back to its association with less vegetated areas discussed above, suggesting that it may not follow the same movement patterns suggested above for *C. pulicaris* s.l.

Culicoides nubeculosus, in contrast to both *C. obsoletus* s.s. and *C. scoticus*, showed no association with woodland habitats with large numbers being collected

from areas of improved grassland (class 1) and only from transitional habitats when traps were placed on the pasture side of the woodland-pasture transition. This indicates that the presence and structure of deciduous woodland plays a much more prominent role in determining adult Obsoletus group species occurrence and abundance than for *C. nubeculosus*, despite these species having similar larval habitats [see Chapter 4 on page 91].

Culicoides obsoletus s.l., *C. pulicaris* s.l. and *C. nubeculosus* species are all considered to have a close association with their host distribution. No association was found between cattle or sheep presence/abundance in determining *Culicoides* species community composition. Areas in which hosts are likely to be abundant i.e. pastures, were however, found to contain large numbers of *C. nubeculosus* and *C. obsoletus* s.l. and the percentage of both improved and calcareous grassland surrounding sampling points was found to significantly effect their abundance when considered as individual species, where the presence of sheep had a significant positive effect on *C. obsoletus* s.s. abundance, while cattle presence had a significant negative effect on *C. nubeculosus* abundance. Within farms the rotational grazing systems often operated in the UK, leads to a high degree of variability in host availability in both space and time. The 'snap-shot' type measure of cattle and sheep availability used in this study may not have reflected the long term availability of hosts in an area and these associations should therefore be interpreted with caution. No attempts have been made to assess how quickly *Culicoides* populations respond to movements of hosts. At the within-farm scale there may be long term impacts on *Culicoides* abundance in subsequent generations, if the average host availability increases or decreases.

Although farm land is considered to provide relatively stable environmental conditions, due to intensive management strategies, seasonal conditions of temperature and moisture levels vary significantly between years. Climatic associations may therefore have been over-looked due to the discrepancy in time between the satellite imagery acquisition dates (2003) and the *Culicoides* abundance data collection (2009). Inter-annual variability in the abundance of the Obsoletus group species, potentially due

to variations in their seasonality (Holmes and Boorman, 1987) may also have lead to species being temporally absent, or present at very low numbers, during the sampling period. The use of higher resolution satellite data that is precisely coincident with field sampling, and where both emerging and adult populations are sampled several times through a season, may enable additional or stronger species-environment associations to be identified.

For the other *Culicoides* species collected, *C. albicans*, *C. stigma* and *C. puncticollis*, habitat associations should be interpreted with caution as no attempts have been made to quantify the suitability of using semiochemical-baited traps (CO_2 and 1-octen-3-ol) for their collection. The association of these species with livestock is also poorly defined. *Culicoides stigma* has been described as being primarily associated with livestock on farms, often occurring association with *C. nubeculosus* Kettle and Lawson (1952). A finding supported by this study, with *C. stigma* collected from areas where livestock are abundant for at least part of the year [improved grassland (class 1), hedgerows surrounding pasture (class 8) and surrounding artificial water sources located in pasture (class 10)]. *Culicoides stigma* has also been collected in small numbers from a sheep baited drop trap (S. Carpenter, personnel communication). *Culicoides puncticollis* is a known vector of *Onchocerca reticulata* Diesing (Nematoda: Onchocercidae) in cattle, and in Cyprus Mellor and Pitzolis (1979b) often found it in close association with livestock particularly cattle. Bluetongue virus, however, has been found not to replicate in an Israeli strain of orally infected *C. puncticollis* (Mellor et al., 1981).

6.5 Conclusions

The development of models of vector abundance in the Palaearctic region have, to date, been restricted by a lack of species-specific datasets for the morphologically cryptic species groups, *C. obsoletus* s.l. and *C. pulicaris* s.l. While species-specific classifications of the Pulicaris group remain elusive. This study presents the first investigation of the climatic and non-climatic ecological drivers of Obsoletus group abundance at the species level. The large number of failed identifications to species

level using the multiplex PCR system in this study, however, highlights the problems of relying on the current system for consistent Obsoletus group species identification. Although unlikely, the high failure rate could be the result of contamination at the sampling point, or the result of some yet undetermined cause. The possibility of additional species within the Obsoletus group in the UK has been postulate and requires further investigation (J. De Gabriel, personnel communication).

The targeting of disease control measures and accurate BTV transmission risk assessment requires, in addition to work on other aspects of vector competence, models that can explain and predict local-scale variation in adult vector abundance, rather than simply occurrence. Despite the low level of variance explained within the individual species model and the ordination model in this study, significant divergence in the ecological requirements of the different Obsoletus group species was observed. To understand the mechanisms underlying the differential impacts of both climatic and non-climatic factors and provide accurate risk assessments, future studies must establish links between ecological correlates at the species rather than the group level for *C. obsoletus* s.l. The potential for future studies to further characterise the species-environment relationships suggested in this study are discussed further in Chapter 7.

Chapter 7

General Discussion

The incidence and prevalence of BTV infection is highly dependent on the distribution and abundance of its *Culicoides* vectors. Quantification of the drivers of vector abundance are therefore required for accurate estimates of transmission risk and for the development of cost-effective targeted vector control strategies. The extremely small size of *Culicoides* and the cryptic nature of the Palaearctic BTV vectors, however, makes investigations into their bionomics and interactions with susceptible hosts and pathogens exceptionally difficult and has resulted in a paucity of detail regarding Palaearctic BTV vector distribution and abundance at the within-farm scale. This thesis proposed to address this issue by using a multidisciplinary approach combining statistical modelling and GIS techniques with detailed ecological surveys of adult and larval Palaearctic BTV vector populations to investigate habitat use by these species in the UK and mainland Europe. As a result of this work, drivers of Palaearctic BTV vector occurrence and abundance at the farm-level were identified.

Maximum catches during summer-time have been shown in previous studies to be consistently related to the annual abundance of *Culicoides* in Morocco (Baylis et al., 1997). The work contained in Chapter 3, however, confirmed the problems identified by Calistri et al. (2003) and Pili et al. (2006), which concluded that climatic models of BTV vector abundance, based on only one or two trap catches per sampling point, still failed to explain a significant level of the observed variation in *Culicoides* populations. This has been the case despite the inclusion of additional landscape, host and soil characteristics, which have improved predictions in other countries (Calistri et al., 2003; Conte et al., 2007a). It is clear from this that despite the use of a spatially extensive, farm-based, surveillance system and the consideration of a good range of biologically plausible environmental relationships, accurate models of BTV vector abundance, in comparison to BTV outbreak occurrence are difficult to obtain using

the type of abundance estimates available for Greece and Bulgaria, where sampling effort was very low with only two trap catches made per site. This is in part due to the generalist requirements of adult *Culicoides* when considered within farm habitats as they occur in abundances relative to the proportion of available resources in an area, for example the Palearctic BTV vectors were found to utilise larval development sites which are ubiquitous across farm land. Studies in non-insect taxa have found that species with narrow, well-defined niches are better modelled than those with broader niches (Boone and Krohn, 1999; Pearce et al., 2001; Kadmon et al., 2003) and that models for specialist species are generally more accurate than models for generalists (Elith et al., 2006; Hepinstall et al., 2002; McPherson and Jetz, 2007; Segurado and Araujo, 2004; Thuiller et al., 2004). Models for species that have narrow distributions in geographical space have also been found to be more accurate than models for species with larger distributions (Brotons et al., 2004; Stockwell and Peterson, 2002; Segurado and Araujo, 2004; Hernandez et al., 2006). The Obsoletus and Pulicaris groups of *Culicoides* are widely distributed and abundant across the entire Palaearctic region adding to the difficulties intrinsic to predicting their abundance. Though final distribution models often had weak explanatory power, the different predictors selected were suggestive of differential responses to environmental factors between the Obsoletus and Pulicaris group and between their constituent species. This is to be expected from their differing ecological characteristics (McPherson and Jetz, 2007) and emphasise the need to investigate relationships at the species, rather than group level. In addition, measures of *Culicoides* abundance between farms resulting from a single trap location will only reflect the abundance at that location, which may or may not be relevant to BTV transmission dependent upon the distribution of susceptible hosts in relation to the trap site. In light of these problems this study aimed to provide the first species-specific quantitative assessment of the local-scale spatial variation in farm-associated Palaearctic *Culicoides* species, in both larval and adult forms, at the within-farm scale, although this objective was achieved for adult *Culicoides*, it was not met with regards to the species-specific identification of larval

forms of live-stock associated *Culicoides* species.

For over 50 years the larval development substrates utilised by the Palaearctic BTV vectors have received little attention (Uslu and Dik, 2007, 2010; Zimmer et al., 2008). The lack of research into larval habitats is a reflection of the complexities of sampling a small and fragile genus, which is present seasonally at a very low density in patchy populations within moist soil habitats that can cover wide areas. These problems are compounded by uncertainty in the identification of specimens to species level by morphological means. Although in this work significant difficulties were still encountered with identification, the use of emergence traps has in part solved this issue. These were deployed across a wide variety of habitats at more than one site, a study that has not previously been attempted and which demonstrated significant between-farm variation in *Culicoides* populations, both in terms of species composition and their overall abundance. The identification of pigmented, newly emerged, nulliparous adults would also not have been possible without the use of emergence traps. This new evidence raises an issue that has already been proposed for other *Culicoides* species (Boorman and Goddard, 1970; Braverman and Mumcuoglu, 2009; Dyce, 1969; Walker and Boreham, 1976), that dispite established scientific wisdom, an increase in abdominal pigmentation is not always associated with parity, potentially leading to errors in the determination of *C. obsoletus* s.s. population age structure.

Accurate BTV risk assessment requires identification of which species are largely responsible for transmission is a goal that has yet to be achieved in northern Europe. It also requires a similarly accurate means of sampling the adult population, in order to derive a clear understanding of their wider vectorial capacity. Light-suction traps have, to date, been the primary tool used for *Culicoides* surveillance due to their ease of use and ability to catch large numbers of individuals. Carpenter et al. (2008d), however, showed that the species composition of Palaearctic BTV vectors in drop-trap catches on sheep differed significantly from those taken from light traps on the same night and the same location. Several *Culicoides* species caught on sheep were not present at all in light trap collections and these included potential BTV vec-

tors, with the numbers of *C. chiopterus* feeding on sheep in particular being severely underestimated by light trapping. In light of these problems, and the difficulties of using a light-based surveillance method at a within-farm scale, the research within Chapter 5 was undertaken to find an alternative trapping method. Although CO_2 alone has been used effectively for the collection of other *Culicoides* species, it was found in preliminary trials in this project to be ineffective at collecting livestock-associated *Culicoides* in south east England. The semiochemical-bait combinations trialled, therefore, focused on the use of a synergistic combination of CO_2 and 1-octen-3-ol and comparing different enantiomeric combinations of 1-octen-3-ol. The number of *C. obsoletus* s.l. collected in CDC traps baited with (R)-(-)-1-octen-3-ol and CO_2, indicated that this would be a suitable method for use as an effective surveillance tool for livestock-associated *Culicoides* species at the 'within-farm' scale in southern England. Preliminary comparisons to a sheep baited drop trap indicated that this semiochemical-bait combination was also accurately reflecting Palaearctic BTV vector population structure attracted to hosts, and due to the host derived nature of this bait is likely to provide host-equivalent estimates of vector abundance. Additionally semiochemical baited traps provide a major advantage over light traps, with a shorter range of attraction allowing for measures of *Culicoides* abundance to be made in discrete habitat patches at the within-farm scale.

The use of this semiochemical-baited trapping system allowed the first species-specific quantitative assessment of the within-farm spatial variation in livestock-associated *Culicoides* abundance to be made in the Palaearctic region. Models of both larval BTV vector occurrence and adult BTV vector abundance developed from data collected within Chapters 4 and 6, have enhanced our understanding of the relative roles of drivers of *Culicoides* abundance. Non-climatic factors were of equal importance as climatic factors in determining livestock-associated *Culicoides* abundance and occurrence at the within-farm scale. Models of adult *Culicoides* abundance revealed a differential sensitivity between the Obsoletus group species that can, at least in part, be explained by their ecological characteristics. The question remains,

however, as to how species-environment relationships can be estimated with sufficient accuracy for inclusion within risk assessments to improve both within and between-farm disease transmission models. Key variables related to the sensitivity of the Obsoletus and Pulicaris groups and between the constituent species of the Obsoletus group were related to measures of vegetation activity, moisture availability and habitat fragmentation both at a landscape level and fragmentation of key land cover classes, in particular grassland and broad-leaved woodland. The examination of the variation in response to these key environmental gradients across a wider area of these Palaearctic species distributions may provide useful information on limitations to their distribution, and therefore their potential to be involved in transmission, within these species thermal tolerances.

This study provides the first attempts to define the spatial variation in Palaearctic BTV vector abundance at the farm level rather than at a 1km pixel or administrative district level. One of the main limitations, however, against extending the results of this project to improving BTV risk assessment, is the 'snap-shot' nature of the *Culicoides* abundance and occurrence estimates made, which do not allow for inter-annual variability in *Culicoides* population sizes to be accounted for. Despite the lack of significant ($P>0.05$) spatial autocorrelation in *Culicoides* larval occurrence (Chapter 4) and adult abundance (Chapter 6) it remains likely that geographic space will influence species abundances in addition to environmental space at the local within-farm scale. The influence of spatial autocorrelation on abundance estimates may become apparent if *Culicoides* abundance estimates are based on more frequent trapping throughout the vector season, as errors in population estimates caused by inter-annual variability in species abundance and occurrence may have obscured influential spatial autocorrelation. Limitations due to the resolution of the Landsat 7 ETM+ imagery used (15m) and the time discrepancy between image acquisition and entomological data collection may also have obscured significant environmental relationships. Efforts were made to acquire very high resolution seasonal imagery (Quickbird - multispectral pansharpened resolution 60 cm) of the farm sites in this

study, however, the tasking capacity of these satellites is in high demand. Successful acquisitions were only achieved during winter, and were blighted by both snow and cloud coverage resulting in clear acquisitions for only a very limited area. The recent launch of additional multispectral very high resolution satellites (e.g. DigitalGlobe's WorldView-2 - pansharpened multispectral resolution 46 cm) and in particular multi-satellite constellation systems (RapidEye's five satellite constellation - resolution 5 m) will allow for a larger amount of high resolution imagery to be collected with quicker revisit times in the case of multi-satellite systems. The increased availability and hopefully the reduced cost of such high resolution imagery should lead to the more frequent use of this type of remotely-sensed data. It is likely, however, to be some time before datasets capturing the seasonality of natural habitats equivalent to that developed for MODIS satellite data at a 1km resolution are available (Scharlemann et al., 2008).

Due to the significant and ongoing financial and disease impact of BTV in Europe, practical strategies for vector control for use alongside vaccination campaigns remain of paramount importance during incursions of new strains. It is noteworthy that vaccines against the recent BTV-8 outbreak in northern Europe took 18 months to develop (Carpenter et al., 2009) although subsequent production of vaccines against other serotypes may be more rapid depending on the size and level of certainty of the potential market. During periods where vaccines are not available to reduce or prevent transmission of BTV an integrated control programme should aim to reduce the vector:host ratio, to levels that will have a significant effect on the transmission cycle of BTV. This could be achieved through increasing the mortality rate of adult/immature vectors and/or through reducing vector to host contact. Knowledge of the biology of candidate vectors is therefore essential for the development of cost-effective, targeted, mitigation techniques that will reduce the demographic rates of vectors and, in turn, their contact rates with hosts. To date, however, there has been a lack of sufficient information on the spatial distribution of the Palaearctic BTV vectors for adequate targeting of control measures and this knowledge gap has

been compounded by a lack of quantitative evidence on the effectiveness of practical vector control measures. The wide range of habitats found to be utilised by both adult and immature species of the Obsoletus and Pulicaris group, however, present problems for the development of effective habitat modification schemes. Larvae of these species were mainly associated with areas of decaying organic matter rather than the top layer of the soil horizon and, in particular, moist areas where the substrate was formed by, or contaminated with, livestock faecal matter. Despite the lack of success in the habitat modification strategy tested in this study the types of habitats identified as being used by *C. obsoletus* s.l. indicate that good water and waste management practices remain key to practical population control of this group. *Culicoides* larval occurrence was found to be significantly associated with increased moisture levels. Areas made permanently moist by artificial sources of water, such as leaking taps and overflowing water troughs also contained an abundance of *Culicoides* larvae. These relatively discrete and compact often man-made habitats can be prolific larval development sites, but that have the potential to be easily and effectively eliminated. It is unlikely, however, that any one control method would have a major effect on local vector populations, instead control measures must be considered as being part of an integrated system and should combine not just larvicidal methods but also methods aimed at controlling the adult population, which may included modifications to husbandry and farm management systems but also the use of repellents and other livestock protection, such as screened accommodation [for review see Carpenter et al. (2008b)]. Further extended trials of the currently recommended control techniques (DEFRA, 2009b) are required, however, before their recommendation can be justified. A key constraint to the effectiveness of any control technique is user uptake, therefore cost, the dissemination of information to, and the motivation of, stakeholder groups is vital.

7.1 Final Comment

This thesis has demonstrated the complexities intrinsic in working with *Culicoides* in the field and laboratory, and has also provided significant amounts of novel data concerning their life history and ecology. The accurate characterisation of drivers of population abundance, which would revolutionise both risk assessment and response to Orbivirus incursion remains a long-term challenge. While the advent of cheap and readily available satellite imagery and computer processing power, combined with molecular assays, has the potential to assist in this process, there remain major gaps in our basic understanding of BTV vector ecology and these are linked to the difficulty of sustaining the long term collection of entomological data. The infrequent appearance of *Culicoides*-borne pathogens in Europe results in research effort in this area being characterised by peaks and troughs in line with funding availability. Recent years, however, have seen an explosion in the interest in *Culicoides* biology, driven almost entirely by the incursion of BTV into Europe. While this has resulted in the collection of data sets that may prove valuable in improving this situation the paucity of sustainable funding for basic, field-based entomological research remains an important issue. In addition, it is vital that such long term datasets collected within individual countries and regions are comparable at an EU level. The comparison of the ecological characteristics of the Obsoletus and Pulicaris groups and how, or if they vary across their range will allow factors that may promote or depress the movement of BTV and other *Culicoides*-borne pathogens across Europe. While steps have been taken to address this through international collaboration in recent years, the journey towards fully integrated, international, datasets has yet to be achieved.

Bibliography

Akaike, H. 1973. Information theory as an extension of the maximum likelihood principle. In *In 2nd International Symposium on Information Theory (edited by B.N. Petrov & F. Csaksi), pp. 267-281 Akademiai Kiado, Budapest, Hungary.*

Akey, D. 1987. Pigmentation associated with oogenesis in the biting fly *Culicoides variipennis* (Diptera: Ceratopogonidae): light and electron microscopy. *Journal of Medical Entomology*, 24(1):106–114.

Akey, D. and Potter, H. 1979. Pigmentation associated with oogenesis in the biting fly *Culicoides variipennis* (Diptera: Ceratopogonidae): Determination of Parity. *Journal of Medical Entomology*, 16(1):67–70.

Alexandridis, T., Zalidis, G., and Silleos, N. 2008. Mapping irrigated area in Mediterranean basins using low cost satellite earth observation. *Computers and Electronics in Agriculture*, 64(2):93–103.

Allan, S., Day, J., and Edman, J. 1987. Visual ecology of biting flies. *Annual Review of Entomology*, 32:297–314.

Andreadis, T., Anderson, J., and Vossbrinck, C. 2001. Mosquito surveillance for West Nile virus in Connecticut, 2000: Isolation from *Culex pipiens*, *Cx. restuans*, *Cx. salinarius*, and *Culiseta melanura*. *Emerging Infectious Diseases*, 7(4):670–674.

ANON 1998a. Bluetongue in Greece: confirmation of diagnosis. *l'Office International des Épizooties Disease Information*, 11:166–167.

ANON 1998b. Disease outbreaks reported to the OIE in November-December 1998. *Office International des Épizooties (OIE) Disease Information, accessed: 22/09/2009*, 110:506.

ANON 2000. Bluetongue in Greece: typing of virus strains isolated in 1998-1999. *l'Office International des Épizooties Disease Information*, 13:33–34.

ANON 2006a. Bluetongue in Algeria, Immediate Notification (23/07/2006). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2006b. Bluetongue in Belgium, Immediate Notification (18/08/2006). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, assessed 22/02/2009.

ANON 2006c. Bluetongue in France, Immediate Notification (31/08/2006). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2006d. Bluetongue in Germany, Immediate Notification (21/08/2006). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2006e. Bluetongue in Italy (06/11/2006). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System.

ANON 2006f. Bluetongue in Luxembourg, Immediate Notification (31/11/2006). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2006g. Bluetongue in Morrocco, Immediate Notification (30/10/2006). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2006h. Bluetongue in Netherlands, Immediate Notification (18/08/2006). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2006i. Press Release No. 114, Ministry of Agriculture, Nature and Food, Safety, The Hague, 17 August 2006.

ANON 2007a. Bluetongue in Denmark, Immediate Notification (13/10/2007). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2007b. Bluetongue in France, (23/11/2007). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2007c. Bluetongue in Portugal, Immediate Notification. *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2007d. Bluetongue in Spain, Immediate Notification (26/07/2007). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2007e. Bluetongue in Switzerland, Immediate Notification (29/10/2007). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2007f. Bluetongue in the Czech Republic, Immediate Notification (29/11/2007). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2007g. Bluetongue in the United Kingdom, Immediate Notification (27/09/2007). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2007h. Bluetongue in Tunisia, Immediate Notification (18/0/2007). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2008a. Bluetongue in Austria, Immediate Notification (07/11/2008). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2008b. Bluetongue in Hungary, Immediate Notification (15/09/2008). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2008c. Bluetongue in Italy, Immediate Notification (15/01/2009). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2008d. Bluetongue in Slovakia (21/04/2008). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 03/10/2009.

ANON 2008e. Bluetongue in Spain - Immediate Notification (17/01/2008). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 03/10/2009.

ANON 2008f. Bluetongue in Sweden, Immediate Notification (10/09/2008). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 22/02/2009.

ANON 2008g. Bluetongue in the Netherlands (27/10/2008). *Bulletin de l'Office International des Épizooties*, World Animal Health Information Database, Assessed: 03/03/2009.

ANON 2009a. Bluetongue in Germany (03/11/2008). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 03/03/2009.

ANON 2009b. Bluetongue in Greece - Immediate Notification (23/03/2009). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information System, accessed: 03/10/2009.

ANON 2009c. Bluetongue in Norway - Immediate Notification (20/02/2009). *Office International des Épizooties (OIE) Disease Information*, World Animal Health Information Database, assessed: 04/03/2009.

Ansari, M., Carpenter, S., and Butt, T. 2010. Susceptibility of *Culicoides* biting midge larvae to the insect-pathogenic fungus, *Metarhizium anisopliae*: Prospects for bluetongue vector control. *Acta Tropical Animal Health and Production*, 113(1):1–6.

Baldet, T., Delácolle, J., Cêtre-Sossah, C., Mathieu, B., Meiswinkel, R., and Gerbier, G. 2008. Indoor activity of *Culicoides* associated with livestock in the bluetongue virus (BTV) affected region of northern France during autumn 2006. *Preventative Veterinary Medicine*, 87(1-2):84–97.

Balin, O. 1979. Transmission de *l'Onchocerca bovine*, *Onchocerca gutturosa*, par *Culicoides*. *Annuals of Parasitology*, 54:483–488.

Bartsch, S., Nauer, B., Wiemann, A., Clausen, P., and Steuber, S. 2009. Feeding patterns of biting midges of the *Culicoides obsoletus* and *Culicoides pulicaris* groups on selected farms in Brandenburg, Germany. *Parasitology Research*, 105(2):373–380.

Battle, F. and Turner, E. 1972. Some nutritional and chemical properties of the larval habitats of certain species of *Culicoides* (Diptera: Ceratopogonidae). *Journal of Medical Entomology*, 9(1):32–35.

Baylis, M. 2002. The re-emergence of bluetongue. *Veterinary Journal*, 164(1):5–6.

Baylis, M., Bouayoune, H., Touti, J., and El Hasnaoui, H. 1998. Use of climatic data and satellite imagery to model the abundance of *Culicoides imicola*, the vector of African horse sickness virus, in Morocco. *Medical and Veterinary Entomology*, 12(3):255–266.

Baylis, M., El Hasnaoui, H., Bouayoune, H., Touti, J., and Mellor, P. S. 1997. The spatial and seasonal distribution of African horse sickness and its potential *Culicoides* vectors in Morocco. *Medical and Veterinary Entomology*, 11(3):203–212.

Baylis, M., Meiswinkel, R., and Venter, G. J. 1999. A preliminary attempt to use climate data and satellite imagery to model the abundance and distribution of *Culicoides imicola* (Diptera: Ceratopogonidae) in southern Africa. *Journal of the South African Veterinary Association*, 70(2):80–9.

Baylis, M., Mellor, P., Wittmann, E. J., and Rogers, D. J. 2001. Prediction of areas around the Mediterranean at risk of bluetongue by modelling the distribution of its vector using satellite imaging. *Veterinary Record*, 149(21):639–43.

Baylis, M. and Mellor, P. S. 2001. Bluetongue around the Mediterranean in 2001. *Veterinary Record*, 149(21):659–659.

Baylis, M. and Rawlings, P. 1998. Modelling the distribution and abundance of *Culicoides imicola* in Morocco and Iberia using climatic data and satellite imagery. *Archives of Virology Supplement*, 14:137–53.

Bekker, J., DeKock, W., and Quinlann, J. 1934. The occurance and identification of bluetongue in cattle, the so-called pseudo foot-and-mouth disease in South Africa. *Onderstepoort Journal of Veterinary Science Animal Industrys*, 2:393–507.

Belton, P. and Pucat, A. 1967. A comparison of different light in traps for *Culicoides* (Diptera: Ceratopogonidae). *Canadian Entomology*, 99:267 – 272.

Bernier, U., Kline, D., Shreck, C., Yost, R., and Barnard, D. 2002. Chemical analysis of human skin emanations: comparision of volities from humans that differ in attraction of *Aedes aegypti* (DipteraL Culicidae). *Journal of The American Mosquito Control Association*, 18:186–195.

Bhasin, A., Mordue (Luntz), A., and Mordue, W. 2000a. Electrophysiological and behavioural identification of host kairomones as olfactory cues for *Culicoides impunctatus* and *C. nubeculosus*. *Physiological Entomology*, 25:6–16.

Bhasin, A., Mordue (Luntz), A., and Mordue, W. 2000b. Responses of the biting midge *Culicoides impunctatus* to actetone, CO_2 and 1-octen-3-ol in a wind tunnel. *Medical and Veterinary Entomology*, 14:300–307.

Bhasin, A., Mordue (Luntz), A., and Mordue, W. 2001. Field studies on efficacy of host odour baits for the biting midge *Culicoides impunctatus* in Scotland. *Medical and Veterinary Entomology*, 15(2):147–156.

Bidlingmayer, W. 1961. Field activity studies of adult *Culicoides furens*. *Annals of the Entomological Society of America*, 54:149–156.

Birkett, M., Agelopoulos, N., Jensen, K., Jespersen, J., Pickett, J., Prijs, H., Thomas, G., Trapman, J., Wadhams, L., and Woodcock, C. 2004. The vole of volatile semichemicals in mediating host location and selection by nuisance and disease-transmitting cattle flies. *Medical and Veterinary Entomology*, 18:313–322.

Birley, M. and Boorman, J. 1982. Estimating the survival and biting rates of haematophagous insects, with particular reference to the *Culicoides obsoletus* group (Diptera, Ceratopogonidae) in southern England. *Journal of Animal Ecology*, 51(1):135–148.

Bishop, A., McKenzie, H., Barchia, I., and Harris, A. 1996. Effect of temperature regimes on the development, survival and emergence of *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae) in bovine dung. *Australian Journal of Entomology*, 35:361–368.

Bishop, A., McKenzie, H., and Spohr, L. 2008. Attraction of *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae) and *Culex annulirostris* Skuse (Diptera: Culicidae) to simulated visual and chemical stimuli from cattle. *Australian Journal of Entomology*, 47:121–127.

Bishop, A., McKenzie, H., Spohr, L., and Barchia, I. 1994. *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae) in different farm habitats. *Australian Journal of Zoology*, 42:379–384.

Bishop, A., Worrall, R., Spohr, L., McKenzie, H., and Barchia, I. 2004. Response of *Culicoides* spp. (Diptera: Ceratopogonidae) to light-emitting diodes. *Australian Journal of Entomology*, 43:184–188.

Bishop, A. L., Bellis, G., McKenzie, H., Spohr, L. J., Worrall, R., Harris, A., and Melville, L. F. 2006. Light Trapping of biting midges *Culicoides* pp. (Diptera: Ceratopogonidae) with green light-emitting diodes. *Australian Journal of Entomology*, 45:202–205.

Bishop, A. L., McKenzie, H., Barchia, I., and Harris, A. 1995. The effects of habitat on the distribution of *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae) during its resting phase. *Australian Journal of Zoology*, 473:531–539.

Blackwell, A. 1997. Diel flight periodicity of the biting midge Culicoides impunctatus and the effects of meteorological conditions. *Medical and Veterinary Entomology*, 11:361–367.

Blackwell, A. 2001. Recent advances on the ecology and the behaviour of *Culicoides* spp. in Scotland and the prospects for control. *Veterinary Bulletin*, 71:1–8.

Blackwell, A., Brown, M., and Mordue, W. 1995. The use of an enhanced ELISA method for the identification of *Culicoides* bloodmeals in host preference studies. *Medical and Veterinary Entomology*, 9:214 – 218.

Blackwell, A., Dyer, C., Mordue (Luntz), A., Wadhams, L., and Mordue, W. 1996. The role of 1-octen-3-ol as a host odour attractant for the biting midge, *Culicoides impunctatus* Goetghebuer and the interactions of 1-octen-3-ol with a volatile pheremone produced by parous female midges. *Physiological Entomology*, 21:15–19.

Blackwell, A. and King, F. 1997. The vertical distribution of *Culicoides impunctatus* larvae (Diptera, Ceratopogonidae). *Medical and Veterinary Entomology*, 11:45 – 48.

Blackwell, A., Lock, K., Marshall, B., Boag, B., and Gorden, S. 1999. The spatial distribution of larvae of *Culicoides impunctatus* biting midges. *Medical and Veterinary Entomology*, 13:362–371.

Blackwell, A., Mordue (Luntz), A., and Mordue, W. 1994. Identification of blood meals of the Scottish biting midge, *Culicoides impunctatus*, by indirect enzyme linked immunosorbent assay (ELISA). *Medical and Veterinary Entomology*, 8:20 – 24.

Blackwell, A., Mordue (Luntz), A., Young, M., and Mordue, W. 1992. Bivoltism, survival rates and reproductive characteristics of the Scottish biting midge, *Culicoides impunctatus*, (Diptera: Ceratopogonidae) in Scotland. *Bulletin of Entomological Research*, 84:295 – 306.

Blanchet, F., Legendre, P., and Borcard, D. 2008. Forward selection of explanatory variables. *Ecology*, 89(9):2623–2632.

Boegh, E., Soegaard, H., Broge, N., Hasager, C. B., Jensen, N. O., Schelde, K., and Thomsen, A. 2002. Airborne multispectral data for quantifying leaf area index, nitrogen concentration, and photosynthetic efficiency in agriculture. *Remote Sensing of Environment*, 81:179–193.

Boone, R. and Krohn, W. 1999. Boone RB, Krohn WB (1999) Modeling the occurrence of bird species: are the errors predictable? *Ecological Applications*, 9:835–848.

Boorman, J. 1986. British *Culicoides* (Diptera: Ceratopogonidae): notes on distribution and biology. *Entomological Gazette*, 37:253–266.

Boorman, J. and Goddard, P. 1970. Observations on the biology of *Culicoides impunctatus* Goetgh. (Dipt., Ceratopogonidae) in southern England. *Bulletin of Entomological Research*, 60:189–198.

Borcard, D. and Legendre, P. 2002. All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. *Ecological Modelling*, 151(1-2):51–68.

Borcard, D., Legendre, P., and Drapeu, P. 1992. Partialling out the spatial component of ecological variation. *Ecology*, 73:1045–1055.

Borkent, A. 2005. *Biology of Disease Vectors 2nd Edition*, chapter The Biting Midges, the Ceratopogonidae (Diptera), pages 113–126. Elsevier.

Braverman, Y., Galun, R., and Ziv, M. 1974. Breeding sites of some *Culicoides* species (Diptera: Ceratopogonidae) in Israel. *Mosquito News*, 34:303 – 308.

Braverman, Y. and Mumcuoglu, K. 2009. Newly emerged nulliparous *Culicoides imicola* Kieffer (Diptera: Ceratopogonidae) with pigmented abdomen. *Veterinary Parasitology*, 160:356–358.

Breard, E., Sailleau, C., Hamblin, C., and Zientara, S. 2005. Bluetongue virus in the French Island of Reunion. *Veterinary Microbiology*, 106(3-4)(3-4):157–165.

Brogdon, W. and McAllister, J. 1998. Insecticide resistance and vector control. *Emerging Infectious Diseases*, 4(4):605–613.

Brotons, L., Thuiller, W., Araújo, M., and Hirzel, A. 2004. Presence-absence versus presence-only modelling methods for predicting bird habitat suitability. *Ecography*, 27:437–448.

Brown, H., Diuk-Wasser, M., Guan, Y., Caskey, S., and Fish, D. 2008. Comparision of three satellite sensors at three spatial scales to predict larval mosquito presence in Connecticut wetlands. *Remote Sensing of Enviroment*, 112:2301–2308.

Brownstein, J., Skelly, D., Holford, T., and Fish, D. 2005. Forest fragmentation predicts local scale hetrogeneity of Lyme disease risk. *Oecologia*, 146:469–475.

Buxton, P. 1960. British Diptera associated with fungi III, Flies of all families reared from about 150 species of fungi. *Entomologists' Monthley Magazine*, 96:61–94.

Cagienard, A., Griot, C., Mellor, P., Denison, E., and Stark, K. 2006. Bluetongue vector species of *Culicoides* in Switzerland. *Medical and Veterinary Entomology*, 20(2):239–247.

Calistri, P., Goffredo, M., Caporale, V., and Meiswinkel, R. 2003. The distribution of *Culicoides imicola* in Italy: application and evaluation of current Mediterranean models based on climate. *Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health*, 50(3):132–138.

Calvete, C., Estrada, R., Miranda, M., Borrás, D., Calvo, J., and Lucientes, J. 2008. Modelling the distributions and spatial coincidence of bluetongue vectors *Culicoides imicola* and the *Culicoides obsoletus* group throughout the Iberian peninsula. *Medical and Veterinary Entomology*, 22(2):124–134.

Calvete, C., Estrada, R., Miranda, M., Borrás, D., Calvo, J., and Lucientes, J. 2009. Ecological correlates of bluetongue virus in Spain: Predicted spatial occurrence and its relationship with the observed abundance of the potential *Culicoides* spp. vector. *The Veterinary Journal*, 182(2):235–243.

Cameron, A. 1946. Insects and other pests of 1945. *Transactions of the Highland Agricultural Society of Scotland*, 57:100–120.

Cameron, A., Downes, J., Morison, G., and Peacock, A. 1946. *The Control of Midges*, chapter A survey of Scottish midges, pages 9–11. HMSO, Edinburgh.

Campano Lopez, A. and Sanchez Botija, C. 1958. L'epizootie de fievre catarrhale ovine en Espagne (bluetongue). *Bulletin de L'Office International des Épizooties*, 50:65–93.

Campbell, J. and Pelham-Clinton, E. 1960. Taxonomic review of the British species of *Culicoides* Latreille (Diptera, Ceratopogonidae). *Proceedings of the Royal Entomology Society of London (B)*, 67:181–302.

Campbell, M. and Kettle, D. 1979. Abundance and temporal and spatial distribution of *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae) on cattle in south-east Queensland. *Australian Journal of Zoology*, 27:251–260.

Capela, R., Purse, B., Pena, I., Wittman, E. J., Margarita, Y., Capela, M., Romao, L., Mellor, P. S., and Baylis, M. 2003. Spatial distribution of *Culicoides* species in Portugal in relation to the transmission of African horse sickness and bluetongue viruses. *Medical and Veterinary Entomology*, 17(2):165–177.

Capela, R., Sousa, C., Pena, I., and Caeiro, V. 1993. Preliminary note on the distribution and ecology of *Culicoides imicola* in Portugal. *Medical and Veterinary Entomology*, 7(1):23–6.

Caracappa, S., Torina, A., Guercio, A., Vitale, F., Calabro, A., Purpari, G., Ferrantelli, V., Vitale, M., and Mellor, P. S. 2003. Identification of a novel bluetongue virus vector species of *Culicoides* in Sicily. *Veterinary Record*, 153(3):71–74.

Carpenter, S. 2001. *Colonisation and Dispersal Studies of the Scottish Biting Midge, Culicoides impunctatus Goetghebuer*. PhD thesis, Department of Zoology, University of Aberdeen.

Carpenter, S., McCArthur, C., Selby, R., Ward, R., Nolan, D., Mordue Luntz, A., Dallas, J., Tripet, F., and Mellor, P. 2008a. Exterimental infection studies of UK *Culicodes* species midges with bluetongue virus serotype 8 and 9. *Veterinary Record*, 163:589–592.

Carpenter, S., Mellor, P., and Torr, S. 2008b. Control techniques for *Culicoides* biting midges and their application in the U.K. and northewestern Palaearctic. *Medical and Veterinary Entomology*, 22:175–187.

Carpenter, S., Mordue, W., and Mordue, J. 2008c. Selection of resting areas by emerging *Culicoides impunctatus* (Diptera: Ceratopogonidae) on downy birch (*Betula pubescens*). *International Journal of Pest Management*, 54(1):39–42.

Carpenter, S., Mordue (Luntz), A., and Mordue, W. 2001. Oviposition in *Culicoides impunctatus* under laboratory conditions. *Entomologia Experimentalis et Applicata*, 101:123–129.

Carpenter, S., Mordue (Luntz), A. J., and Mordue, W. 2006. Oogenesis and laboratory survival in the Scottish biting midge *Culicoides impunctatus*. *Physiological Entomology*, 31(1):89–93.

Carpenter, S., Szmaragd, C., Barber, J., Labuschange, K., Gubbins, S., and Mellor, P. 2008d. An assessment of *Culicoides* surveillance techniques in northern Europe: have we underestimated a potential bluetongue virus vector. *Journal of Applied Ecology*, 45:1237–1245.

Carpenter, S., Wilson, A., and Mellor, P. 2009. *Culicoides* and the emergence of bluetongue in northern Europe. *Trends in Microbiology*, 17(4):172–178.

Carstensen, B., Plummer, M., Laara, E., and Hills, M. 2009. *R Package 'Epi': A package for statistical analysis in epidemiology.*

Casati, S., Racloz, V., Delécolle, J., Kuhn, M., Mathis, A., Griot, C., Stark, K., and Vanzetti, T. 2010. Entomological monitoring of bluetongue vectors at sentinel sites in southern Switzerland: an investigation on the *Culicoides* population composition. *Medical and Veterinary Entomology*, 23(2):93–98.

Chavez, P. J. 1996. Image-based atmospheric corrections-revisited and revised. *Photogrammetric Engineering and Remote Sensing*, 62(9):1025–1036.

Chen, Q., Gong, P., Baldocchi, D., and Tian, Y. 2006. Estimating Basal Area and Stem Volume for Individual Trees from LIDAR data. *Photogrammetric Engineering & Remote Sensing*, 73:1355–1365.

Chevan, A. and Sutherland, M. 1991. Hierarchical partitioning. *American Statician*, 45:90–96.

Cilek, J. and Hallmon, C. 2005. The effectivness of the mosquito magnet trap for reducing biting midge (Dptera: Ceratopogonidae) populations in coastal residential backyards. *Journal of the American Mosquito Control Association*, 21(2):218–221.

Cilek, J. and Kline, D. 2002. Adult biting midge response to trap type, carbon dioxide, and an octenol-phenol mixture in northwestern Florida. *Journal of the American Mosquito Control Association*, 18(3):228–231.

Cilek, J. E., Kline, D., and Hallmon, C. 2003. Evaluation of a novel removal trap system to reduce *Culicoides* (Diptera: Ceratopogonidae) populations in Florida backyards. *Journal of Vector Ecology*, 28:23–30.

Clements, A., Pfeiffer, D., Otte, M., Morteo, K., and Chen, L. 2002. Global livestock production and health atlas (GLiPHA) for interactive presentation, integration and analysis of livestock data. *Preventative Veterinary Medicine*, 56(1):19–32.

Clements, B. and Rogers, A. 1967. Tests of larvicides for control of salt marsh sandflies (*Culicoides*). *Mosquito News*, 28:529–534.

Coetzer, J., Thomson, G., and Tustin, R. 1994. *Infectious Diseases of Livestock with Special Reference to Southern Africa*. Oxford University Press, Cape Town.

Coggon, D. 2002. Work with pesticides and organophosphate sheep dips. *Occupational Medicine*, 52(8):467–470.

Cohen, W. and Goward, S. 2004. Landsat's role in ecological applications of remote sensing. *BioScience*, 54(6):535–545.

Cohen, Y. and Shoshany, M. 2002. A national knowledge-based crop recognition in Mediterranean environment. *International Journal of Applied Earth Observation and Geoinformation*, 4(1):75–87.

Conte, A., Giovannini, A., Savini, L., Goffredo, M., Calistri, P., and Meiswinkel, R. 2003. The effect of climate on the presence of *Culicoides imicola* in Italy. *Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health*, 50(3):139–147.

Conte, A., Goffredo, M., Ippoliti, C., and Meiswinkel, R. 2007a. Influence of biotic and abiotic factors on the distribution and abundance of *Culicoides imicola* and the Obsoletus Complex in Italy. *Veterinary Parasitology*, 150(4):333–344.

Conte, A. ande Ippoliti, C., Savini, L., Goffredo, M., and Meiswinkel, R. 2007b. Novel environmental factors influencing the distribution and abundance of *Culicoides imicola* and the Obsoletus Complex in Italy. *Veterinaria Italiana*, 43(3):571–580.

Crawley, M. 2002. *Statistical computing: an introduction to data analysis using S-Plus*. Wiley, Chichester, UK.

Crist, E. 1986. The tasseled cap de-mistified. *Photogrammetric Engineering and Remote Sensing*, 52:81–86.

Crist, E. and Cicone, R. 1984. A physically-based transformation of Thematic Mapper data - the TM Tasseled Cap. *IEEE Transactions on Geosciences and Remote Sensing*, GE-22:256–263.

Crist, E., Laurin, R., and Cicone, R. 1986. Vegetation and soils information contained in transformed Thematic Mapper data. In *Proceedings of IGARSS' 86 Symposium, 1465-70. Ref. ESA SP-254. Paris: European Space Agency.*

Cross, E., Newcomb, W., and Tucker, C. 1996. Use of weather data and remote sensing to predict the geographic and seasonal distribution of *Phlebotomus papatasi* in South-West Asia. *American Journal of Tropical Medicine and Hygiene*, 54(5):530–536.

Dal Pozzo, F., De Clercq, K., Guyot, H., Vandemeulebroucke, E., Sarradin, P., Vandenbussche, F., Thiry, E., and Saegerman, C. 2009. Experimental reporduction of bluetongue virus serotype 8 clinical disease in calves. *Veterinary Microbiology*, in press.

Dale, P. 2008. Assessing impacts of habitat modification on a subtropical salt marsh: 20 years of monitoring. *Wetlands Ecology and Management*, 16(1):77–87.

Danson, F., Craig, P., Man, W., Shi, D., and Giraudoux, P. 2004. Landscape dynamics and risk modeling of human alveolar echinococcosis. *Photogrammetric Engineering and Remote Sensing*, 70(3):359–366.

Darpel, K., Batten, C., Veronesi, E., Shaw, A., Anthony, S., Bachanek-Bankowska, K., Kgosana, L., Bin-Tarif, A., Carpenter, S., Mller-Doblies, U., Takamatsu, H., Mellor, P. S., and Mertens, P. P. C. 2007. Clinical signs and pathology shown by British sheep and cattle infected with bluetongue virus serotype 8 derived from the 2006 outbreak in northern Europe. *Veterinary Record*, 161:253–261.

Davidson, G. 1954. Estimation of the survival-rate of *Anopheline* mosquitoes in nature. *Nature*, 174:792–793.

De Clercq, K., Mertens, P., De Leeuw, I., Oura, C., Houdart, P., Potgieter, A., Maan, S., Hooyberghs, J., Batten, C., Vandemeulebroucke, E., Wright, I., Maan, N., Riocreux, F., Sanders, A., Vanderstede, Y., Nomikou, K., Raemaekers, M., Bin-Tarif, A., Shaw, A., Henstock, M. Bréard, E., Dubois, E., Gastaldi-Thiéry, C., Zientara, S., Verheyden, B., and Vandenbussche, F. 2009. Emergence of Bluetongue Serotypes in Europe, Part 2: The Occurrence of a BTV-11 Strain in Belgium. *Transboundary Emerging Diseases*, 56(9-10):355–361.

De Deken, G., Madder, M., Deblauwe, I., De Clercq, K., Fassotte, C., Losson, B., Haubruge, E., and De Deken, R. 2008. Vector monitoring at Belgian outbreak sites during the bluetongue epidemic of 2006. *Preventive Veterinary Medicine*, 87(1-2):64–73.

De Liberato, C., Purse, B. V., Goffredo, M., Scholl, F., and Scaramozzino, P. 2003. Geographical and seasonal distribution of the bluetongue virus vector, *Culicoides imicola*, in central Italy. *Medical and Veterinary Entomology*, 17(4):388–394.

De Silva, H., Samarawickrema, N., and Wickremasinghe, A. 2006. Toxicity due to organophosphorus compounds: what about chronic exposure? *Trasactions of the Royal Scociety of Tropical Medicine and Hygiene*, 100(9):803–806.

deBarjac, H. 1990. *Bacterial Control of Mosquito and Blackflies*, chapter Characterisation and prospective view of *Bacillus thuringiensis israelensi*, pages 10–15. Rutgers University Press.

DEFRA 2007. Initial epidemiological report on the outbreak of Bluetongue in East Anglia and South East England from Investigations completed to 19 October 2007, publised 19th October 2007. Technical report, Department for Enviroment, Food and Rural Affairs.

DEFRA 2008a. Bluetongue in Northern France: BTV-1, Preliminary Outbreak Assessment 28th October 2008, accessed: 22/02/2009. Technical report, Food and Farming Group, International Animal Health, International Disease Monitoring, Department for Enviroment, Food and Rural Affairs.

DEFRA 2008b. Guidence for Keepers in England - Rules for identifying sheep and goats - January 2008. Technical report, Department for Enviroment, Food and Rural Affairs.

DEFRA 2008c. Report on the distribution of bluetongue infection in Great Britain on 15 March 2008, Nobel House, 17 Smith Square, London, SW1P 3JR, United Kingdom, Version 1, Released 18th April 2008. Technical report, Food and Farming Group, Veterinary Sciences Core Team, Department for Enviroment Food and Rural Affairs.

DEFRA 2009a. Bluetongue: 2008 confirmed cases, avaliable at *www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/bluetongue/latest/cases.htm*, updated: 03/11/2008, assessed: 20/12/2009. Technical report, Department of Enviroment, Food and Rural Affairs.

DEFRA 2009b. Bluetongue: Advise to farmers, avaliable at */www.defra.gov.uk/foodfarm/farmanimal/diseases/atoz/bluetongue/farmers*, updated: 4th Spetember 2009, Assessed: 22nd September 2009.

DEFRA 2009c. CTSonline - British Cattle Movement Service, Department of Enviroment, Food and Rural Affairs, avaliabe at: *https : //secure.services.defra.gov.uk/wps/portal/ctso.*

Dekker, T. and Takken, W. 1998. Differential responses of mosquito sibling species *Anopheles arabiensis* and *An. quadriannulatus* to carbon dioxide, a man or a calf. *Medical and Veterinary Entomology*, 12:136–140.

Delecolle, J. 1985. *Nouvelle contribution a letude systacuteematique et iconographique des espèces du genre Culicoides (Diptera: Ceratopogonidae) du Nord-Est de la France.* PhD thesis, These dUniversite, Universite Louis Pasteur de Strasbourg UER Sciences Vie et Terre.

Detinova, T. 1962. Age-grouping methods in Diptera of medical importance, with special reference to some vectors of malaria. Technical report, World Heath Organisation Monograph Series No. 47.

Dijkstra, F. and Wiken, T. 1976. Studies on mushroom flavours. I Organoleptic significance of constituents of the cultivated mushroom *Agaricus bisporous. Zeitschrift fr Lebensmittel-Untersuchung und -Forschung A*, 160:255–262.

Dister, S. W., Fish, D., Bros, S. M., Frank, D. H., and Wood, B. L. 1997. Landscape characterization of peridomestic risk for Lyme disease using satellite imagery. *American Journal of Tropical Medicine and Hygiene*, 57(6):687–692.

Diuk-Wasser, M., Bagayoko, M., Sogoba, N., Dolo, G., Touré, and Taylor, C. 2004. Mapping rice field *Anopheline* breeding habitats in Mali, West Africa, using Landsat ETM+ sensor data. *International Journal of Remote Sensing*, 25(2):359–376.

Djuricic, B., Nedic, D., Lausevic, D., and Pavlovic, M. 2004. The epizootiological occurrence of bluetongue in the central Balkans. *In Proceedings of the Third International Symposium on Bluetongue 26-29 October , 2003 Taormina, Sicily. Veterinaria Italiana*, 40:105–107.

Dohnal, J., Blinn, J., Wahl, G., and Schulz-key, H. 1990. Distribution of microfilariae of *Onchocerca lienalis* and *Onchocerca gutturosa* in the skin of cattle in Germany and their development in *Simulium ornatum* and *Culicoides nubeculosus* following artificial infestation. *Veterinary Parasitology*, 36(3-4):325–332.

Downes, J. 1955. The swarming flight and mating of *Culicoides nubeculosus* (Diptera: Ceratopogonidae). *Transcripts of the Royal Entomological Society London*, 106:213–236.

Dragonas, P. 1981. Evolution of bluetongue in Greece. *OIE monthly epizootic circular*, 9(417):10–11.

Du Toit, R. 1944. The transmission of bluetongue and horse sickness by *Culicoides. Onderstepoort Journal of Veterinary Research*, 19:7–16.

Dyce, A. 1969. The recognition of nulliparous and parous *Culicoides* (Diptera: Ceratopogonidae) without dissection. *Journal of the Australian Entomology Society*, 8:11–15.

Dyce, A., Standfast, H., and Kay, B. 1972. Collection and preparation of biting midges (Fam. Ceratopogonidae) and other small Diptera for virus isolation. *Journal of the Australian Entomological Society*, 11:91–96.

Dzhafarov, S. M. 1960. The fauna of bloodsucking midges (Diptera, Heleidae) of the Tugai forest in the Kura valley in Azerbaidzhan. *Zoologicheskii Zhurnal*, 39(8):1180–1184.

Dzhafarov, S. M. 1962. New species of bloodsucking midges (Diptera, Heleidae) from the valley of the Kura river. *Transcaucasus Entomologicheskoe Obozrenie*, 41:206–219. [in Russian]. Translation in: Entomological Review, 41: 121–131.

EDINA 2002. Digimap online mapping service ©Crown Copy-right, EDINA a JISC National Data Centre, avaliable at-satellite $http://edina.ac.uk/digimap/$.

Edwards, F. 1926. On the British biting midges (Diptera, Ceratopogonidae). *Transactions of the Royal Entomological Society, London*, 74(2):389–426.

Edwards, F. W. 1928. The Nematocerous diptera of Corsica. *Encyclopida Entomology (B)*, 4(3-4):157–189.

Edwards, F. W., Oldroyd, H., and Smart, J. 1939. *British blood-sucking flies*. British Museum (Natural History), London.

Edwards, P. 1980. Effect of season and sampling method on *Culicoides* (Diptera: Ceratopogonidae) and other small Diptera for virus isolation. *Journal of the Australian Entomological Society*, 19:201–209.

EEA 2004. Corine Land Cover 2000 - Mapping a decade of change. Technical report, European Enviroment Agency, Copenhagen.

EFSA 2007. Report on Epidemiological analysis of the 2006 bluetongue virus serotype 8 epidemic in north-western Europe, European Food Safety Authority, Parma, Italy.

EFSA 2010. EFSA Map of Bluetongue restriced zones as of 22 January 2010, Updated 22/01/2010, Accessed 12/02/2010, Avaliable from $www.ec.europa.eu/food/animal/diseases/controlmeasures/bt_restrictedzones-map.jpg$. Technical report, European Food Safety Authority.

Ehler, M. 2004. *Remote Sensing for Enviromental Monitoring, GIS Applications and Geology: IV (Proceedings of SPIE)*. Bellingham, WA.

Elbers, A. R. W., Backx, A., Meroc, E., Gerbier, G., Staubach, C., Hendrickx, G., van der Spek, A., and Mintiens, K. 2008a. Field observations during the bluetongue serotype 8 epidemic in 2006: I. Detection of first outbreaks and clinical signs in sheep and cattle in Belgium, France and the Netherlands. *Preventive Veterinary Medicine*, 87(1-2):21–30.

Elbers, A. R. W., Backx, A., Mintiens, K., Gerbier, G., Staubach, C., Hendrickx, G., and van der Spek, A. 2008b. Field observations during the Bluetongue serotype 8 epidemic in 2006: II. Morbidity and mortality rate, case fatality and clinical recovery in sheep and cattle in the Netherlands. *Preventive Veterinary Medicine*, 87(1-2):31–40.

Elith, J., Graham, C., Anderson, R., Dudk, M., Ferrier, S., Guisan, A., Hijmans, R., Huettmann, F., Leathwick, J., Lehmann, A., Li, J., Lohmann, L., Loiselle, B., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., McC, J., Overton, M., Peterson, A., Phillips, S., Richardson, K.R. Scachetti-Pereira, R., Schapire, R., Sobern, J., Williams, S., Wisz, M., and Zimmermann, N. 2006. Novel methods improve prediction of species distributions from occurrence data. *Ecography*, 29:129–151.

Elith, J. and Leathwick, J. R. 2009. Species distribution models: Ecological explanation and prediction across space and time. *Annual Review of Ecology and Evolution, and Systematics*, 40:677–697.

ERDAS 2008. *Leica Photogrametary Suite (LPS), version 9.3.* ©ERDAS Inc, 1991-2008, Norcross, Georgia.

ESRI 2006a. *ArcGIS Desktop ArcInfo [GIS software] Version 9.2.* ©Environmental Systems Research Institute Inc., 1992-2006, Redlands California.

ESRI 2006b. *ESRI Map Manager, Version 9.1.* ©Environmental Systems Research Institute Inc., Redlands California.

ESRI 2009. *ArcGIS Spatial Analyst.* ©Environmental Systems Research Institute Inc., 1992-2006, Redlands California.

Estrada-Pena, A. and Venzal, J. 2006. High-resolution predictive mapping for *Boophilus annulatus* and *B. microplus* (Acari: ixodidae) in Mexico and Southern Texas. *Veterinary Parasitology*, 143(3-4):350–358.

EU 2009. *Agricultural output, price indices and income, avaliable from http : //epp.eurostat.ec.europa.eu, updated on 09/12/2009, accessed on 19/01/2010.* European Commission.

EU-JRC 2009. *European Soil Database - FAO/UNESCO 1km World Reference Base (WRB) for Soil Resource, Avaliable from http : //eusoils.jrc.ec.europa.eu/$ESDB_A$rchive/ESDB/index.htm, a service supplied by the EU-JRC Land Management and Natural Hazards Unit, Euorpean Soil Data Centre.* European Commission - Joint Research Centre, Institute for Environment and Sustainability.

European Commission 1993. CORINE land cover; guide technique, Report EUR 12585EN. Technical report, Office for Official Publications of the European Communities, Luxembourg.

European Commission 2000a. COUNCIL DIRECTIVE 2000/75/EC of 20 November 2000 - laying down specific provisions for the control and eradication of bluetongue. Technical report, European Commission (EU).

European Commission 2000b. Regulation (EC) No 1760/2000 of the European Parliament and of the Council of 17 July 2000 establishing a system for the identification and registration of bovine animals and regarding the labelling of beef and beef productsand repealing Council Regulation (EC) No 820/97. Technical report, Official Journal of the European Communities, European Commission (EU).

European Commission 2003. COUNCIL REGULATION (EC) No 21/2004 of 17 December 2003 establishing a system for the identification and registration of ovine and caprine animals and amending Regulation (EC) No 1782/2003 and Directives 92/102/EEC and 64/432/EEC. Technical report, Official Journal of the European Union, European Commission (EU).

European Commission 2007. Commission Regulation (EC) No 1266/2007 (26th October 2007) - on implementing rules for Council Directive 2000/75/EC as regards the control, monitoring, surveillance and restrictions on movements of certain animals of susceptible species in relation to bluetongue. Technical report, European Union.

FAO 2005. *Global Livestock Distribution Maps, Avaliable from: The Environmental Research Group, University of Oxford, http : //ergodd.zoo.ox.ac.uk/agaagdat/index.htm.* Food and Agriculture Organization of the United Nations.

Fassotte, C., Delécolle, J. C., Cors, R., Defrance, T., De Deken, R., Haubruge, E., and Losson, B. 2008. *Culicoides* trapping with Rothamsted suction traps before and during the bluetongue epidemic of 2006 in Belgium. *Preventive Veterinary Medicine*, 87(1-2):74–83.

FAVV 2009. *Bluetongue - situatie , 16 februari 2009: reeds 8 rundveebedrijven besmet met het serotype BTV-11.* Federaal Angentschap voor de veiligheid van de voedselketen [in German].

Ferrier, S. and Guisan, A. 2006. Spatial modelling of biodiversity at the community level. *Journal of Applied Ecology*, 43:393–404.

Fichet-Calvet, E. and Rogers, D. 2009. Risk Maps of Lassa Fever in West Africa. *PLoS Neglected Tropical Diseases*, 3(3) (e388).

Flasse, S., Walker, C., Biggs, H., Stephenson, P., and Hutchinson, P. 1998. Using remote sensing to predict outbreaks of *Oestris ovis* in Namibia. *Preventive Veterinary Medicine*, 33:31–38.

Foil, L. D. and Hribar, L. J. 1995. Evaluation of Tsetse attractants as aaits for horse flies and deer flies (Diptera: Tabanidae) in Louisiana. *The Florida Entomologist*, 78(1):129–133.

Foster, N., Jones, R., and McCrory, B. 1963. Preliminary investigation on insect transmission of bluetongue virus in sheep. *American Journal of Veterinary Research*, 24:1195–1200.

Fox, J. 2009. *R Package 'car': Companion to Applied Regression Version.*

Franklin, J. 2009. *Mapping Species Distributions Spatial Inderence and Prediction.* Cambridge University Press.

Fredeen, F. 1969. *Culicoides* (Selfia) *denningi*, a unique river breeding species. *Canadian Entomologist*, 101:539–544.

Frolich, K., Hamblin, C. Jung, S., Ostrowski, S., Mwanzia, J., Streich, W., Anderson, J., Armstrong, R., and Anajariyah, S. 2005. Serologic surveillance for selected viral agents in captive and free-ranging populations of Arabian oryx (*Oryx leucoryx*) from Saudi Arabia and the United Arab Emirates. *Journal of Wildlife Diseases*, 41(1)(1):67–79.

Fuller, D., Troyo, A., and Beier, J. 2009. El Nino southern oscillation and the vegetation dynamics as predictors of dengue fever cases in Costa Rica. *Environmental Research Letters*, 4(4):140111–140118.

Fuller, R., Smith, G., Sanderson, J., Hill, R., and Thomson, A. 2002a. The UK Land Cover Map 2000: construction of a parcel-based vector map from satellite images. *The Cartographic Journal*, 39:15e25.

Fuller, R., Smith, G., Sanderson, R., Hill, A., Thonson, R., Cox, R., Brown, N., Clarke, R., Rothery, P., and Gerard, F. 2002b. *Countryside Survey 2000 Module 7 - Land Cover Map 2000 Final Report*. Centre for Ecology and Hydrology (Natural Environment Research Council).

Galli-Valerio, B. 1925. Boebachtungen *ü*ber Culiciden, nebst Bemerkungen *ü*ber Tabaniden, Simuliden und Chironomiden [in German]. *Zbl. Bakt. Abt. Orig*, 95(5):309–313.

Galuzo, I. 1975. Landscape epidemiology (epizootiology). *Advances in Veterinary Science and Comparative Medicine*, 19:73–96.

Gambles, R. 1949. Bluetongue of sheep in Cyprus. *Journal of Comparative Pathology*, 59:176–190.

Gazeau, L. and Messersmith, D. 1970. Rearing and distribution of Maryland *Culicoides* (Diptera: Ceratopogonidae). *Mosquito News*, 30(30):30–34.

Geier, M., Bosch, O., and Boeckh, J. 2000. Influence of odour plume structure on upwind flight of mosquitoes toward hosts. *Journal of Experimental Biology*, 202:1639–1648.

Gelman, A. and Rubin, D. 1992. Inference from iterative simulation using multiple sequences. *Statistical Science*, 7:457–511.

Gerry, A. and Mullens, B. 1998. Response of male *Culicoides variipennis sonorensis* (Diptera : Ceratopogonidae) to carbon dioxide and observations of mating behavior on and near cattle. *Journal of Medical Entomology*, 35(3):239–244.

Gerry, A. and Mullens, B. A. 2000. Seasonal abundance and survivorship of *Culicoides sonorensis* (Diptera: Ceratopogonidae) at a southern Californian dairy, with reference to potential bluetongue virus transmission and persistence. *Journal of Medical Entomology*, 37:675–688.

Gerry, A., Sarto I Monteys, V., Moreno Vidal, J., Francino, O., and Mullens, B. 2009. Biting rates of *Culicoides* midges (Diptera: Ceratopogonidae) on sheep in Northeastern Spain in relation to midge capture using UV light and carbon dioxide baited traps. *Journal of Medical Entomology*, 46(3):615–624.

Gibbs, E. and Greiner, E. 1994. The epidemiology of bluetongue. *Comparative Immunology, Microbiology and Infectious Diseases*, 17:207 – 220.

Gibbs, E., Lawman, M., and Herniman, K. 1979. Preliminary observations on transplacental infection of bluetongue virus in sheep - a possible overwintering mechanism. *Research in Veterinary Science*, 27(1):118–120.

Gibson, G. and Torr, S. 1999. Visual and olfactory responsed of haematophagous Diptera to host stimuli. *Medical and Veterinary Entomology*, 13:2–23.

Gillies, M. 1980. The role of carbon dioxide in host-finding by mosquitoes (Diptera: Culicidae): a review. *Bulletin of Entomological Research*, 70:220–235.

Gillies, M. and Wilkes, T. 1969. A comparision of the range of attraction of animal baits and of carbon dioxide for some West African mosquitoes. *Bulletin of Entomological Research in Veterinary Science*, 59:441–456.

Gillies, M. and Wilkes, T. 1970. The range of attraction of single baits for some West African mosquitoes. *Bulletin of Entomological Research*, 60:225–235.

Ginsberg, J. 1945. Toxicity of DDT to fish. *Journal of Ecconomic Entomology*, 38:323–326.

Gleiser, R., Schelotto, G., and Gorla, D. 2002. Spatial pattern of abundance of the mosquito *Ochlerotatus albifasciatus*, in relation to habitat characteristics. *Medical and Veterinary Entomology*, 16:364–371.

Gloster, J., Burgin, L., Witham, C., Athanassiadou, M., and Mellor, P. 2008. Bluetongue in the United Kingdom and northern Europe in 2007 and key issues for 2008. *Veterinary Record*, 162:298–302.

Gloster, J., Mellor, P., Burgin, L., Sanders, C., and Carpenter, S. 2007a. Will bluetongue come on the wind to the United Kingdom in 2007? *Veterinary Record*, 160(13):422–6.

Gloster, J., Mellor, P., Manning, A., Webster, H., and Hort, M. 2007b. Assessing the risk of windborne spread of bluetongue in the 2006 outbreak of disease in northern Europe. *Veterinary Record*, 160(2):54–56.

Glukhova, V. 1968. Systematic review of larvae of the genus *Culicoides* (Diptera: Ceratopogonidae). *Parazitologiya*, 2:559–567.

Glukhova, V. 1969. Description of larvae of midges of the genus *Culicoides* (ceratopogonidae). *Parazitologiya*, 3:461–467.

Glukhova, V. 1971. On interspecific relations, variability and specific composition of bloodsucking midges of the nubeculosus group of the genus *Culicoides* (DIptera, Ceratopogonidae). *Parazitologiya*, 5:499–511.

Glukhova, V. and Dubrovskaya, O. 1974. On the swarming flight and mating of blood sucking midges. *Parazitologiya*, 8:432–437.

Goetghebuer, M. 1919. Métamorphoses et Moeurs du *Culicoides pulicaris*, Linné [in french]. *Annales de la Socieété Entomologique de Belgique*, 59:25–30.

Goetghebuer, M. 1936. Les ceratopogonides et les chironomides de Belgique au point de vue hydrobiologique [in french]. *Bulletin de la Socieété Entomologique de Belgique*, 76(1-2):69–76.

Goffredo, M., Romeo, G., Monaco, F., Di Gennaro, A., and Savini, G. 2004. Laboratory survival and blood feeding response of wild-caught *Culicoides obsoletus* Complex (Diptera: Ceratopogonidae) through natural and artificial membranes. *Veterinaria Italiana*, 40(3):282–285.

Gomulski, L. M., Meiswinkel, R., Delécolle, J., Goffredo, M., and Gasperi, G. 2006. Phylogeny of the subgenus *Culicoides* and related species in Italy, inferred from internal transcribed spacer 2 ribosomal DNA sequences. *Medical and Veterinary Entomology*, 20:229–238.

Graham, A., Atkinson, P., and Danson, F. 2004. Spatial analysis for epidemiology. *Acta Tropica*, 91(3):219–225.

Grant, A. and Kline, D. 2003. Electrophysiological responses from *Culicoides* (Diptera: Ceratopogonidae) to stimulation with Carbon Dioxide. *Journal of Medical Entomology*, 40(3):284–293.

Grimes, J., Burroughs, J., Gouet, P., Diprose, J. M., Malby, R., Zientara, S., Mertens, P., and Stuart, D. 1998. The atomic structure of the bluetongue virus core. *Nature*, 395(6701):470–478.

Grubb, P. J. 1977. The maintenance of species-richness in plant communities: the importance of the regeneration niche. *Biological Reviews*, 52:107–145.

Gubbins, S., Carpenter, S., Baylis, M., Wood, J., and Mellor, P. 2008. Assessing the risk of bluetongue to UK livestock: uncertainty and sensitivity analyses of a temperature-dependent model for the basic reproduction number. *Joural of the Royal Society Interface*, 5:363–371.

Guerenstein, P. and Hildebrand, J. 2008. Roles and effects of environmental carbon dioxide in insect life. *Annual Review of Entomology*, 53:161–178.

Guerra, C., Gikadi, P., Tatem, A., Noor, A., Smith, D., Hay, S., and Snow, R. 2008. The limits and intensity of *Plamodium faciparum* transmission: implications for malaria control and elimination worldwide. *PLoS Medicine*, 5(2) (e38):300–310.

Guis, H., Tran, A., de La Rocque, S., Baldet, T., Gerbier, G., Barrague, B., Biteau-Coroller, F., Roger, F., Viel, J. F., and Mauny, F. 2007. Use of high spatial resolution satellite imagery to characterize landscapes at risk for bluetongue. *Veterinary Research*, 38(5):669–683.

Hall, D., Beevor, P., Cork, A., Nesbitt, B., and Vale, G. 1984. 1-octen-3-ol a potent olfactory stimulant and attractant isolated from cattle odours. *Insect Science and its Applications*, 5:335–339.

Harrell, F. 2001. *Regression Modeling Strategies with Applications to Linear Models, Logistic Regression, and Survival Analysis*. Springer, New York.

Harrell, F. J. 2009. *R Package 'rms' - Regression Modeling Stratagies, Version 2.0-0*.

Hastie, T. 1992. *Statistical Models in s*, chapter Generalized Additive Models, pages 249–308. Chapman & Hall with CRS Press USA.

Hastie, T. 2008. *R Package 'gam': Generalized Additive Models*.

Hastie, T. and Tibshirani, R. 1987. Generalized additive model: some applications. *Journal of American Statistical Applications*, 82:371–386.

Hay, S., Snow, R., and Rogers, D. 1998. From predicting mosquito habitat to malaria seasons using remotely sensed data: practice, problems and perspectives. *Parasitology Today*, 14(8):306–313.

Hemingway, J., Hawkes, N., McCarroll, L., and Ranson, H. 2004. The molecular basis of insecticide resistance in mosquitoes. *Insect Biochemistry and Molecular Biology*, 34:653–665.

Hepinstall, J., Krohn, W., and Sader, S. 2002. *Predicting species occurrences: issues of accuracy and scale*, chapter Effects of niche width on the performance and agreement of avian habitat models, pages 593–606. Island Press, Washington.

Hernandez, P., Graham, C., Master, L., and Albert, D. 2006. The effect of sample size and species characteristics on performance of different species distribution modeling methods. *Ecography*, 29:773–785.

Hill, M. 1947. The life-cycle and habitats of *Culicoides impunctatus* Goetghebuer and *Culicoides obsoletus* meigen, together with some observeations on the life-cycle of *Culicoides odibilis* Austen, *Culicoides pallidicornis* Kieffer, *Culicoides cubitalis* Edwards and *Culicoides chiopterus* Meigen. *Annuals of Tropical Medicine and Parasitology*, 41(1):55–115.

Hill, M. and Gauch, H. 1980. Detrended correspondence analysis: an improved ordination technique. *Vegetatio*, 42:47–58.

Hoar, B., Carpenter, T., Singer, R., and Gardner, I. 2004. Probability of introduction of exotic strains of bluetongue virus into the US and into California through importation of infected cattle. *Preventive Veterinary Medicine*, 66(1-4):79–91.

Hoff, G. and Hoff, D. 1976. Bluetongue and epizootic hemorrhagic disease: A review of these diseases in non domestic artiodactyles. *Journal of Zoo Animal Medicine*, 7:26–30.

Holloway, M. and Phelps, R. 1991. The responses of *Stomoxys* spp. (Diptera: Muscidae) to traps and artificial host odours in the field. *Bulletin of Entomological Research*, 81:51–55.

Holmes, P. and Boorman, J. 1987. Light and suction trap catches of *Culicoides* midges in southern England. *Medical and Veterinary Entomology*, 1(4):349–359.

Hoogendarn, K. 2007. International study on the ecconomic consequences of outbreaks of bluetongue sertotype 8 in north-western European Biophysics Journal with Biophysics Letters. *International study on the ecconomic consequences of outbreaks of bluetongue sertotype 8 in north-western European Biophysics Journal with Biophysics Letters, Van Hall Institute, Leeuwarden*.

Hothorm, T., Zeileis, A., Millo, G., and Mitchell, D. 2009. *R Package 'lmtest' - Testing Linear Regression Models*.

Hothorn, T., Bretz, and Westfall 2008. Simultaneous inference in general parametric models. *Biometrical Journal*, 50(3):346–363.

Hothorn, T. and Hornik, K. 2009. *R Package - 'exactRankTests': Exact Distributions for Rank and Permutation Tests*.

Howell, P. 1960. A preliminary antigenic classification of strains of bluetongue virus. *Onderstepoort Journal of Veterinary Research*, 28:357–363.

Howell, P. 1970. The antigenic classification and distribution of naturally occuring strains of bluetongue virus. *Journal of the South African Veterinary Medicine Association*, 41:215–223.

Hribar, L. 1990. A review of methods for recovering biting midge larvae (Diptera: Ceratopogonidae) from substrate samples. *Journal of Agricultural Entomology*, 7(1):71–77.

Huang, C., Wylie, B., Yang, L., and Homer, C. Zylstra, G. 2002a. Derivation of a tasselled cap transformation based on Landsat 7 at-satellite reflectance. *International Journal of Remote Sensing*, 23:1741–1748.

Huang, C., ylie, B., Yang, C., Homer, C., and Zylstra, G. 2002b. *Erdas Model Maker Model: Landsat 7 Tasseled Cap Transformation for Reflectance Data*. The University of Arizona, Arizona Remote Sensing Center (ARSC).

Huete, A., Didan, K., Miura, T., Rodriguez, E., Gao, X., and Ferreira, L. 2002. Overview of the radiometric and biophysical performance of the MODIS vegetation indices. *Remote Sensing of Environment*, 83(1-2):195–213.

Huete, A., Didan, K., Shimabukuro, Y., Ratana, P., Saleska, C., Hutyra, L., Yang, W., Nemani, R., and Myneni, R. 2006. Amazon rainforests green-up with sunlight in dry season. *Geophysical Research Letters*, 33:L064505, doi:10.1029/2005GL025583.

Huete, A. and Justice, C. 1999. *MODIS vegetation index (MOD13) algorithm theoretical basis document Version 3.*

Hutcheon, D. 1880. Fever or epizootic catarrh. *Royal College of Veterinary Surgons*, pages 12–15.

Hutcheon, D. 1902. Malaria catarrh fever of sheep. *Veterinary Record*, 14:629–633.

International Society for Infectious Diseases 2006. Bluetongue - Netherlands, Belgium, Germany (06): BTV-8. *ProMED-Mail*, 28th August 2006, Archive Number: 20060828.2448.

International Society for Infectious Diseases 2008a. Bluetobgue - Europe (69): Netherlands, BTV-6 Vaccine Strain Update. *ProMED-Mail, 31st october 2008, Archive Number 20081031.3431.*

International Society for Infectious Diseases 2008b. Bluetongue - Europe (64): Netherlands, BTV-6. *ProMED-Mail, 24th October 2008, Archive Number: 20081024.3364.*

International Society for Infectious Diseases 2008c. Bluetongue - Europe (66): Netherlands, BTV-6 vaccine Strain Suspected. *ProMED-Mail, 28th October 2008, Archive Number: 20081028.3410.*

International Society for Infectious Diseases 2008d. Bluetongue - Europe (73): Germany (Lower Saxony), BTV-6. *ProMED-Mail, 7th November 2008, Archive Number: 2081107.3494.*

International Society for Infectious Diseases 2009. Bluetongue - Europe (04): Belgium, BTV-11, Laboratory Report. *ProMED-Mail, 11 February 2009, Archive Number: 20090211.0622.*

Irish, R. 2000. *Landsat 7 science data user's handbook, Report 430-15-01-003-0.* National Aeronautics and Space Administration.

Jackman, S. 2008. *R Package 'pscl': Classes and Methods for R Developed in the Political Science Computational Laboratory, Stanford University.* Department of Political Science, Stanford University, Stanford, California.

Jackson, L., Hilborn, E., and Thomas, J. 2006. Towards landscape design guidlines for reducing lyme disease risk. *International Journal of Epidemiology*, 35:315–322.

Jamnback, H. 1965. The *Culicoides* of New York State (DIptera: Ceratopogonidae). *Bulletin of the New York State Museum Science Service*, 399:1–154.

Jennings, D. and Mellor, P. 1988. The vector potential of British *Culicoides* species for bluetongue virus. *Veterinary Microbiology*, 17:1 – 10.

Jia-Gang, G., Vounatsou, P., Chun-Li, C., Utzinger, J., Hong-Qing, Z., Anderegg, D., Rong, Z., Zhan-Ying, H., Dong, L., Fei, H., Ming-Gand, C., and Tanner, M. 2005. A geographic information and remote sensing based model for prediction of *Oncomelania hupensis* habitats in the Poyang Lake area, China. *Acta Tropical Animal Health and Production*, 96:213–222.

Jiang, Z., Huete, A., Didan, K., and Miura, T. 2008. Development of a two-band enhanced vegetation index without a blue band. *Remote Sensing of Environment*, 112:3833–3845.

JNCC 2003. *Handbook for Phase 1 Habitat Survey. A technique for environmental audit.* Joint Nature Conservation Committee.

Jobling, B. 1953. On the blood-sucking midge *Culicoides vexans* Staeger, including the description of its eggs and the first stage larvae. *Parasitology*, 43:148–159.

Johnson, D. and Hoeting, J. 2003. Autoregressive models for capture-recapture data: a Bayesian approach. *Biometrics*, 59:341–350.

Jones, R. 1961. Some observations on biting flies attacking sheep. *Mosquito News*, 21:113–115.

Jones, R. 1977. Control of *Culicoides variipennis* on farms and ranches - water management. *Proceedings of the West Central Mosquito and Vector Control Association*, 3:17–28.

Jones, R. 1978. In *VI Culicoides and Leptoxonops (Family Ceratopogonidae)*, pages 31–35. USDA Agricultural Handbook.

Jones, R. and Foster, N. 1971. Transovarian transmission of bluetongue virus unlikely for *Culicoides variipennis*. *Journal of the American Mosquito Control Association*, 31:434–437.

Kadmon, R., Farber, O., and Danin, A. 2003. A systematic analysis of factors affecting the performance of climatic envelope models. *Ecological Applications*, 13:853–867.

Kamanyire, R. and Karalliedde, L. 2004. Organophosphate toxicity and occupational exposure. *Occupational Medicine*, 54(2):69–75.

Kardatzke, J. and Rowley, W. 1971. Comparison of *Culicoides* larval breeding habitats and populations in Central Iowa. *Annuals of the Entomological Society of America*, 64(1):215–218.

Kay, B. 1973. Seasonal studies of a population of *Culicoides marmoratus* (Skuse) (Diptera: Ceratopogonidae) at Deception Bay, Queensland. *Journal of the Australian Entomological Society*, 12:42–58.

Kelson, R., Colwell, A., and McKluskey, D. 1980. Studies of *Culicoides occidentalis* at Borax Lake, California. *Proceedings of the Californian Mosquito Control Association*, 48:130–135.

Kettle, D. 1951. The spatial distribution of *Culicoides impunctatus* Goet. under woodland and moorland conditions and its flight range through woodland. *Bulletin of Entomological Research*, 42:239 – 291.

Kettle, D. 1957. Preliminary observations on weather conditions and the activity of biting flies. *Procedings of the Royal Entomological Society, London (A)*, 32:13–20.

Kettle, D. 1961. A study of the association between moorland vegetation and breeding sites of *Culicoides* (Diptera, Ceratopogonidae). *Bulletin of Entomological Research*, 52:381 – 411.

Kettle, D. 1962. The bionomics and control of *Culicoides* and *Leptoconops* (Diptera, Ceratopogonidae = Heleidae). *Annual Review of Entomology*, 7:401 –418.

Kettle, D. 1977. Biology and Bionomics of bloodsucking Ceratopogonids. *Annual Review of Entomology*, 22:33–51.

Kettle, D. 1984. Ceratopogonidae (biting midges). In *Medical and Veterinary Entomology*, pages 137 – 158. Croom Helm, Sydney, Sydney.

Kettle, D., Edwards, P., and Barnes, A. 1998. Factors affecting numbers of *Culicoides* in truck traps in coastal Queensland. *Medical and Veterinary Entomology*, 12:367–377.

Kettle, D. and Elson, M. 1976. The immature stages of some Australian *Culicoides* Latreille (Diptera: Ceratopogonidae). *Journal of the Australian Entomology Society*, 15:303–352.

Kettle, D. and Lawson, J. 1952. The early stages of British biting midges *Culicoides* latreille (Diptera:Ceratopogonidae) and allied genera. *Bulletin of Entomological Research*, 43:421 – 467.

Killick-Kendrick, R., Wilkes, T., Alexander, J., Bray, R., Rioux, J., and Bailly, M. 1985. The distance of attraction of CDC light traps to *phlebotomine* sand flies. *Annales de Parasitologie et Humaine*, 60(6):763–767.

Kinsman, R., Sauer, F., Jackson, H., and Wolynetz 1995. Methane and carbon dioxide emissions from dairy cows in full lactation monitored over a six-month period. *Journal of Dairy Science*, 78:2760–2766.

Kirchgessner, M., Windisch, W., Mueller, H., and Kreuzer, M. 1991. Release of methane and of carbon dioxide by dairy cattle. *Agribiological Research*, 44(2-3):91–102.

Kirkland, P. and Hawkes, R. 2004. A comparison of laboratory and 'wild' strains of bluetongue virus - is there any difference and does it matter? *Veterinaria Italiana*, 40:448–455.

Kitaoka, S. and Morii, T. 1963. Observation on the breeding habitats of some biting midges and seasonal population dynamics in the life cycle of *Culicoides furens* Poey (Diptera: Ceratopogonidae) in Kenya. *Bulletin of Entomological Research*, 66:421–425.

Kitron, U. and Kazmierczak, J. 1997. Spatial analysis of the distribution of Lyme disease in Wisconsin. *American Journal of Epidemiology*, 145:558–566.

Kitron, U., Otieno, L., Hungerford, L., Odulaja, A., Bringham, W., Okello, O., and Joselyn, M. 1996. Spatial analysis of the distribution of tsetse flies in the Lambwe Valley Kenya using Landsat TM satellite imagery and GIS. *Journal of Animal Ecology*, 65:371 – 380.

Kline, D., Allan, S., Bernier, U., and Welch, C. 2007. Evaluation of the enantiomers of 1-octen-3-ol and 1-octyn-3-ol as attractents for mosquitoes associated with freshwater swamp in Florida, USA. *Medical and Veterinary Entomology*, 21:323–331.

Kline, D. and Axtell, R. 1975. *Culicoides melleus* (COQ) (Diptera: Ceratopogonidae): seasonal abundance and emergence from sandy intertidal habitats. *Mosquito News*, 35(3):328–334.

Kline, D., Hagan, D., and Wood, J. 1994. *Culicoides* responses to 1-octen-3-ol and carbon dioxide in salt marshes near Sea Island, Georgia, USA. *Medical and Veterinary Entomology*, 8:25–30.

Kline, D., Takken, W., Wood, J., and D.A., C. 1990. Field studies on the potential of butanone, carbon dioxide, honey extract, 1-octen-3-ol, L-lactic acid and phenols as attractants for mosquitoes. *Medical and Veterinary Entomology*, 4(4):383–391.

Klopatek, J. and Gardner, R., editors 1999. *Landscape Ecological Analysis Issues and Applications*. Springer.

Konurbayev, E. 1965. Biting midges (Diptera: Heleidae) of the Issyk-kul' depression in Kirgizia. *Entomological Review*, 44:75–78.

Kremer, M. 1965. *Contrubution a l'étude du genre Culicoides Latreille, particlieèrement en France [in French]*. Paul Lechevalier, Paris.

Lacey, L. 2007. *Bacillus thuringiensis serovariety israelensis* and *Bacillus sphaericus* for mosquito control. *Journal of the American Mosquito Control Associtation*, 23 (Supplement 2):133–163.

Legendre, K. amd Legendre, L. 1998. *Numerical ecology: Developments in environmental modelling*. Elsevier Scientific, Amsterdam, The Netherlands.

Legendre, P. 1993. Spatial autocorrelation: trouble or new paradigm? *Ecology*, 74:1659–1673.

Legendre, P. and Gallagher, E. 2001. Ecologically meaningful transformations for ordination of species data. *Ocecologica*, 89:107–138.

Letham, G. 2004. GISuser Introduction to the new ETM+ SLC-off to SLC-off Gap-filled Product, 27th December 2004. Technical report, GISuser.

Lillie, T., Kline, D., and Hall, D. 1985. The dispersal of *Culicoides mississippiensis* (Diptera: Ceratopogonidae) in a salt marsh near Yankeetown, Florida. *Journal of the American Mosquito Control Association*, 1(4):463–467.

Lillie, T., Marquardt, W., and Jones, R. 1981. The flight range of *Culicoides variipennis* (Diptera: Ceratopogonidae). *Canadian Entomologist*, 113(5):419–426.

Linley, J. 1965. The ovarian cycle in *Culicoides barbosai* Wirth and Blanton and *C. furens* (Poey) (Diptera: Ceratopogonidae). *Bulletin of Entomological Research*, 57:1–17.

Linley, J. 1983. Autogeny in the Ceratoponidae. *Florida Entomologist*, 66:228–234.

Linley, J. and Braverman, Y. 1984. The tergal pigmentation patterns of *Culicoides Variipennis* and *Culicoides Furens* (Diptera: Ceratopogonidae). *Journal of Medical Entomology*, 21(6):636–647.

Linley, J. and Braverman, Y. 1986. The lateral abdominal pigmentation in *Culicoides variipennis* and *Culicoides furens* (Diptera: Ceratopogonidae): quantitative measurement of its relationship to age and oogenesis. *Journal of Medical Entomology*, 23(1):51–63.

Linthicum, K., Bailey, C., Tucker, C., Mitchell, K., Logan, T. M., Davies, F. G., Kamau, C., Thande, P., and Wagateh, J. 1990. Application of polar-orbiting, meteorological satellite data to detect flooding of Rift Valley Fever virus vector mosquito habitats in Kenya. *Medical and Veterinary Entomology*, 4:433–438.

Linzen, B. 1974. *Advances in Insect Physiology*, chapter The tryptophan ommochrome pathway in insects. Academic Press, New York.

Lloyd, A., Kline, D., Hogsette, J., and Kaufman, Allan, S. 2008. Evaluation of two commercial traps for the collection of *Culicoides* (Diptera: Ceratopogonidae). *Journal of the American Mosquito Control Association*, 24(2):253–262.

Logan, J., Seal, N., Cook, J., Stanczyk, N., Birkett, M., Clark, S., Gexan, S., Wadhams, L., Pickett, J., and Mordue (Luntz), A. 2009. Identification of human-derived volatile chemicals that interfere with attraction of the scottish biting midge and their potential use as repellents. *Journal of Medical Entomology*, 46(2):208–219.

Lopez, A. and Sanchez, C. B. 1958. l'epizootie de fievre catarrhale en espagne (bluetongue). *Bulletin Office Internationale des Épizootices*, 50:65–93.

Lukacik, G., Anand, M., Shusas, E., Howard, J., Oliver, J., Chen, H., Backenson, P., Kauffman, E., Bernard, K., Kramer, L., and White, D, J. 2006. West Nile Virus surveillance in mosquitoes in New York State, 2000-2004. *Journal of the American Mosquito Control Association*, 22(2):264–271.

Lunn, D., Thomas, A., Best, N., and Spiegelhalter, D. 2000. WinBUGS - a Bayseian modelling framework: concepts, structure and extensibility. *Statistics and Computing*, 10:325–337.

Lysyk, T. 2007. Seasonal abundance, parity, and survival of adult *Culicoides sonorensis* (Diptera: Ceratopogonidae) in southern Alberta, Canada. *Journal of Medical Entomology*, 44(6):959–969.

Ma, M. 2008. *Wind Rose 26th Nov 2007 (updated 14th May 2008), Matlab Central File Exchange file:17748, assessed: 11th October 2009*. University Federal da Bahia, Salvador, Brazil.

Maan, S., Maan, N., Nomikou, K., Anthony, S., Ross-Smith, N., Singh, K., Samuel, A., Shaw, A., and Mertens, P. 2008a. *Bluetongue*, chapter Molecular epidemiology studies of bluetongue virus, pages 134–166. Elsevier.

Maan, S., Maan, N., Ross-Smith, N., Batten, C., Shaw, A., Anthony, S., Samuel, A., Darpel, K., Veronesi, E., Oura, C., Singh, K., Nomikou, K., Potgieter, A., Attoui, H., van Rooij, E., van Rijn, P., De Clercq, K., Vandenbussche, F., Zientara, S., Breard, E., Sailleau, C., Beer, M., Hoffman, B., Mellor, P., and Mertons, P. 2008b. Sequence analysis of bluetongue virus serotype 8 from the Netherlands 2006 and comparison to other European strains. *Virology*, 377:308–318.

Mac Nally, R. 2000. Regression and model-building in conservation biology, biogeography and ecology: The distinction between - and reconciliation of - 'predictive and 'explanatory models'. *Biodiversity and Conservation*, 9:655–671.

Mac Nally, R. and Walsh, C. 2004. Hierarchical partitioning public-domain software. *Biodiversity and Conservation*, 13:659–660.

MacDonald, G. 1952. The analysis of equilibrium in malaria. *Tropical Diseases Bulletin*, 49(9):813–829.

Mackney, D., Hodgson, J., Hollis, J., and Staines, S. 1983. *Legend for the 1:250,000 Soil Map of England and Wales*. Harpenden.

MacLachlan, N. 2006. Impact of bluetongue virus infection on the international movement and trade of ruminants. *Journal of the American Veterinary Medical Association*, 228(9):1346–1349.

MacLachlan, N. and Gard, G. 2009. *Biology of Animal Infections - Bluetongue*, chapter Clinical signs and pathology, pages 285–294. Elsevier.

Mair, J. and Blackwell, A. 1998. Mating behaviour of *Culicoides nubeculosus* (Diptera: Ceratopogonidae). *Journal of Medical Entomology*, 33 (5):856–858.

Malone, J., Abdel-Rahman, M., El Bahy, M., Huh, O., Shafik, M., and Bavia, M. 1997. Geographic information systems and the distribution of *Schistosoma mansoni* in the Nile Delta. *Parasitology Today*, 13:112–119.

Mands, V., Kline, D., and Blackwell, A. 2004. *Culicoides* midge trap enhancement with animal odour baits in Scotland. *Medical and Veterinary Entomology*, 18:336–342.

Manel, S., Dias, J.-M., and Ormerod, S. 1999. Comparing discriminant analysis, neural networks and logistic regression for predicting species distributions: a case study with a Himalayan river bird. *Ecological Modelling*, 120(2-3):337–347.

Manso Ribeiro, J. and Noronha, F. 1958. Fievre catarrhale du mouton au Portugal (Blue Tongue). *Bulletin de l'Office International des Épizooties*, 50:46–64.

Manso-Ribeiro, J., Rosa-Azevedo, J., Noronha, F., Braco-Forte, M., Grave-Periera, C., and Vasco-Fenandes, M. 1957. Fievre catarrhale du mouton (blue-tongue). *Bulletin de l'Office International des Épizooties*, 48:350–367.

Markham, B. and Barker, J. 1986. Landsat MSS and TM post-calibration dynamic ranges, exoatmospheric reflectances and at-satellite temperatures. *EOSAT Landsat Technical Notes*, 1:3–8.

Masuoka, P., Claborn, D., Andre, R., Nigro, J., Gordon, S., Klein, T., and Kim, H. 2003. Use of IKONOS and Landsat for malaria control in the Republic of Korea. *Remote Sensing of Environment*, 88:187–194.

Mboera, L., Takken, W., and Sambu, E. 2000. The response of *Culex quinquefasciatus* (Diptera: Culicidae) to traps baited with carbon dioxide, 1-octen-3-ol, actone, butyric acid and human foot odour in Tanzania. *Bulletin of Entomological Research*, 90:155–159.

McCall, P. and Trees, A. 1993. Onchocerciasis in British cattle: a study of the transmission of *Onchocerca* sp. in North Wales. *Journal of Helminthology*, 67:123–135.

McFadden, D. 1979. *Behavioural travel modelling*, chapter Quantitative methods for analysing travel behaviour of individuals: some recent developements, pages 279–318. Croom Helm, London.

McGarigal, K. and Marks, B. 2002. *FRAGSTATS: Spatial Pattern Analysis Program for Categorical Maps, Computer Software Program produced by the authors at the University of Massachustts, Amherst, available from http : //www.umass.edu/landeco/research/fragstats/fragstats.html.* University of Massachusetts, Amherst.

McLachlan, N., Barratt-Boyes, S., and Brewer, A. 1992. *Bluetongue, African horse sickness and related Orbiviruses*, chapter Bluetongue virus infection of cattle, pages 725–736. CRC Press, Boca Raton.

McPherson, J. and Jetz, F. 2007. Effects of species ecology on the accuracy of distribution models. *Ecography*, 30:135–151.

Meiswinkel, R. 1989. Afrotropical *Culicoides*: a redescription of *C.* (avaritia) *imicola* Kieffer, 1913 (Diptera: Ceratopogidae) with description of the closely allied *C.* (a.) *bolitinos* sp. Nov. reared from the dung of the African buffalo, blue wildebeest and cattle in South Africa. *Onderstepoort Journal of Veterinary Research*, 56:23–39.

Meiswinkel, R. 1998. The 1996 outbreak of African horse sickness in South Africa the entomological perspective. *Archives of Virology (Supplement)*, 14:69–83.

Meiswinkel, R., Baldet, T., de Deken, R., Takken, W., Delácolle, J. C., and Mellor, P. S. 2008a. The 2006 outbreak of bluetongue in northern Europe - the entomological perspective. *Preventive Veterinary Medicine*, 87(1-2):55–63.

Meiswinkel, R., Baylis, M., and Labuschange, K. 2000. Stabling and the protection of horses from *Culicoides bolitinos* (Diptera: Ceratopogonidae), a recently identified vector of African horse sickness. *Bulletin of Entomological Research*, 90:509 – 515.

Meiswinkel, R., Goffredo, M., Dijkstra, E., van der Ven, I., Baldet, T., and Elbers, A. 2008b. Endophily in *Culicoides* associated with BTV-infected cattle in the province of Limburg, south-eastern Netherlands, 2006. *Preventative Veterinary Medicine*, 87 (1-2):182–195.

Meiswinkel, R., Goffredo, M., Leijs, P., and Conte, A. 2008c. The *Culicoides* 'snapshot': A novel approach used to assess vector densities widely and rapidly during the 2006 outbreak of bluetongue (BT) in the Netherlands. *Preventive Veterinary Medicine*, 87(1-2):98–118.

Meiswinkel, R., Gomulski, L., Delécolle, J., Goffredo, M., and Gasperi, G. 2004a. The taxonomy of *Culicoides* vector complexes - unfinished business. *Veterinaria Italiana*, 40:151–159.

Meiswinkel, R., Nevill, E. M., and Venter, G. J. 1994. *Infectious Diseases of Livestock with Special Reference to Southern Africa*, chapter Vectors: *Culicoides* spp., pages 68 –89. Oxford University Press, Cape Town.

Meiswinkel, R., Van Rijn, P., Leijs, P., and Goffredo, M. 2007. Potential new *Culicoides* vector of bluetongue in northern Europe. *Veterinary Record*, 161:564–565.

Meiswinkel, R., Venter, G. ., and Nevill, E. . 2004b. *Infectious Diseases of Livestock with special reference to South Africa , 2nd ed. , Vol. 1*, chapter Vectors. *Culicoides* spp ., page 93 136. Oxford University Press, Cape Town.

Mellor, P. 1974. Studies on *Onchocerca cervicalis* Railliet and Henry 1910: IV. Behaviour of the vector *Culicoides nubeculosus* in relation to the transmission of Onchocerca cervicalis. *Journal of Helminthology*, 48(4):283–288.

Mellor, P. 1975. Studies on *Onchocerca cervicalis* Railliet and Henry 1910: V. The development of *Onchocerca cervicalis* larvae in the vectors. *Journal of Helminthology*, 49(1):33–42.

Mellor, P. 2000. Replication of Arboviruses in insect vectors. *Journal of Comparative Pathology*, 123:231 – 247.

Mellor, P. 2002. Bluetongue and African Horse Sickness. Technical report.

Mellor, P. and Boorman, J. 1995. The transmission and geographical spread of African horse sickness and bluetongue virus. *Annals of Tropical Medicine and Parasitology*, 89:1 – 15.

Mellor, P., Boorman, J., and Baylis, M. 2000. *Culicoides* biting midges, their role as arbovirus vectors. *Annual Review of Entomolgy*, 45:307–340.

Mellor, P., Carpenter, S., Harrup, L., Baylis, M., Wilson, A., and Mertons, P. 2008. *Bluetongue*, chapter Bluetongue in Europe and the Mediterranean Basin, pages 235–264. Elsevier.

Mellor, P. and Hamblin, C. 2004. African Horse Sickness. *Veterinary Research*, 35:445–466.

Mellor, P., Jennings, D., Braverman, Y., and Boorman, J. 1981. Infection of Israeli *Culicoides* with African horse sickness bluetongue and akabane viruses. *Acta Virologica*, 25(6):401–407.

Mellor, P., Osborne, R., and Jennings, D. 1984. Isolation of bluetongue and related viruses from *Culicoides* spp. in the Sudan. *Journal of Hygiene (London)*, 93(3):621–628.

Mellor, P. and Pitzolis, G. 1979a. Observations on breeding sites and light-trap collections of *Culicoides* during an outbreak of bluetongue in Cyprus. *Bulletin of Entomological Research*, 69:229 – 234.

Mellor, P. and Pitzolis, G. 1979b. The transmission and geographical spread of African horse sickness and bluetongue viruses. *Annals of Tropical Medicine and Parasitology*, 89:1–15.

Mellor, P., Tabachnick, W., Baldet, T., Baylis, M., Bellis, G., Calistri, P., Delécolle, J., Goffredo, M., Meiswinkel, R., Mullens, B., Scaramozzino, P., Torina, A., Venter, G., and White, D. 2004. Conclusions of Working Groups. Group 2. Vectors. Proceedings of the 3rd OIE Bluetongue International Symposium, 26-29th October 2003, Taormina, Italy. *Veterinaria Italiana*, 40:715–717.

Mellor, P. and Wittmann, E. 2002. Bluetongue virus in the Mediterranean Basin 1998-2001. *Veterinary Journal*, 164(1):20–37.

Melville, L., Weir, R., Harmsen, M., Walsh, S., Hunt, N., and Daniels, P. 1995. Characteristics of naturally occuring bluetongue viral infection of cattle. In *Bluetongue disease in southeast Asia and the Pacific: proceedings of the first southeast Asia and Pacific regional bluetongue symposium, 22-24 August 1995*, volume no 66, pages 245–250, Greenlake Hotel, Kunming, P.R. China. ACIAR Proceedings.

Menzies, F., McCullough, S., McKeown, I., Forster, J., Jess, S., Batten, C., Murchie, A., Gloster, J., Fallows, J., Pelgrim, W., Mellor, P., and Oura, C. 2008. Evidence for transplacental and contact transmission of bluetongue virus in cattle. *Veterinary Record*, 163(7):203–209.

Mercer, D., Spinelli, G. R., Watts, D., and Tesh, R. 2003. Biting rates and developmental substrates for biting midges (Diptera: Ceratopogonidae) in Iquitos, Peru. *Journal of Medical Entomology*, 40(6):807–812.

Mertens, P., Attoui, H., Ducon, R., and Dermody, T. 2005. *Virus taxonomy, Eighth Report of the International Committee on Taxonomy of Viruses*. Elsevier & Academic Press.

Mertens, P. and Mellor, P. 2003. Bluetongue. *State Veterinary Journal*, 13:18–25.

Microsoft Corporation 2003. *Microsoft Office Excel 2003*.

Miller, M., Hui, S., and Tierney, W. 1991. Validation techniques for logistic regression models. *Statistics in Medicine*, 10:1213–1226.

Mintiens, K., Meroc, E., Mellor, P. S., Staubach, C., Gerbier, G., Elbers, A. R. W., Hendrickx, G., and De Clercq, K. 2008. Possible routes of introduction of bluetongue virus serotype 8 into the epicentre of the 2006 epidemic in northwestern Europe. *Preventive Veterinary Medicine*, 87(1-2):131–144.

Minton, N. and Murray, V. 1988. A review of organophosphate poisoning. *Medical Toxicology Adverse Drug Exposure*, 3(5):350–375.

Miura, T., Huete, A., and van Leeuwen, W. 1998. Vegetation detection through smoke-filled AVHRIS images: An assessment using MODIS band passes. *Journal of Geophysical Research*, 103(D24):32,001–32,011.

Moncayo, A., Edman, J., and Finn, J. 2000. Application of geographic information technology in determining risk of eastern equine encephalomyelitis virus transmission. *Journal of the American Mosquito Control Association*, 16(1):28–35.

Montgomery, D. and Peck, E. 1992. *Introduction to Linear Regression Analysis*. Wiley, New York.

Moran, P. 1950. Notes on continuous stochastic phenomena. *Biometrika*, 37:17–33.

Mulhern, F. 1985. Economic impact of bluetongue and related orbiviruses: Western Hemisphere. *Progress in Clinical Biology Research*, 178:21–25.

Mullens, B. 1991. Integrated management of *Culicoides varipennis* a problem of applied ecology. In *Bluetongu, African Horse Sickness and Related Orbiviruses, Proceedings of the Second International Symposium*, pages 896–905, Paris France. CRS Press.

Mullens, B. 1995. Flight activity and response to Carbon Dioxide of *Culicoides variipennis sonorensis* (Diptera: Ceratopogonidae) in Southern California. *Journal of Medical Entomology*, 32(3):310–315.

Mullens, B. and Gerry, A. 1998. Comparison of bait cattle and carbon dioxide-baited suction traps for collecting *Culicoides variipennis sonorensis* (Diptera : Ceratopogonidae) and *Culex quinquefasciatus* (Diptera : Culicidae). *Journal of Medical Entomology*, 35(3):245–250.

Mullens, B., Gerry, A., Lysyk, T., and Schmidtmann, E. 2004. Enviromental effects on vector competence and virogenesis of bluetongue virus in *Culicoides*: interpreting laboratory data in a field context. *Veterinaria Italiana*, 40:160–166.

Mullens, B., Owen, J., Heft, D., and Sobeck, R. 2005. *Culicoides* and other biting flies on the Palos Verdes Peninsula of Southern California, and their possible relationship to Equine Dermatitis. *Journal of the American Mosquito Control Association*, 21(1):90–95.

Mullens, B. and Rodriguez, A. 1988. Colonization and response of *Culicoides variipennis* (Diptera: Ceratopogonidae) to pollution levels in experimental dairy wastewater ponds. *Journal of Medical Entomology*, 25:441–451.

Mullens, B. A., Tabachnick, W., Holbrook, F. R., and Thompson, L. H. 1995. Effect of temperature on virogenesis of bluetongue virus serotype 11 in *Culicoides variipenis sonorensis*. *Medical and Veterinary Entomology*, 9:71–76.

Muller, M. and Murray, M. 1977. Blood-sucking flies feeding on sheep in eastern Australia. *Australian Journal of Zoology*, 25:75–85.

Murlis, J., Elkinton, J., and Card, R. 1992. Odor plumes and how insects use them. *Annual Review of Entomology*, 37:505–532.

Murlis, J., Willis, M., and Carde, R. 2000. Spatial and temporal structures of pheromone plumes in fields and forests. *Physiological Entomology*, 25:211–222.

Mushinzimana, E., Munga, S., Minakawa, N., Li, L., Feng, C., Bian, L., Kitron, U., Schmidt, C., Beck, L., Zhou, G., Githeko, A., and Yan, G. 2006. Landscape determinants and remote sensing of *Anopheline* mosquito larval habitats in the western Kenya highlands. *Malaria Journal*, 5(13):5–13.

Nagelkerke, N. J. D. 1991. A note on a general definition of the coefficient of determination. *Biometrika*, 78:691–692.

NASA 1973. The use of remote sensing in mosquito control Report No. NASA-TM-X-70293. Technical report, National Aeronatutics and Space Administration, Health Applications Office, Lyndon B. Johnson Space Centre, Texas, USA.

NASA 2003. Preliminary Assessment of the Value of Landsat 7 ETM+ Data following Scan Line Corrector Malfunction, 16^{th} July 2003. Technical report, USGS, NASA and the Landsat 7 Science Team.

NASA 2009. Landsat 7 Science Data Users Handbook, Updated: 06/06/2009, Assessed:09/12/2009.

Nelson, R. L. 1965. Carbon dioxide as an attractant for *Culicoides*. *Journal of Medical Entomology*, 2(1):56–57.

Nielsen, B. 1964. Studies on the Danish Biting Midges, *Culicoides* Latreille (Dipt., Ceratopogonidae). *Entomologiske Meddelelser*, XXXII:261–276.

Nolan, D. V., Carpenter, S., Barber, J., Mellor, P., Dallas, J., Mordue, A., and Piertney, S. 2007. Rapid diagnostic PCR assays for members of the *Culicoides obsoletus* and *Culicoides pulicaris* species complexes, implicated vectors of bluetongue virus in Europe. *Veterinary Microbiology*, 124(1-2):82–94.

Nomikou, K., Mangana-Vougiouka, O., and Panagiotataos, D. 2003. Overview of Bluetongue in Greece. Bluetongue, Part II. In *Proceedings of the Third OIE Bluetongue International Symposium*, volume 40, pages 108–115, Taormina. Veterinaria Italiana.

Nomikou, K., Mangana-Vougiouka, O., and Panagiotatos, D. 2004. Overview of bluetongue in Greece. *Veterinaria Italiana*, 40(3):108–115.

NSRI 2009. NATMAP Soilscapes: Soils Data ©Cranfield University (NSRI) and for the Controller of HMSO [2009]. Technical report, National Soil Resources Institute (NSRI), Cranfield University.

Nunamaker, R., Sieburh, P., Dean, V., Wigington, J., Nunamaker, C., and Mecham, J. 1990. Absence of transovarial transmission of bluetongue virus in *Culicoides variipennis*: immunogold labelling of bluetongue virus antigen in developing oocytes from *Culicoides variipennis* (Coquillett). *Comparative Biochemistry and Physiology Part A: Physiology*, 96:19–31.

Ogden, N., Barker, I., Beauchamp, G., Brazeau, S., Charron, D., Maarouf, A., Morshed, M., OCallaghan, C., Thompson, R., Waltner-Toews, D., Waltner-Toews, M., and Lindsay, L. 2006. Investigation of ground level and remote-sensed data for habitat classification and prediction of survival of *Ixodes scapularis* in habitats of Southeastern Canada. *Journal of Medical Entomology*, 43(2):403–414.

OIE 2006. OIE Listed Diseases, last modified: 23/01/2006, accessed: 04/03/2009.

OIE 2007. World Animal Health Information Database (WAHID) Interface.

OIE 2009a. *Summary of immediate notifications and follow-ups - 2009, accessed: 03/10/2009*. Office International des Épizooties (OIE), World Animal Health Information Database (WAHID) - Version: 1.2.

OIE 2009b. *Terrestrial Animal Health Code - Chapter 1.2. Criteria for Listing Diseases, avaliable from www.oie.int/eng/normes/mcode/en_chapitre_1.1.2.pdf , assessed: 04/10/2009,*. Office International des Épizooties (OIE).

OIE 2009c. World Animal Health Information Database (WAHID) Interface, Summary of Immediate notifications and Follow-up, Assessed: 20^{th} December 2009.

Oksanen, J., Blanchet, F., Kindt, R., Legendre, P., OHara, R., Simpson, G., Solymos, P., Henry, M., Stevens, H., and Wagner, H. 2010. *R Package 'Vegan': Community Ecology Package*.

Ordnance Survey 2009. OS MasterMap Topography Layer [GML geospatial data], updated 07/01/2009, Ordnance Survey, GB using: EDINA Digimap Ordnance Survey Service, avaliable at: *http : //edina.ac.uk/digimap*, Downloaded: June 2009. Technical report, Ordnance Survey.

Ortega, M., Mellor, P., Rawlings, P., and Pro, M. 1998. The seasonal and geographical distribution of *Culicoides imicola*, *C. pulicaris* group and *C. obsoletus* group biting midges in central and southern Spain. *Archives of Virology*, Supplement 14:85–91.

Osburn, B. I. 1994. The impact of bluetongue virus on reproduction. *Comparative Immunology Microbiology and Infectious Diseases*, 17(3-4):189–96.

Overgaard Nielsen, B. 1971. Some observations on biting midges (Diptera: Ceratopogonidae) attacking grazing cattle in Denmark. *Entomologica Scandanavia*, 2:94–98.

Oya Alpar, H., Bramwell, V., Veronesi, E., Darpel, K., Pastoret, P., and Mertens, P. 2009. *Biology of Animal Infections - Bluetongue*, chapter Bluetongue virus vaccines past and present, pages 397–428. Elsevier.

Pages, N., Muñoz-Muñoz, F., Talavera, S., Sarto, V., Lorca, C., and Nèńez, J. 2009. Identification of cryptic species of *Culicoides* (Diptera: Ceratopogonidae) in the subgenus *Culicoides* and development of species-specific PCR assays based on barcode regions. *Veterinary Parasitology*, 165(3-4):298–310.

Pajor, I. T. 1987. A collapsible, semi-automatic tent-type, emergence trap, suitable for sampling *Culicoides* (Diptera: Ceratopogonidae) from a wide range of habitats. *Onderstepoort Journal of Veterinary Research*, 54:99 –101.

Paradis, E., Claude, J., and Strimmer, K. 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20:289–290.

Parker, A. 1949. Observations on the seasonal and daily incidence of certain biting midges (*Culicoides* Latrielle - Diptera, Ceratopogidae) in Scotland. *Transcripts of the Royal Entomological Society*, 100:179 – 190.

Parker, A. 1950. Studies on the eggs of certain biting midges (*Culicoides* Latreille) occurring in Scotland. *Proceedings of the Royal Entomology Society of London*, 38:191–193.

Parsonson, I. 1990a. Pathology and pathogenesis of bluetongue infections. *Current Topics in Microbiology and Immunology*, 162:119–141.

Parsonson, I. 1990b. *Pathology and pathogenesis of BT infections*. Springer-Verlag.

Patakakis, M., Papazahariadou, M., Wilson, A., Mellor, P., Frydas, S., and Papadopoulos, O. 2009. Distribution of *Culicoides* in Greece. *Journal of Vector Ecology*, 34(2):242–251.

Pavloskiy, E. 1966. *Natural Nidality of Transmissible Diseases with Special Reference to the Landscape Ecology of Zooanthroponses*. University of Illinois Press, Urbana and London.

Pearce, J., Ferrier, S., and Scotts, D. 2001. An evaluation of the predictive performance of distributional models for flora and fauna in north-east New South Wales. *Journal of Environmental Management*, 62:171–184.

Peres-Neto, P., Legendre, P., Dray, S., and Bocrcard, D. 2006. Variance partitioning of species data matrices: estimation and comparision of fractions. *Ecology*, 7(10):2614–2625.

Pierce, A. M., Pierce, H., Borden, J., and Oehlschlager, A. 1989. Production dynamics of cucujolide pheromones and identification of 1-octen-3-ol as a new aggregation pheromone for *Oryzaephilus surinamensis* and *O . mercator*. *Enviromental Entomology*, 18:747–755.

Pili, E., Ciucce, S., Culurgioni, J., Figus, V., Pinna, G., and Marchi, A. 2006. Distribution and abundance of Bluetongue vectors in Sardinia: Comparison of field data and prediction maps. *Journal of Veterinary Medicine Series B-Infectious Diseases and Veterinary Public Health*, 53:312–316.

Pinares-Patino, C., Hour, P., Jouany, J.-P., and Martin, C. 2007. Effects of stocking rate on methane and carbon dioxide emissions from grazing cattle. *Agriculture, Ecosystems and Enviroment*, 121:30–49.

Pinto, M., Campbell-Lendrum, D., Lozovei, A., Teodoro, U., and Davies, C. 2001. *Phlebotomine* sandfly responsed to carbon dioxide and human odour in the field. *Medical and Veterinary Entomology*, 15:132–139.

Potter, H. and Akey, D. 1978. Pigmentation associated with oogenesis in the biting mide, *Culicoides variipennis* changes in abdominal tergite patterns. *Mosquito News*, 38:499–504.

Prasad, G., Malik, P., Malik, P., and Minakshi, R. 1998. Serological survey of bluetongue virus antibody in domestic and wild ruminants in and around Sariska tigar reserve, Rajasthan. *Indian Journal of Virology*, 14:51–53.

Proctor, M., Siddons, P., Jones, R., Bellamy, P., and Keay, C. 1998. *Land Information Systems: Developments for planning the sustainable use of land resources*, chapter Land Information Systems: Developments for planning the sustainable use of land resources, pages 219–234. Office for Official Publications of the European Communities, Luxembourg.

Protopopoff, N., Van Bortel, W., Marcotty, T., Van Herp, M., Maes, P., Baza, D., D'Alessandro, U., and Coosemans, M. 2007. Spatial targeted vector control in the highlands of Burundi and its impact on malaria transmission. *Malaria Journal*, 6:158.

Protopopoff, N., Van Bortel, W., Marcotty, T., Van Herp, M., Maes, P., Baza, D., D'Alessandro, U., and Coosemans, M. 2008. Spatial targeted vector control is able to reduce Malaria preverlence in the highlands of Burundi. *American Journal of Tropical Medicine and Hygiene*, 79(1):12–18.

Purse, B., Baylis, M., Tatem, A., Rogers, D., Mellor, P., Van Ham, M., Chizov-Ginzburg, A., and Braverman, Y. 2004a. Predicting the risk of bluetongue through time: climate models of temporal patterns of outbreaks in Israel. *Revue Scientifique et Technique de L'Office International Des Épizooties*, 23(3):761–75.

Purse, B., Brown, H., Harrup, L., Mertens, P., and Rogers, D. 2008. Invasion of bluetongue and other orbivirus infections into Europe: the role of biological and climatic processes. *Revue Scientifique et Technique de L'Office International Des Épizooties*, 27(2):427–442.

Purse, B., McCormick, B., Mellor, P., Baylis, M., Boorman, J., Borras, J., Burgu, I., Capela, R., Caracappa, S., Collantes, F., De Liberato, C., Delgado, J., Denison, E., Georgiev, G., El Harak, M., De La Rocque, S., Lhor, Y., Lucientes, J., Mangana, O., Miranda, M., Nedelchev, N., Nomikou, K., Ozkul, A., Patakakis, M., Pena, I., Scaramozzino, P., Torina, A., and Rogers, D. J. 2007. Incriminating bluetongue virus vectors with climate envelope models. *Journal of Applied Ecology*, 44:1231–1242.

Purse, B., Mellor, P., Rogers, D., Samuel, A., Mertens, P., and Baylis, M. 2005. Climate change and the recent emergence of bluetongue in Europe. *Nature Reviews Microbiology*, 3(2):171–81.

Purse, B., Nedelchev, N., Georgiev, G., Veleva, E., Boorman, J., Denison, E., Veronesi, E., Carpenter, S., Baylis, M., and Mellor, P. 2006. Spatial and temporal distribution of bluetongue and its *Culicoides* vectors in Bulgaria. *Medical and Veterinary Entomology*, 20(3):335–344.

Purse, B., Tatem, A., Caracappa, S., Rogers, D., Mellor, P., Baylis, M., and Torina, A. 2004b. Modelling the distributions of *Culicoides* bluetongue virus vectors in Sicily in relation to satellite-derived climate variables. *Medical and Veterinary Entomology*, 18(2):90–101.

R Development Core Team 2009. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, version 2.9.1 edition. ISBN 3-900051-07-0.

Racloz, V., Presi, P., Vounantsou, P., Schwermer, H., Casati, S., Vanzetti, T., Griot, C., and Stärk, K. 2007. Use of mapping and statistical modelling for the prediction of Bluetongue occurence in Switzerland based on vector biology. *Veterinaria Italiana*, 43(3):513–518.

Racloz, V., Straver, R., Kuhn, M., Thur, B., Vanzetti, T., Stark, K., and Griot, C. 2006. Establishment of an early warning system against bluetongue virus in Switzerland. *Schweizer Archiv fuer Tierheilkunde*, 148:593–598.

Rahman, A., Sims, D., Cordova, V., and El-Masri, B. 2005. Potential of MODIS EVI and surface temperature for directly estimating per-pixel ecosystem C fluxes. *Geophysical Research Letters*, 32:L19404. doi:10.1029/2005GL024127.

Raich, T., Jacobson, M., Holbrook, F. R., Babion, R., Blair, C., and Beaty, B. 1997. *Culicoides variipennis* (Diptera: Ceratopogonidae) host selection in Colorado. *Journal of Medical Entomology*, 34(2):247–249.

Randolph, S. 2000. *Remote Sensing and GIS in Public Health, Advances in Parasitology, vol. 47*, chapter Ticks and tick-borne disease systems in space and from space, pages 217–243. Academic Press, San Diego, US.

Rangel, T., Diniz-Filho, J., and Bini, L. 2006. Towards an integrated computational tool for spatial analysis in macroecology and biogeography. *Global Ecology and Biogeography*, 15:321–327.

Rao, D., Mani, T., Rajendran, R., Joseph, A., Gajanana, A., and Reuben, R. 1995. Development of a high level of resistance to *Bacillus sphaericus* in a field population of *Culex quinquefasciatus* from Kochi, India. *Journal of the American Mosquio Control Association*, 11:1–5.

Rawlings, P. 1997. A key based on wing patterns of biting midges (genus Culicoides-Latreille-Diptera-Ceratopogonidae) in the Iberian Peninsula, for use in epidemiological studies. *Graellsia*, 52:57–71.

Reisen, W. 2010. Landscape epidemiology of vector-borne diseases. *Annual Review of Entomology*, 55:461–483.

Reisen, W. and Lothrop, H. 1999. Effects of sampling design on the estimation of adult mosquito abundance. *Journal of the American Mosquito Control Association*, 15:104–114.

Rejmankova, E., Pope, K., Roberts, D., Lege, M., Andre, R., and Greico, J. 1998. Characterization and detection of *Anopheles vestitipennis* and *Anopheles punctimacula* (Diptera: Culicidae) larval habitats in Belize with field survey and SPOT satellite imagery. *Journal of Vector Ecology*, 23:74–88.

Rejmankova, E., Roberts, D., Pawley, A., Manguin, S., and Polanco, J. 1995. Predicitions of adult *Anopheles albimanus* densities in villages based on distances to remotely sensed larval habitats. *American Journal of Tropical Medicine and Hygiene*, 53:482–488.

Richards, J. and Jia, X. 2006. *Remote Sensing Digital Image Analysis - An Introduction, 4th Edition*, chapter Supervised Classification Techniques, pages 193–248. Springer.

Ritchie, A., Van Essen, P., Kemme, J., Kay, B., and Allaway, D. 1994. Responses of biting midges (Diptera: Ceratopogonidae) to carbon dioxide, octenol and light in southeastern Queensland, Australia. *Journal of Medical Entomology*, 31(5):645–648.

Roberts, D., Paris, J., Manguin, S., Harbach, R., Woodruff, R., Rejmankova, E., Polanco, J., Wullschleger, B., and Legters, L. J. 1996. Predictions of malaria vector distribution in Belize based on multispectral satellite data. *American Journal of Tropical Medicine and Hygiene*, 54:304–308.

Rodriguez, H. 2004. *Erdas Model Maker Model: Simultaneous Vegetation Index Computation Model (VIM)*. The University of Arizona, Arizona Remote Sensing Center (ARSC).

Rogers, D. 2000. *Remotes Sensing and Geographical Information Systems in Epidemiology, Advances in Parasitology Volume 47*, chapter Satellites, space, time and the African trypanosomiase, pages 129–171. Academic Press.

Rogers, D. and Randolph, S. 1991. Mortality rates and population density of Tsetse flies correlated with satellite imagery. *Nature (London)*, 351:739–741.

Rogers, D., Randolph, S., Snow, R., and Hay, S. 2002. Satellite imagery in the study and forecast of malaria. *Nature*, 415:710–715.

Rogers, D. J. and Randolph, S. 2003. Studying the global distribution of infectious diseases using GIS and RS. *Nature Reviews Microbiology*, 1:231 – 236.

Rogers, S. and Mather, T. 2006. Evaluating satellite sensor-derived indices for Lyme disease risk prediction. *Journal of Medical Entomology*, 43(2):337–343.

Rotela, C., Fouque, F., Lamfri, M., Sabatier, P., Introini, V., Zaidenberg, M., and Scavuzzo, C. 2007. Space-time analysis of the dengue spreading dynamics in the 2004 Tartagal outbreak, Northern Argentina. *Acta Tropica*, 103:1–13.

Rowley, W. 1967. Observations on larval habitats and the winter bionomics of some common species of *Culiocides* (Diptera: Ceratopogonidae) in the Central Colombian Basin. *Mosquito News*, 27(4):499–505.

Rowley, W. and Jorgensen, N. 1967. Relative effectiveness of three types of light traps in collecting adult *Culicoides*. *Journal of Economic Entomology*, 60(5):14 78–1479.

Ruiz-Fons, F., Reyes-Garcia, A., Alcaide, V., and Gortazar, C. 2008. Spatial and temproal evolution of bluetongue virus in wild ruminants, Spain. *Emerging Infectious Diseases*, 14:6.

Sandholzer, L. 1945. The effect of DDT upon the Chesapeake Bay Blue Crab (Callinectes sapidus. *Fish Market News*, 7:2–4.

Satta, G., Goffredo, M., Vento, L., Cubeddu, G., and Mascherpa, E. 2004. Field disinfestation trials against *Culicoides* in north-west Sardinia. *Veterinaria Italiana*, 40(3):329–335.

Savini, G., Goffredo, M., Monaco, F., de Santis, P., and Meiswinkel, R. 2003. Transmission of bluetongue virus in Italy. *Veterinary Record*, 152(4):119–119.

Savini, G., Goffredo, M., Monaco, F., Di Gennaro, A., Cafiero, M. A., Baldi, L., de Santis, P., Meiswinkel, R., and Caporale, V. 2005. Bluetongue virus isolations from midges belonging to the Obsoletus complex (*Culicoides*, Diptera: Ceratopogonidae) in Italy. *Veterinary Record*, 157(5):133–9.

Scharlemann, J., Benz, D., Hay, S., Purse, B., Tatem, A., Wint, G., and Rogers, D. 2008. Global data for ecology and epidemiology: a novel algorithm for temporal Fourier processing MODIS data. *PLoS ONE*, 3(1):e1408.

Schmidtmann, E., Holbrook, F., Day, E., Taylor, T., and Tabachnick, W. 1998. *Culicoides variipennis* (Diptera : Ceratopogonidae) complex in Virginia. *Journal of Medical Entomology*, 35(5):818–824.

Schmidtmann, E., Jones, C., and Gollands, B. 1980a. Comparative host-seeking activity of *Culicoides* (Diptera: Ceratopogonidae) attracted to pastured livestock in central New York State, USA. *Journal of Medical Entomology*, 17(3):221–231.

Schmidtmann, E., Mullens, B., Schwager, S., and Spear, S. 1983. Distribution, abundance and, a probability model for larval *Culicoides variipennis* on dairy farms in New York State. *Environmental Entomology*, 12:768.

Schmidtmann, E., Valla, M., and Abend, J. 1980b. Nocternal blood-feeding from pastured calves by the Ceratopogonid midge, *Culicoides venustus*, in New York State. *Mosquito News*, 571-577.

Schott, J. 1988. Thematic Mapper, band 6, radiometric calibration assessment. In *Proceedings of SPIE No.924, Orlando, USA*, pages 72–88.

Schwenkenbecher, J., Mordue (Luntz), A., Switek, K., and Piertney, S. 2009. Discrimination of *Culicoides* midge larvae using multiplex polymerase chain reaction assays based on DNA sequence variation at the Mitochondrial Cytochrome C Oxidase I Gene. *Journal of Medical Entomology*, 46(3):610–614.

Segurado, P. and Araujo, M. 2004. An evaluation of methods for modelling species distributions. *Journal of Biogeography*, 31:155–1568.

Sellers, R., Pedgley, D., and Tucker, M. R. 1979. Possible windborne spread of bluetongue to Portugal, June-July 1956. *Journal of Hygiene, Cambridge*, 81:189–196.

Sellers, R. F. and Mellor, P. 1993. Temperature and persistence of viruses in Culicoides during adverse conditions. *OIE Scientific Technical Review*, 12:733 – 755.

Sellers, R. F. and Taylor, W. P. 1980. Epidemiology of bluetongue and the import and export of livestock, semen and embryos. *Bulletin of Entomological Research*, 92:587–592.

Service, M. 1968. Blood digestion and oviposition in *Culicoides impunctatus* Goetghebuer and *C. obsoletus* (Meigen) (Diptera, Ceratopogonidae). *Annuals of Tropical Medicine and Parasitology*, 62(3):325–330.

Service, M. 1969. Studies on the biting habits of *Culicoides impunctatus* Goetghebuer, *C. obsoletus* (Meigen) and *C. punctatus* (Meigen) (Diptera: Ceratopogonidae) in sourthern England. *Proceedings of the Royal Entomological Society, London (A)*, 44:1–10.

Service, M. 1971. Adult flight activities of some British *Culicoides species*. *Medical Entomology*, 8(5):605–609.

Service, M. 1974. Further results of catches of *Culicoides* (Diptera: Ceratopgonidae) and Mosquitoes from suction traps. *Journal of Medical Entomology*, 11(4):471–479.

Shapiro, S. S. and Wilk, M. B. 1965. An analysis of variance test for normality (complete samples). *Biometrika*, 52(3-4):591–611.

Sharma, V. and Srivastava, A. 1997. Role of geographic information system in malaria control. *Indian Journal of Medical Research*, 106:198–204.

Silver, J. 2007. *Mosquito Ecology: Field Sampling Methods*. Springer.

Sims, D. A., Rahman, A. F., Cordova, V. D., El-Masri, B. Z., Baldocchi, D. D., Flanagan, L. B., Goldstein, A. H., Hollinger, D., Misson, L., Monson, R., Oechel, W., Schmid, H., Wofsy, S., and Xu, L. 2006. On the use of MODIS EVI to assess gross primary productivity of North American ecosystems. *Journal of Geophysical Research*, 111:G04015, doi:10.1029/2006JG000162.

Sithiprasasna, R., Ja Lee, W., Usgsang, D., and Linthicum, K. 2005. Identification and characterization of larval and adult *Anopheline* mosquito habitats in the Republic of Korea: potential use of remotely sensed data to estimate mosquito distributions. *International Journal of Health Geographics*, 4(17):4–17.

Smith, B. 2007. boa: An R package for MCMC output convergence assesemnt and posterior inference. *Journal of Statistical Software*, 21(11):1–37.

Smith, C. and Davies, A. 1959. Insecticide resistance in the salt marsh sandfly, *Culicoides furens*. *Journal of Ecconomic Entomology*, 52:352–353.

Sollai, G., Solari, P., Masala, C., Crnhar, R., and Liscia, A. 2007. Effects of avermectins on olfactory responses of *Culicoides imicola* (Diptera: Ceratopogonidae). *Journal of Medical Entomology*, 44(4):656–659.

Somers, R. 1962. A new asymmetric measure of association for ordinal variables. *American Sociological Review*, 27:799–811.

Southwood, T. and Henderson, P. 2000. *Ecological Methods*. Wiley Blackwell.

Spreull, J. 1905. Malarial catarrhal fever (bluetongue) of sheep in South Africa. *Journal of Comparative Pathology Therapy*, 18:321–337.

Steward, J. 1933. *Onchocerca cervicalis* (Railliet and Henry, 1910) and its development in *Culicoides nubeculosus* Meigen. Technical report, 3rd Report of the Cambridge University Institute of Animal Pathology.

Stockwell, D. and Peterson, A. 2002. Effects of sample size on accuracy of species distribution models. *Ecological Modelling113.*, 148:1–13.

Takken, W. and Kline, D. 1989. Carbon dioxide and 1-octen-3-ol as mosquito attractants. *Journal of the American Mosquito Control Association*, 5(3):311–316.

Takken, W. and Knols, B. G. J. 1999. Odor-mediated behavior of Afrotropical malaria mosquitoes. *Annual Review of Entomology*, 44:131–157.

Takken, W., Verhulst, N., Ernst-Jan, S., Jacobs, F., Jongema, Y., and van Lammeren, R. 2008. The phenology and population dynamics of *Culicoides* spp. in different ecosystems in The Netherlands. *Preventive Veterinary Medicine*, 87(1-2):41–54.

Tatem, A., Baylis, M., Mellor, P., Purse, B., Capela, R., Pena, I., and Rogers, D. 2003. Prediction of bluetongue vector distribution in Europe and north Africa using satellite imagery. *Veterinary Microbiology*, 97(1-2):13–29.

Taylor, L. 1962. The absolute efficiency of insect suction traps. *Annals of Appled Biology*, 50(3):405 – 421.

Taylor, W., Sellers, R., Gumm, I., Herniman, K., and Owen, L. 1985. Bluetongue epidemiology in the Middle East. In *Bluetongue and Related Orbiviruses*, pages 527–30. Alan R. Liss Inc. New York.

The MathWorks 2006. *Matlab (RS14 SP3).* Natick, Massachusetts, USA.

Thompson, R., Begtrup, K., Cuamba, N., Dgedge, M., Mendis, C., Gamage-Mendis, A., Enosse, S., Barreto, J., Sinden, R., and Hogh, B. 1997. The matola malaria project: a temporal and spatial study of malaria transmission and disease in a suburban area of Maputo Mozambique. *American Journal of Tropical Medicine and Hygiene*, 57:550 – 559.

Thomson, M., Connor, S., Milligan, P., and Flasse, S. 1996. The ecology of malaria - as seen from earth observation satellites. *Annals of Tropical Medicine and Parasitology*, 90:243–264.

Thomson, M., Elnaiem, D., Ashford, R., and Connor, S. 1999. Towards a kala-azar risk map for Sudan: mapping the potential distribution of *Phlebotomus orientalis* using digital data of environmental variables. *Tropical Medicine and International Health*, 4:105–113.

Thuiller, W., Lavorel, S., Midgley, G., Lavergne, S., and Rebelo, T. 2004. Relating plant traits and species distributions along bioclimatic gradients for 88 Leucadendron taxa. *Ecology*, 85:1688–1699.

Torina, A., Caracappa, S., Mellor, P., Baylis, M., and Purse, B. V. 2004. Spatial distribution of bluetongue virus and its *Culicoides* vectors in Sicily. *Medical and Veterinary Entomology*, 18(2):81–89.

Torr, S. 1990. Dose-responses of tsetse flies (Glossina) to carbon dioxide, acetone and octenol in the field. *Physiological Entomology*, 15:93–103.

Tschuor, A., Kaufmann, C., Schaffner, F., and Mathis, A. 2009. Occurrence of biting midges (*Culicoides* spp.) at three different altitudes in an alpine region of Switzerland. *Schweizer Archiv fuer Tierheilkunde*, 151(5):215–221.

Tucker, J., Pinzon, E., Brown, E., Slayback, A., Pak, W., Mahoney, R., E.F., V., and El Saleous, N. 2005. An extended AVHRR 8-km NDVI dataset compatible with MODIS and SPOT vegetation NDVI data. *International Journal of Remote Sensing*, 26:4485–4498.

Turner, E. 1972. An animal baited trap for the collection of *Culicodies* Spp. (Diptera: Ceratopogonidae). *Mosquito News*, 32(4):527–334.

Tyndale-Biscoe, M. 1984. Age-grading methods in adult insects: a review. *Bulletin of Entomological Research*, 74:341–377.

Unkles, S., Marriott, C., Kinghorn, J., Panter, C., and Blackwell, A. 2004. Efficacy of the entomopathogenic fungus, *Culicinomyces clavisporus* against larvae of the biting midges, *Culicoides nubeculosus* (Diptera: Ceratopogonidae). *Biocontrol and Technology*, 14:397–401.

USGS 2007. *USGS Global 30 Arc-Second Elevation (GTOPO30) (avaliable from http://eros.usgs.gov/products/elevation/gtopo30.php)*. USGS Earth Resources Observation and Science (EROS) center.

USGS 2009. *USGS Global Visualization Viewer: Online Service, available at http : //glovis.usgs.gov/*. United States Geological Survey (USGS), Earth Resources Observation and Science Center (EROS) supplied service.

Uslu, U. and Dik, B. 2006. Vertical distribution of *Culicoides* larvae and pupae. *Medical and Veterinary Entomology*, 20(3):350–352.

Uslu, U. and Dik, B. 2007. Description of breeding sites of *Culicoides* species (Diptera: ceratopogonidae) in Turkey. *Parasite*, 14:173–177.

Uslu, U. and Dik, B. 2010. Chemical characteristics of breeding sites of *Culicoides* species (Diptera: Ceratopogonidae). *Veterinary Parasitology, in press.*

Vale, G. and Hall, D. 1985. The role of 1-octen-3-ol, acetone and carbon dioxide in the attraction of tsetse flies, *Glossina* spp. (Diptera: Glossinidae), to ox odour. *Bulletin of Entomological Research*, 75:209–217.

Van Den Bosch, R. and Stern, V. 1962. The integration of chemical and biological control of arthropod pests. *Annual Review of Entomology*, 7:367–386.

Van den Hurk, A. F., Montgomery, B. L., Zborowski, B. L., Beebe, N. W., Cooper, R. D., and Ritchie, S. A. 2006. Does 1-octen-3-ol enhance trap collections of Japanese encephalitis virus mosquito vectors in Northern Australia? *Journal of the American Mosquito Control Association*, 22(1):15–21.

Van Essen, P., Kemme, J., Ritchie, S., and Kay, B. 1994. Differential responses of *Aedes* and *Culex* mosquitoes to octenol or light in combination with carbon dioxide in Queensland, Australia. *Medical and Veterinary Entomology*, 8:63–67.

Vassalos, M. 1980. Cas de fievre catarrhale du mouton dan i' ile de Lesbos (Greece). *Bulletin de l'Office International des Épizooties*, 92:547–555.

Venter, G. and Hermanides, K. 2006. Comparision of black and white light for collecting *Culicoides imicola* and other livestock associated *Culicoides* species in South Africa. *Veterinary Parasitology*, 142(3-4):383–385.

Venter, G., Hermanides, K., Boikanyo, S., Majatladi, D., and Morey, L. 2009a. The effect of light trap height on the numbers of *Culicoides* midges collected under field conditions in South Africa. *Veterinary Parsitology*, 166 (3-4):343–345.

Venter, G., Labuschagne, K., Hermanides, K., Boikanyo, S., Majatladi, D., and Morey, L. 2009b. Comparison of the efficiency of five suction light traps under field conditions in South Africa for the collection of *Culicoides* species. *Veterinary Parasitology*, 166 (3-4):299–307.

Veronesi, E., Mertons, P., Shaw, A.E. amd Brownlie, J., Mellor, P., and Carpenter, S. 2008. Quantifying bluetongue virus in adult *Culicoides* biting mideges (Diptera: Ceratopogonidae). *Journal of Medical Entomology*, 45(1):129–132.

Verwoerd, D. and Erasmus, B. 2004. *Infectious Diseases of Livestock*, chapter Bluetongue, page 1201 1230. Cape Town, Oxford University Press.

Vitale, G., Wirth, W., and Aitken, T. 1981. New species and records of *Culicoides* reared from arboreal habitats in panama, with a synopsos of the Debilipalpis group (Diptera: Ceratopogonidae). *Proceedings of the Entomological Society of Washington*, 83(1):140–159.

Vuong, Q. H. 1989. Likelihood ratio tests for model selection and non-nested hypotheses. *Econometrica*, 57(2):307–333.

Walker, A. 1977. Seasonal fluctuations of *Culicoides* species (Diptera: Ceratopogonidae). *Bulletin of Entomological Research*, 67:217 – 233.

Walker, A. and Boreham, P. 1976. Blood feeding of *Culiocides* (Diptera, Ceratopogonidae) in Kenya in relation to the epidemiology of bluetongue and ephemeral fever. *Bulletin of Entomological Research*, 66:181–188.

Ward, M. P. 1996. Seasonality of infection of cattle with bluetongue viruses. *Preventive Veterinary Medicine*, 26(2):133–141.

Ward, S. and Brown, R. 2004. A framework for incorporating the prevention of Lyme disease transmission into the landscape planning and design process. *Landscape and Urban Planning*, 66:91–106.

Waring, R. H., Milner, K. S., Jolly, W. M., Phillips, L., and McWethy, D. 2006. Assessment of site index and forest growth capacity across the Pacific and Inland Northwest U.S.A. with a MODIS satellite-derived vegetation index. *Forest Ecology and Management*, 228:285–291.

Weisberg, S. 1980. *Applied Linear Regression*. Wiley, New York.

White, J. R., Wilson, W., Blair, C., and Beaty, B. 2005. Studies on overwintering of bluetongue viruses in insects. *Journal of General Virology*, 86:453–462.

Whitten, J. 1960. The tracheal pattern in selected diptera Nematocera. *Journal of Morphology*, 107(3):233–257.

Wilson, A., Darpel, K., and Mellor, P. 2008a. Where does bluetongue virus sleep in winter? *PLoS Biology*, 6(8) e210:1612–1617.

Wilson, A. D., Heesom, K. J., Mawby, W. J., Mellor, P. S., and Russell, C. L. 2008b. Identification of abundant proteins and potential allergens in *Culicoides nubeculosus* salivary glands. *Veterinary Immunology and Immunopathology*, 122(1-2):94–103.

Wittmann, E. 1999. *Temperature and the transmission of arboviruses by Culicoides*. PhD thesis, University of Bristol.

Wittmann, E. J. and Baylis, M. 2000. Climate change: effects on *Culicoides*-transmitted viruses and implications for the UK. *Veterinary Journal*, 160(2):107 – 117.

Wittmann, E. J., Mellor, P. S., and Baylis, M. 2001. Using climate data to map the potential distribution of *Culicoides imicola* (Diptera: Ceratopogonidae) in Europe. *Revue Scientifique et Technique de L'Office International Des Épizooties*, 20(3):731–740.

Wittmann, E. J., Mellor, P. S., and Baylis, M. 2002. Effect of temperature on the transmission of orbiviruses by the biting midge, *Culicoides sonorensis*. *Medical and Veterinary Entomology*, 16(2):147–156.

Wood, B. L., Beck, L., Hibbard, K., and Salute, J. 1992. Estimating high mosquito-producing rice fields using spectral and spatial data. *International Journal of Remote Sensing*, 13:2813–2826.

Wood, B. L., Beck, L., Washino, R., Palchick, S., and Sebesta, P. 1991a. Spectral and spatial characterisation of rice field mosquito habitat. *International Journal of Remote Sensing*, 12:621–626.

Wood, B. L., Washino, R. K., and Beck, L. 1991b. Distinguishing high and low anopeline-producing rice fields using remote sensing and GIS technologies. *Preventative Veterinary Medicine*, 11:277–288.

Worwa, G., Hilbe, M., Ehrensperger, F., Chaignat, V., Hofmann, M., Griot, C., Maclachlan, N., and Thuer, B. 2009. Experimental transplacental infection of sheep with bluetongue virus serotype 8. *Veterinary Record*, 164(16):499–500.

WWF 2009. Terrestrial Ecoregions of the World Database, World Wildlife Fund, avaliable at *http : //www.worldwildlife.org/science/data/item*1872.*html*, accessed: 05/01/2010.

Xiao, X., Braswell, B., Zhang, Q., Boles, S., Frolking, S., and Moore, B. 2003. Sensitivity of vegetation indices to atmospheric aerosols: continental-scale observations in northern Asia. *Remote Sensing of Environment*, 84:385–392.

Xiao, X., Hollinger, D., Aber, J., Goltz, M., Davidson, E. A., and Zhang, Q. 2004. Satellite-based modeling of gross primary production in an evergreen needleleaf forest. *Remote Sensing of Environment*, 89:519–534.

Zeileis, A. and Hothorn, T. 2002. Diagnostic checking in regression relationships. *R News*, 2(3):7–10.

Zeileis, A., Kleiber, C., and Jackman, S. 2008. Regression models for count data in R. *Journal of Statistical Software*, 27(8).

Zhengming, W. 1999. MODIS Land-Surface Temperature Algorithm Theoretical Basis Document (LST ATBD), Version 3.3, Contract Number: NAS5-31370. Technical report, Institute for Computational Earth System Science, University of California, Santa Barbara.

Zhi-Ying, Z., Xu, D.-., Zhou, X., Zhouand, Y., and Liu, S. 2005. Remote sensing and spatial statistical analysis to predict the distribution of *Oncomelania hupensis* in the marshlands of China. *Acta Tropica*, 96(2-3):205–212.

Zimmer, J., Haubruge, E., Francis, F., Bortels, J., Simonon, G., Losson, B., Mignon, B., Paternostre, J., De Deken, R., De Deken, G., Deblauwe, I., Fassotte, C., Cors, R., and Defrance, T. 2008. Letter: Breeding sites of bluetongue vectors in northern Europe. *Veterinary Record*, 162(4):131.

Zimmerman, R. and Turner, E. J. 1983. Seasonal abundance and parity of common *Culicoides* collected in blacklight traps in Virginia pastures. *Mosquito News*, 43(1):63–69.

Zollner, G., Torr, S., Ammann, C., and Meixner, F. 2004. Dispersion of carbon dioxide plumes in African woodland: implications for host-finding by Tsetse flies. *Physicological Entomology*, 29:381–394.

Zou, L., Miller, S., and Schmidtmann, E. 2006. Mosquito larval habitat mapping using remote sensing and GIS: implications of coalbed methane development and West Nile virus. *Journal of Medical Entomology*, 43(5):1034–1041.

Appendix A

Supplementary Information for Chapter 3

A.1 BTV Outbreak Occurrence in Greece and Bulgaria - Landscape Only Models

Variable	$\mathbf{LANDSCAPE}_{1\,km}$	$\mathbf{LANDSCAPE}_{2\,km}$	$\mathbf{LANDSCAPE}_{5\,km}$
PLAND.12	0.68(0.57;0.84)	0.44(0.31;0.63)	1.87(1.18;2.95)
PLAND.25	-	0.86(0.74;1.00)	-
PD.12	0.74(0.60;0.91)	0.41(0.23;0.74)	-
ED.12	-	2.05(1.00;4.20)	0.29(0.16;0.53)
ED.18	0.20(0.06;0.73)	0.95(0.90;1.00)	-
ED.25	0.94(0.88;1.00)	-	-
COHESION.18	-	-	
Low Values [0.00 - 5.9]			2.88(2.27;3.48)
Medium Values [5.9 - 84.8]			1.56(0.81;2.30)
High Values [84.8 - 95.8]			1.58(0.88;2.28)
COHESION.23	-	-	
Low Values [0.0 - 90.2]			2.88(2.27;3.48)
Medium Values [90.2 - 96.3]			1.93(1.39;2.46)
High Values [96.3 - 100.0]			2.57(2.00;3.13)
COHESION.25	0.79(0.59;1.07)	0.41(0.23;0.73)	-

- Indicates variable not included in final landscape model

Table A.1. Variables selected in the final landscape models, selected by stepwise selection of variables, for the prediction of BTV outbreak occurrence in Greece and Bulgaria with Odds Ratios (OR) and their 95 % confidence intervals (OR <1 negative effect OR >1 positive effect). Land cover classes: 12 - non-irrigated arable land; 18 - pastures; 23 - broad-leaved forest; 25 - mixed forest

A.2 BTV Vector Abundance in Greece and Bulgaria - Landscape Only Models

Variable Suite	Selected Variables	AIC	D^2
Host	log(CATTLE+1)	769.01	3.02
Landscape 1 km	s(LSI, 4) + PD.23 + PLAND.25	745.04	19.29
Landscape 2 km	s(LSI, 4) + PLAND.23 + PLAND.25,	738.85	21.94
Landscape 5 km	s(LSI, 4) + MEAN.GYRATE + PLAND.12	729.33	28.23
	+ COHESION.12 + PLAND.23 + PLAND.25		
Climate	dLST.A1 + nLST.A1 + nLST.A2	730.62	30.03
	+ s(MIR.MEAN,4) + s(MIR.A2, 4) + dLST.P1		
Terrain/Soil	SLOPE + log(ALTITUDE+1)	739.00	18.43

Null model AIC = 772.68

Table A.2. Goodness of fit statistics and Akaike's information criterion (AIC) for the final set of variables selected by forwards stepwise selection of variables within each variable suite for *C. obsoletus* s.l. [Fitted smooth terms indicated as *s(name of the predictor, numbers of degrees of freedom)*]. Land cover classes: 12 - non-irrigated arable land; 20 - complex cultivation patterns; 23 - broad-leaved forest; 25 - mixed forest

Variable Suite	Selected Variables	AIC	D^2
Host	log(SHEEP+1)	725.03	3.27
Landscape 1 km	PD.20+PLAND.18	727.24	3.17
Landscape 2 km	PLAND.26 + COHESION.20 + PLAND.18	726.64	4.52
Landscape 5 km	COHESION.20	727.77	1.83
Climate	s(EVI.A3, 4) + MIR.A1	723.20	8.28
Terrain/Soil	SOIL.TEXTURE	724.86	6.45

Null model AIC = 729.19

Table A.3. Goodness of fit statistics and Akaike's information criterion (AIC) for the final set of variables selected by forwards stepwise selection of variables within each variable suite for *C. pulicaris* s.l. [Fitted smooth terms indicated as *s(name of the predictor, numbers of degrees of freedom)*]. Land cover classes: 18 - pastures; 20 - complex cultivation patterns; 23 - broad-leaved forest; 26 - natural grasslands

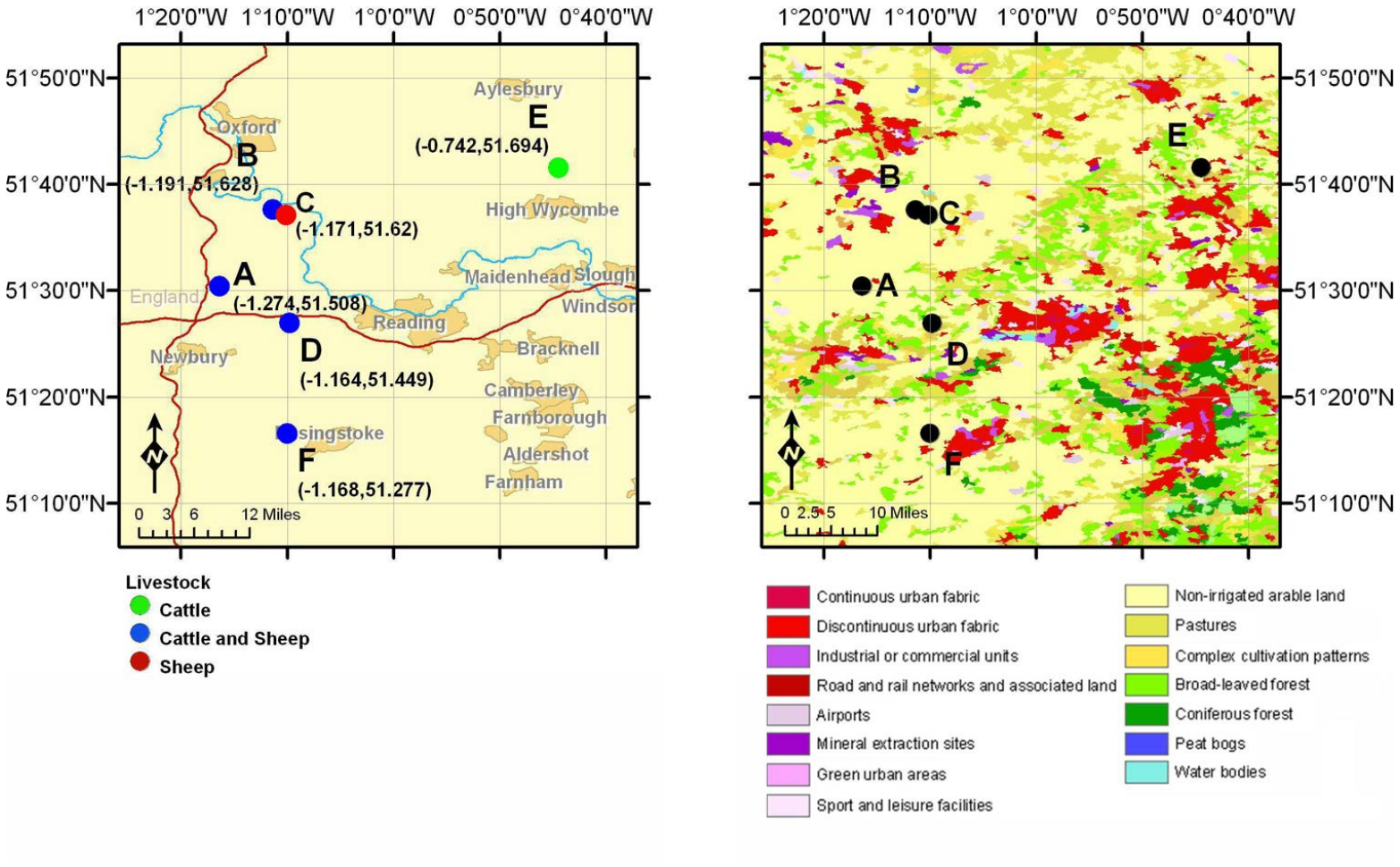
Variable Suite	Selected Variables	AIC	D^2
Host	s(SHEEP,4)	483.64	10.92
Landscape 1 km	s(LSI,4)+s(PLAND.12,4)+s(PD.21)+PLAND.23	464.04	27.30
Landscape 2 km	LSI+MEAN.GYRATE+PLAND.12+COHESION.12	481.90	11.76
Landscape 5 km	MEAN.GYRATE+PLAND.12+PLAND.23	484.02	9.76
Climate	s(EVI.A2, 4) + s(MIR.MEAN, 4) + s(MIR.A2 4) + MIR.A3	459.20	29.18
Terrain/Soil	TEXTURE+SLOPE+log(ALTITUDE+1)	475.16	16.73

Null model AIC = 497.03

Table A.4. Goodness of fit statistics and Akaike's information criterion (AIC) for the final set of variables selected by forwards stepwise selection of variables within each variable suite for *C. imicola*. [Fitted smooth terms indicated as *s(name of the predictor, numbers of degrees of freedom)*]. Land cover classes: 12 - non-irrigated arable land; 20 - complex cultivation patterns; 21 - land principally occupied by agriculture, with significant areas of natural vegetation; 23 - broad-leaved forest

Appendix B

Sampling Point Distribution Maps



(a) (b)

Figure B.1. Location of study area and farms [latitude and longitude shown in parentheses in (a), CORINE land cover classification (CLC2000) (EEA, 2004) for area shown in (b)]

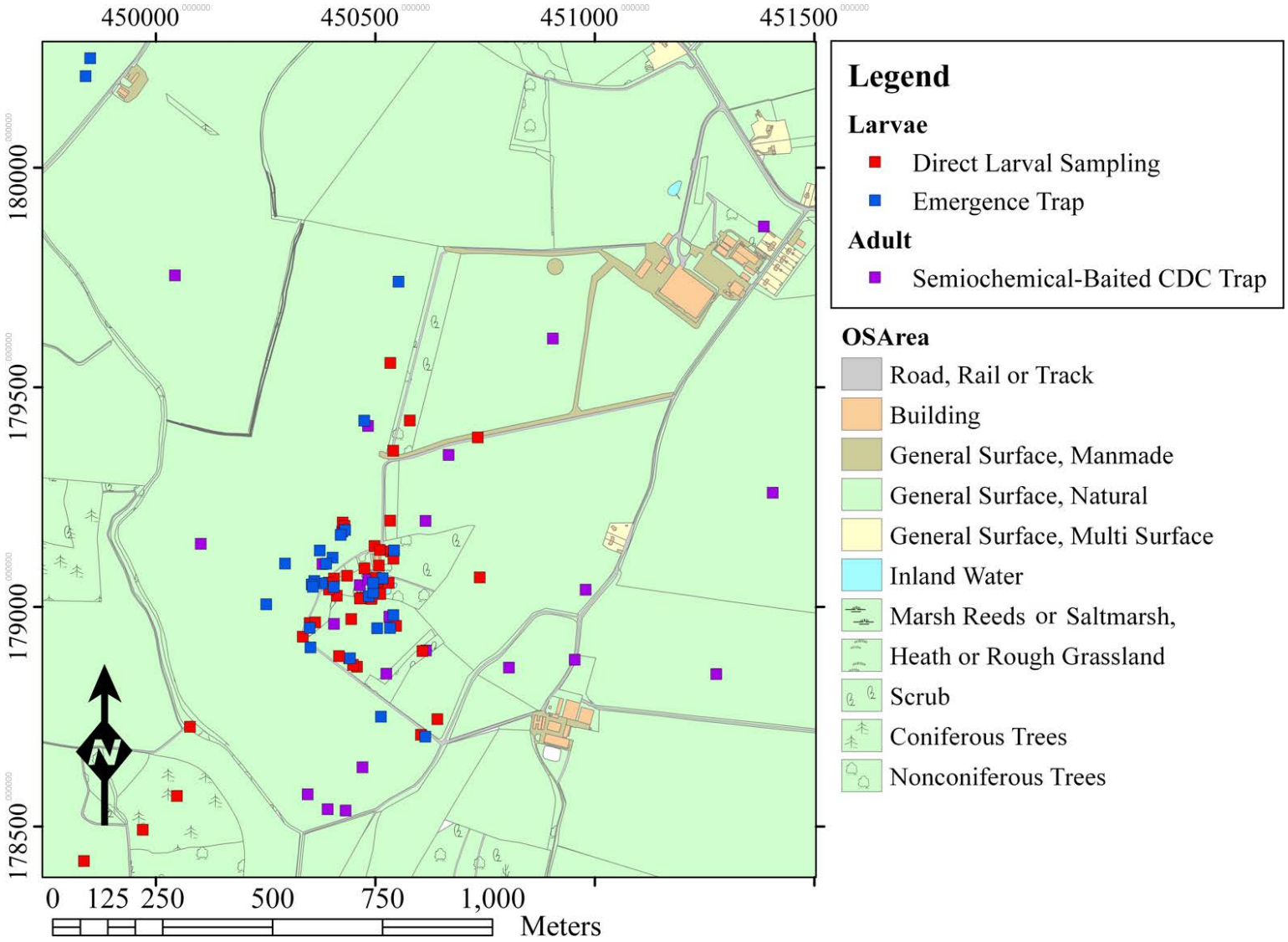


Figure B.2. Spatial distribution of sampling points used for surveillance of adult and immature *Culicoides* at farm A [OS Mastermap basemap (Ordnance Survey, 2009) and British National Grid graticles]

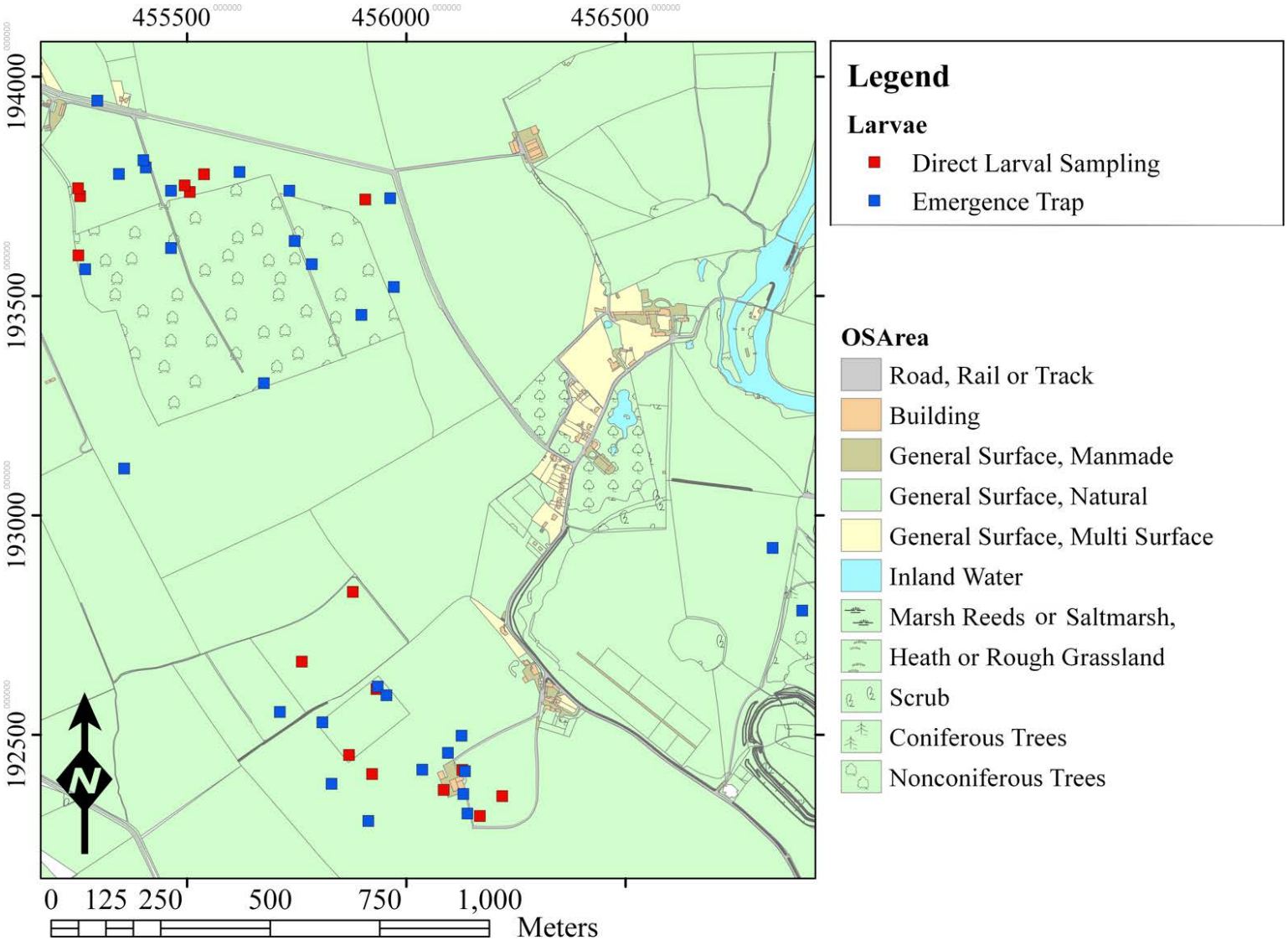


Figure B.3. Spatial distribution of sampling points used for surveillance of immature *Culicoides* at farm B [OS Mastermap basemap (Ordnance Survey, 2009) and British National Grid graticles]

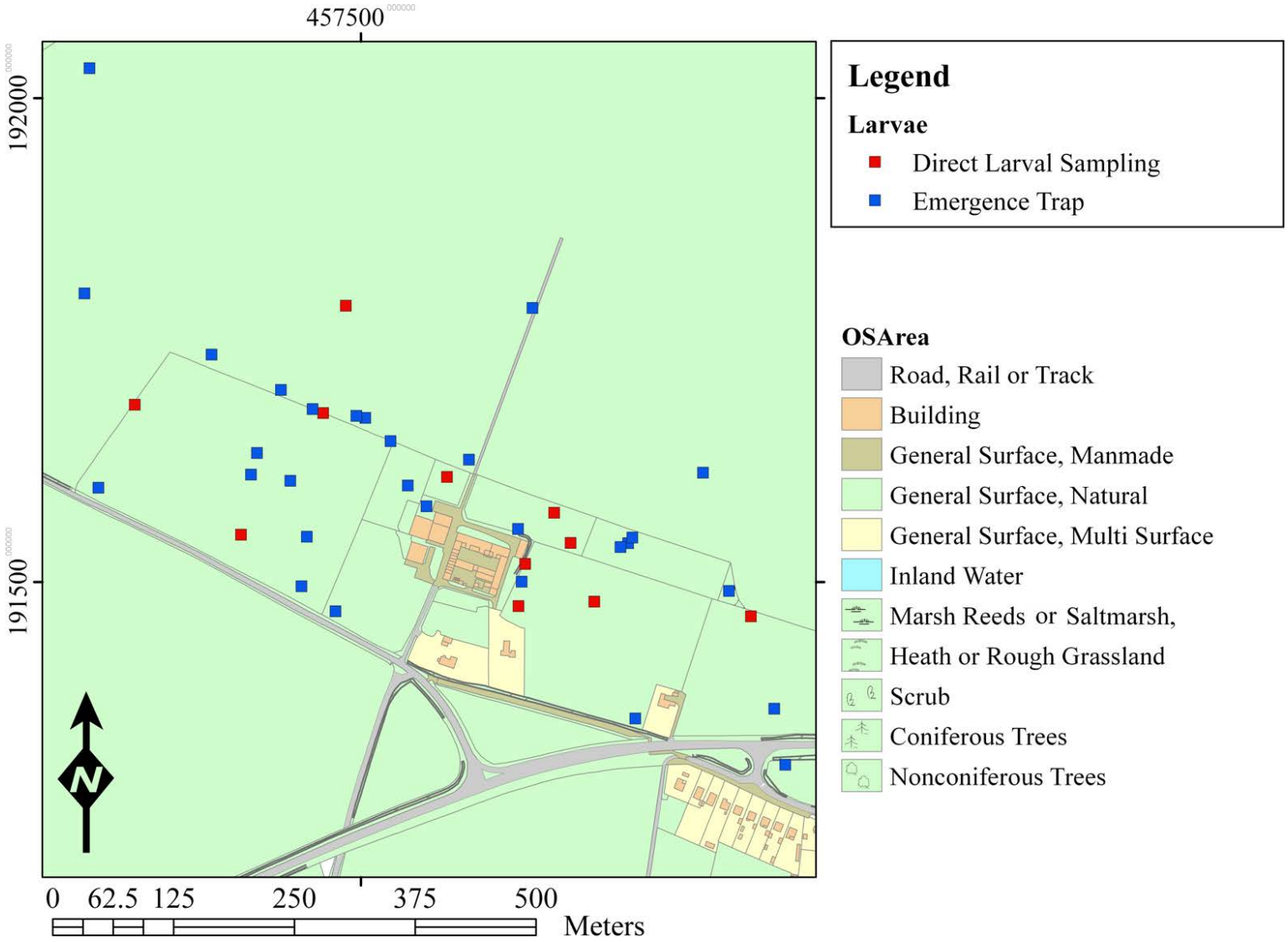


Figure B.4. Spatial distribution of sampling points used for surveillance of immature *Culicoides* at farm C [OS Mastermap basemap (Ordnance Survey, 2009) and British National Grid graticles]

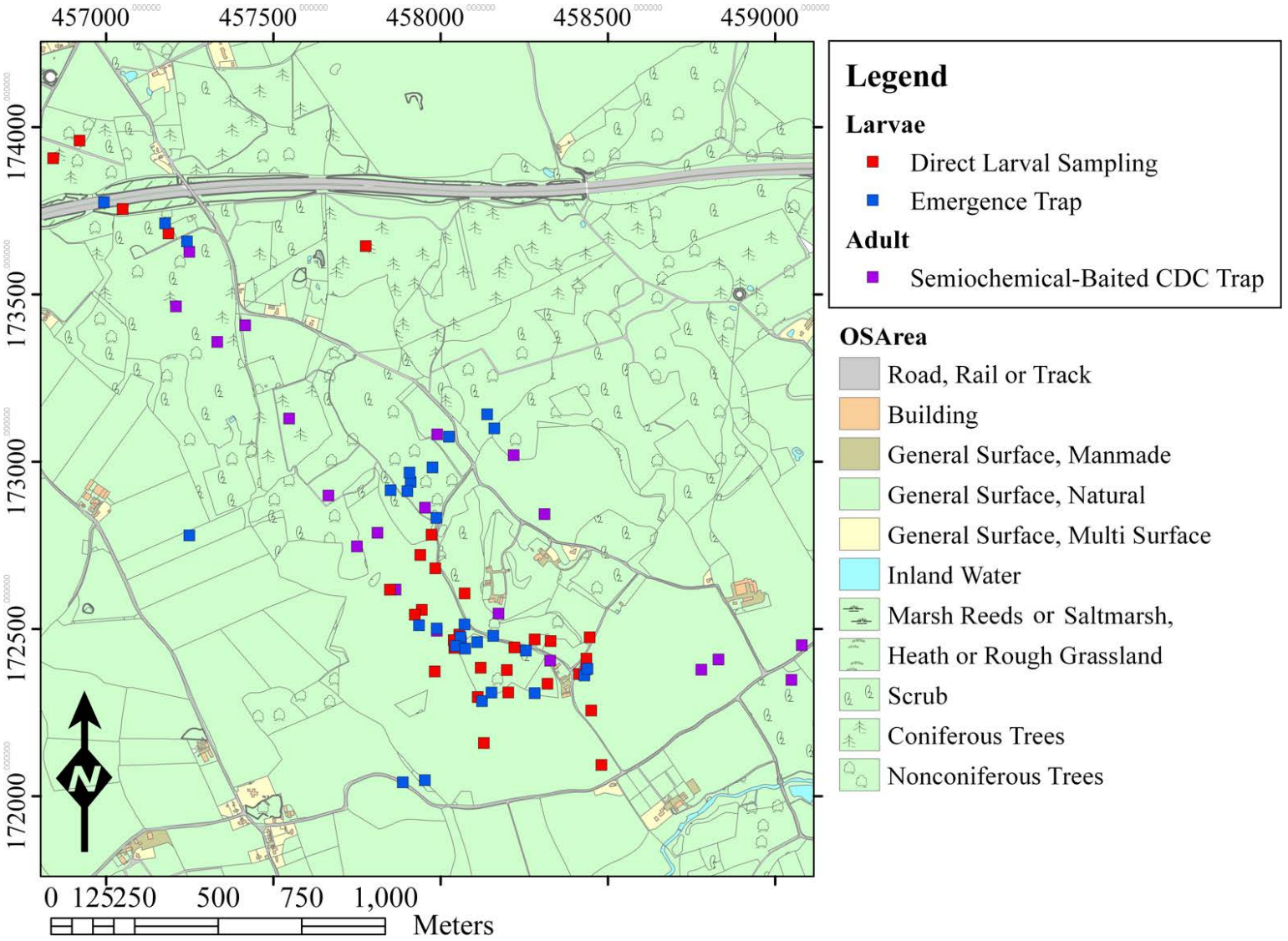


Figure B.5. Spatial distribution of sampling points used for surveillance of adult and immature *Culicoides* at farm D [OS Mastermap basemap (Ordnance Survey, 2009) and British National Grid graticles]

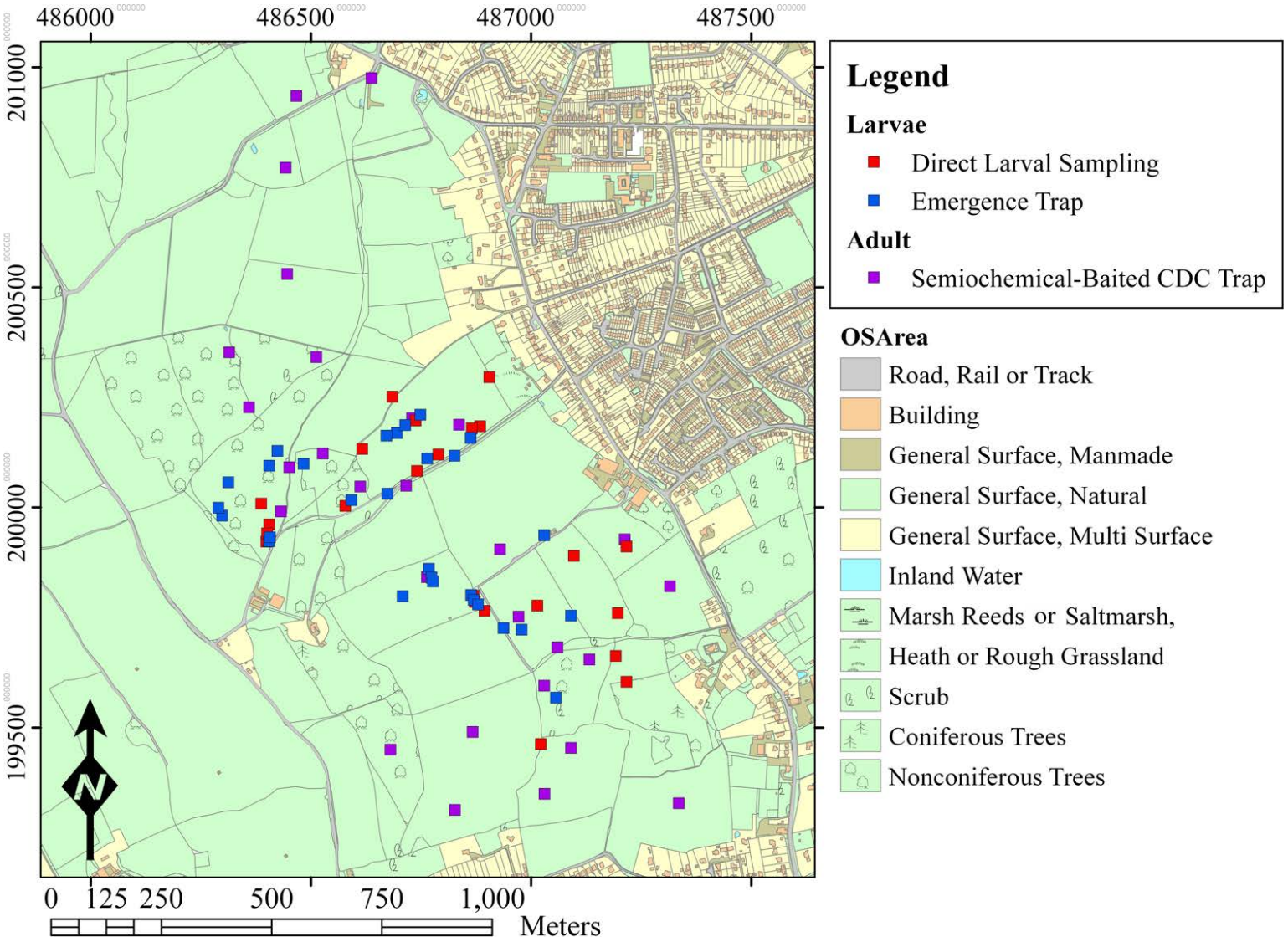


Figure B.6. Spatial distribution of sampling points used for surveillance of adult and immature *Culicoides* at farm E [OS Mastermap basemap (Ordnance Survey, 2009) and British National Grid graticles]

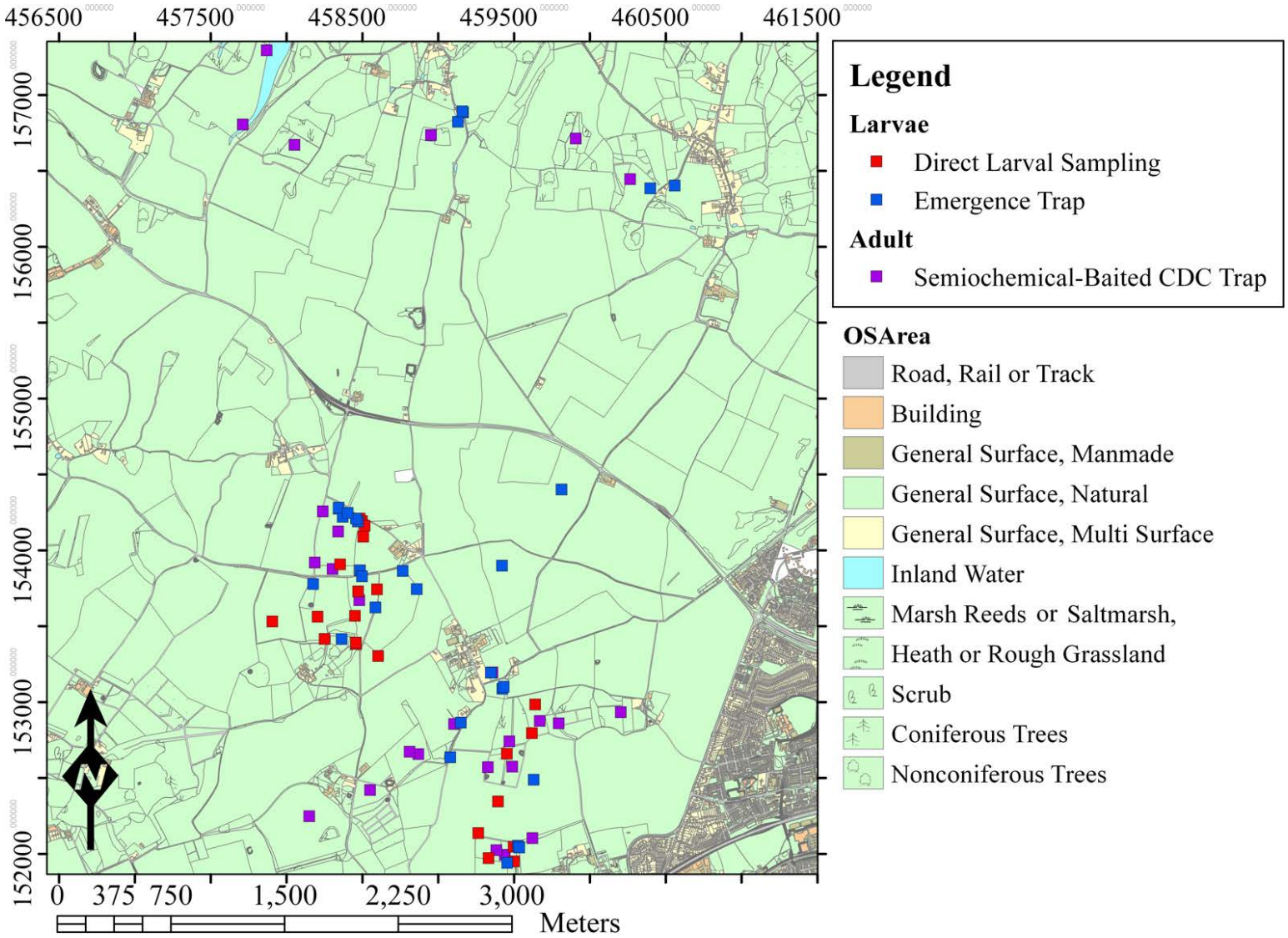


Figure B.7. Spatial distribution of sampling points used for surveillance of adult and immature *Culicoides* at farm F [OS Mastermap basemap (Ordnance Survey, 2009) and British National Grid graticles]

Appendix C

List of Presentations

C.1 Oral

'Exploiting Semiochemicals for Vector Surveillance: The Case of *Culicoides* and Bluetongue Virus', Fifth Asia Pacific Conference on Chemical Ecology (APACE 2009), 25th October 2009, Honolulu **(Oahu, Hawaii)**

'The Influence of Farm Level Factors on Midge Abundance and Transmission of Arboviruses', Institute for Animal Health Student Day, 30th October 2008, Compton **(UK)**.

'Techniques for *Culicoides* spp. (Diptera: Ceratopogonidae) Habitat Identification, Integrating Remote Sensing and Ecological Surveys for Bluetongue Risk Assessment and Control', European Society of Vector Ecology Annual Conference (SOVE 2008), 25th-28th March 2008, Cambridge **(UK)**.

'Field Studies: Local Scale Variation in Habitat Usage by Bluetongue Virus Vectors', Controlling Veterinary Diseases of Livestock Project Steering Group Meeting, 17th April 2008, Pirbright **(UK)**.

'The Influence of Farm Level Factors on Midge Abundance and Transmission of Arboviruses', Institute for Animal Health Student Day, 31st October 2007, Pirbright **(UK)**.

'Landscape Ecology: Its Influence on Bluetongue Virus Outbreaks and Vectors', Internal Seminar Series, Institute for Animal Health, 2nd May 2007, Pirbright **(UK)**.

'The Influence of Farm Level Factors on Midge Abundance and Transmission of Arboviruses', Internal Seminar Series, Institute for Animal Health, Pirbright, 2nd May 2006, Pirbright **(UK)**.

C.2 Poster

'Integrating Remote Sensing and Ecological Surveys for Bluetongue Risk Assessment and Control', Royal Entomological Society Annual Conference (ENT'06), 20th-22nd September 2006, Bath **(UK)**

Appendix D

List of Publications

This appendix contains a list of papers published by the author, relevant to the thesis, details of the appendices containing copies of the papers are stated.

D.1 Journal Articles

Purse, B.V., Brown, H., **Harrup, L.E.**, Rogers, D.J. (2008) Bluetongue and other orbivirus infections: The role of biological and climatic processes, OIE Scientific and Technical Review - Climate Change: the impact on the epidemiology and control of animal diseases, 27 (2), 427-442 [see Appendix E on page 268]

Mellor, P.S., Carpenter, S., **Harrup, L.**, Baylis, M. and Mertens, P.P.C. (2008) Bluetongue in Europe and the Mediterranean Basin: History of occurrence prior to 2006, Preventative Veterinary Medicine, 87 (1-2), 4-20 [see Appendix F on page 285]

D.2 Book Chapters

Mellor, P.S., Carpenter, S., **Harrup, L.**, Baylis, M., Wilson, A. and Mertens, P.P.C. (2008) Bluetongue in Europe and the Mediterranean Basin IN: Mellor, P.S., Baylis, M. and Mertens, P.P.C. (Eds) Bluetongue, Biology of Animal Infections, Elsevier, London [see Appendix G on page 303]

Appendix E

Rev. sci. tech. Off. int. Epiz., 2008, **27** (2), 427-442

Invasion of bluetongue and other orbivirus infections into Europe: the role of biological and climatic processes

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Summary

The invasion of multiple strains of the midge-borne bluetongue virus into southern Europe since the late 1990s provides a rare example of a clear impact of climate change on a vector-borne disease. However, the subsequent dramatic continent-wide spread and burden of this disease has depended largely on altered biotic interactions with vector and host communities in newly invaded areas. Transmission by Palearctic vectors has facilitated the establishment of the disease in cooler and wetter areas of both northern and southern Europe. This paper discusses the important biological and climatic processes involved in these invasions, and the lessons that must be drawn for effective risk management of bluetongue and other midge-borne viruses in Europe.

Keywords

Bluetongue virus – Climate change – Culicoides – Environmental envelope – Europe – Invasion – Virus-vector interactions.

Introduction

Recent unprecedented climate change is widely predicted to increase the incidence and intensity of vector-borne disease transmission (11, 36), largely through direct effects of climate on vector biology, abundance and distribution. However, there is little direct evidence that recently observed changes in vector-borne diseases have been precipitated by climate change (33, 51, 62) and there is a growing recognition of the influence of other non-climatic abiotic and biotic factors on disease distributions (34, 35, 59, 61). Against this background, the invasion of a midge-borne disease of livestock called bluetongue (BT) into southern Europe constitutes a rare example of a clear impact of climate change on a vector-borne disease (56). However, the subsequent continent-wide spread and burden of this disease has depended largely on its biotic interactions with vector and host communities in newly invaded areas (55).

In this paper, we describe how the pattern (and impact) of BT epidemics has altered in Europe and set out the main strands of evidence linking this invasion to climate change. The biotic factors underlying this response to climate change are discussed. These include a northward shift in the range of the traditional African-Asian vector, *Culicoides imicola*, and, beyond this vector's range, the involvement of indigenous European *Culicoides* vector species (56). We demonstrate that the subsequent spread of bluetongue into cooler and wetter areas of Europe was facilitated by these new vectors that carried infection far beyond the range of the traditional vector (55). Understanding the relative role of biotic and environmental processes in such disease invasions is essential for development of early warning systems for vector-borne diseases. We finally consider how such knowledge is best integrated into risk assessment and early warning systems for bluetongue and other orbiviruses.

Bluetongue virus and its *Culicoides* vectors

Bluetongue virus (BTV) is a double-stranded RNA virus (family Reoviridae: genus *Orbivirus*) that can replicate in all ruminant species. Severe clinical signs are usually restricted to improved breeds of sheep and some species of deer (71) whilst in cattle, BTV usually causes long-lived sub-clinical infections, making these ruminants the main reservoir host. Bluetongue virus is transmitted between its ruminant hosts, primarily by certain species of *Culicoides* biting midges (Diptera: Ceratopogonidae) and is, in turn, restricted to areas where these competent vector species occur – broadly, the tropical and sub-tropical parts of the world, between latitudes 35°S and 40°N. *Culicoides* populations can build up to high abundances under suitable conditions, and adults can be transported by the wind for several kilometres within one night, leading to rapid spread of the diseases they carry (66). In Europe, BTV reaches its northern range margin and until recently this continent contained large populations of naïve and susceptible ruminants (particularly fine wool and mutton breeds of sheep).

The shifting pattern and impact of European bluetongue epidemics

Bluetongue virus has circulated on Europe's fringes for decades – in sub-Saharan Africa, Turkey and the Middle East (19, 24, 60, 72). Throughout this period, these fringe areas have been connected to Europe by synoptic wind systems and by traditional livestock trade routes. The potential for BTV to enter Europe has therefore long existed – either by the movement of infected ruminants or by the wind-dispersal of infected midges. Yet, historically, this disease has made only brief sporadic incursions into Europe. Outbreaks were confined to southern Iberia, Cyprus and some Greek islands and occurred wholly within the range of the major African-Asian vector *Culicoides imicola* Kieffer (Fig. 1, dark red hatched area and blue line). On each of these incursions, only one or two countries were affected at a time and only a single BTV serotype was involved.

Between 1998 and 2005, however, six strains (of five serotypes) of BTV entered Europe more-or-less

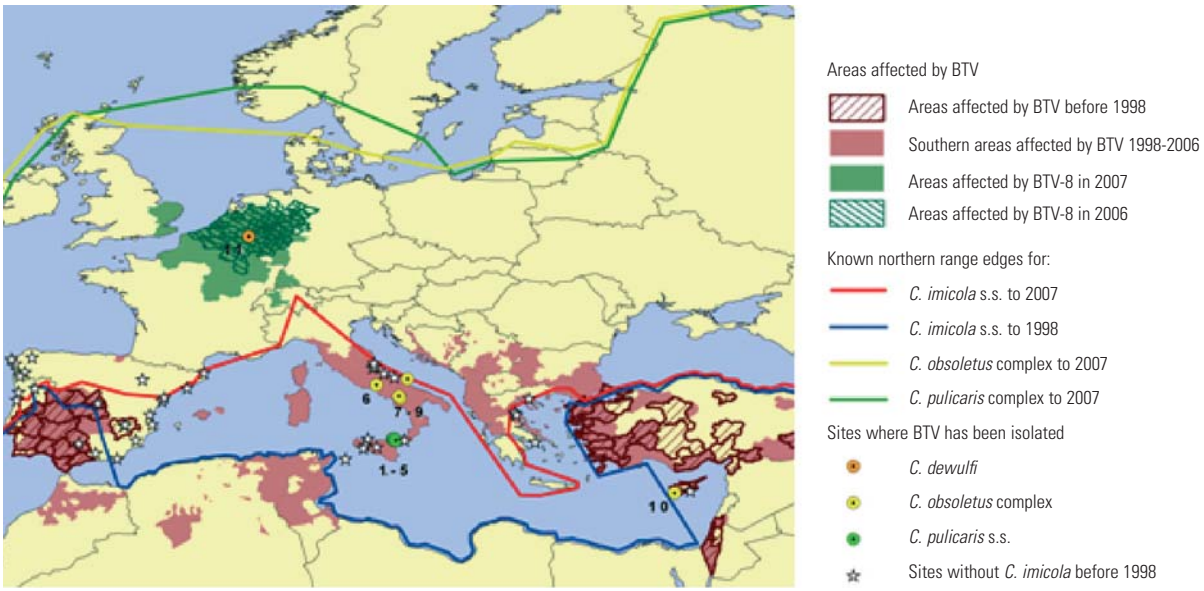

Fig. 1

The changed distribution of bluetongue virus and its vectors in Europe: map showing the distribution of BTV in southern Europe (prior to 1998 and since 1998) and in northern Europe (2006 to 2007)

The distribution of BTV prior to 1998 in North Africa and the Middle East is likely to have been extensive, but much transmission in these endemic areas occurs silently in disease-resistant host animals. The reported outbreaks mapped here, therefore, vastly underestimate the extent of historical transmission in these fringe areas. The green and yellow lines indicate the current known northern range limit of the major indigenous European species complexes. The blue and red lines show the northern range limit of the African-Asian vector midge species, *Culicoides imicola* up to 1998 (blue) and up to the present day (red). Dotted circles indicate sites where BTV has been isolated from wild caught non-engorged individuals from Palearctic vector groups. Mehlhorn *et al.* (37) also made isolations from *C. obsoletus* complex species in Germany at unspecified sites. Stars indicate sites where *Culicoides imicola* was found to be absent before 1998, sites in Bulgaria were surveyed, but not geo-referenced, so are not shown)

simultaneously from at least two origins (Fig. 2, east, via Turkey and Cyprus and west, via north Africa) and spread across 12 countries up to 800 km further north in Europe than ever before (Fig. 1, pink shaded area) (46, 56). This has lead to the death of over 1.5 million sheep and has caused major disruption to trade in livestock and livestock products (7, 46). Not only did the distribution of BTV expand dramatically northward, but so too did that of *C. imicola* (i.e. into the Balearic Islands, mainland France, Switzerland, central and eastern Spain, mainland Greece, Sicily and mainland Italy (Fig. 1, red line) (see refs listed in [56]). Furthermore, early in the epidemic, transmission occurred beyond even the expanded range of *C. imicola* (in north-west Greece, Bulgaria and the Balkans [Fig. 1]) – indicating a vector role for indigenous European *Culicoides* species in these areas. Disease burdens have been highly dependent on particular strain–host type (or breed) interactions, with the severity of clinical signs caused by the same BTV serotype varying considerably across wide geographical areas (e.g. BTV-9 high severity in Greece and Croatia, low severity [<2% sick animals] in Bulgaria, Albania, Bosnia and Herzegovina and eastern Serbia [57]).

In August 2006, an African strain of a new serotype (Fig. 2), BTV-8, arrived in northern Europe by an unknown route (5° latitude further north than any BT outbreaks in the south of the continent and 7° latitude further north than the known range edge of the African-Asian vector – Fig. 1, dark green hatched area). This strain spread rapidly, infecting over 2,000 herds across five countries and unusually caused severe clinical signs and low level mortality in some cattle herds (16). This strain then successfully overwintered (at around 53° N [78]) and reappeared across most of the affected area in 2007, spreading to a further five countries (Fig. 1, green shaded area). By October 2007 it had caused around 25,000 outbreaks (27).

Evidence linking patterns in bluetongue to climate change

Examining responses to recent, unprecedented climate change across a range of biological systems, Walther *et al.*

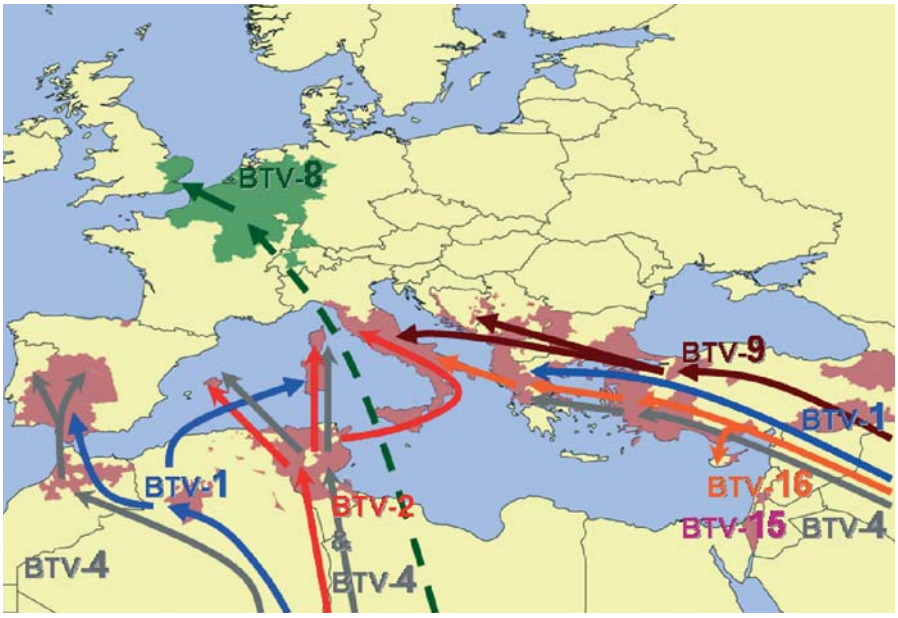

Fig. 2
The molecular epidemiology of bluetongue virus in Europe
Sequence analysis of the 6 European BTV serotypes has identified at least 8 lineages arriving into Europe via at least four distinct routes (47). Two separate introductions of BTV-1 into Europe have occurred. One strain (Greece2001/01) belonging to an 'eastern' group of viruses (being most closely related to Indian isolates) entered Greece from the east whilst another strain, belonging to a 'western' group of viruses, entered via Morocco (MOR2006/01) and Algeria (ALG2006/01) probably from further south in Africa. The European strain of BTV-2, which first appeared in Tunisia in 1998, also belongs to a 'western' group of viruses and is similar to strains from South Africa and Nigeria and probably entered Europe from further south. Both this BTV-2 strain and the 2006 strain of BTV-1 spread further northwards in Europe – into the west Mediterranean islands and mainland Italy (BTV-2) and mainland Spain (BTV-1 in 2007). European BTV-9, which spread extensively in the Balkans, and BTV-16 both belong to 'eastern' groups, while the European type 4, initially isolated in Greece (in 2000), is very similar to viruses that have been periodically isolated in the region (in Cyprus and Turkey) since 1969. However, in late 2003, a distinct western strain of BTV-4 arrived in Corsica and the Balearics, probably from North Africa, and subsequently caused outbreaks in Morocco and Spain. The BTV-8 strain affecting Northern Europe is from a western lineage in sub-Saharan Africa. It did not spread gradually from further south in Europe (it has not been detected there) but 'jumped' into Northern Europe by an unknown mechanism. Importantly, distinct strains are still entering southern Europe on an annual/bi-annual basis, whilst strains from distinct episystems can 'parachute in' and go on to establish in Europe

(76) stated that the 'clearest evidence for a climate trigger occurs where a suite of species, with different histories of introduction, spread *en masse* during periods of climate amelioration' – a situation shown, we suggest, by the near simultaneous entry of six BTV strains into Europe (Fig. 2). Other changes, such as agricultural land use changes, changes in animal health systems, increases in livestock trade, and increases in host density, cannot be shown to follow a similar geographical pattern (56).

Non-climatic (biotic and abiotic) factors unlikely to influence bluetongue distribution in Europe

Alterations in the distribution and movements of susceptible hosts can be ruled out. Although the opening up of trade routes between Europe and the Middle East may have slightly increased the number of host animal movements, the total density of ruminants has actually declined in Europe since the 1980s, particularly in central areas (18). The sudden spread of bluetongue is unlikely to have been due to circulation of new, perhaps more virulent strains of BTV, since the large, naïve populations of European sheep were probably highly susceptible to the entry of any BTV strain in the late 1990s – not only novel ones. This high susceptibility also means that any previous incursions would not have gone unreported and that sudden widespread detection of the disease across the region cannot be due to improvements in disease surveillance or changes in control strategies. Other non-climatic, abiotic factors (socio-economy, land use, animal health systems) also appear unlikely to be responsible. Since *Culicoides* are habitat generalists, breeding in a range of moist microhabitats that are ubiquitous across many farmyards (irrigation channels, drainage pipes, dung heaps [40]), any recent changes in agricultural practice or land-use are unlikely to have had a substantial and sudden impact on *Culicoides* vector distributions all across the continent.

Biological sensitivity of bluetongue virus and *Culicoides* to climate

Having discounted competing explanations, a direct causal link between BT emergence and climate change is suggested due to close adherence of the observed ecological response to criteria set out by peers (33, 52, 59, 62), namely:

– research along several axes (theoretical model systems, laboratory experiments, field manipulations and observations) demonstrates the biological sensitivity of both *Culicoides* vectors and BTV to climate (50)

– there is meteorological evidence of climate change with sufficient measurements in the study region

– significant changes in the climatic drivers of infection in Europe have occurred at the same times and in the same places as the changes in the incidence of BT.

In order to pin down the climatic drivers of BTV transmission, we must consider the various (independent and sometimes opposing) responses of the biological processes involved in the life-cycle of *Culicoides* and in the transmission cycle of BTV to climate. In common with vector-borne disease systems, key events in both cycles are modulated by temperature and moisture availability, as reviewed extensively elsewhere (40, 43, 79). Broadly speaking, warm temperatures enhance the recruitment, development, activity and survival rates of *Culicoides* vectors (45, 80). Significantly, the competence of *Culicoides* vectors, both the degree of transmission by 'traditional vectors' and the extension of transmission to historically 'non-vector' species, is enhanced by warm temperatures (53, 80). Within traditional vectors, warm temperatures increase viral replication rates (optimal temperatures 28°C to 29°C [74]) and may reduce the efficiency of heritable barrier mechanisms that constrain virus dissemination through a vector individual at various stages following oral infection. Bluetongue virus can persist at low temperatures (<10°C) for up to 35 days inside adult vectors and later replicate and be transmitted when the temperature increases (45). In a 'non-vector' species, *C. nubeculosus*, competence can be induced when larvae are reared at high temperatures, with 10% of emerging adults being infectable when reared at 33°C to 35°C, compared to 0% at 30ºC (45, 80). This phenomenon has been attributed to the leakage of virus directly into the haemocoel, bypassing the midgut barriers, allowing virus replication and dissemination. Considering both vector and non-vector species together then, an increase in the cumulative frequency of either warm or hot periods in summer/autumn or whilst overwintering as larvae or adults will increase their transmission potential for BTV.

Moisture availability is the second most important extrinsic variable affecting *Culicoides* vectors and, in turn, BTV transmission. Precipitation governs the size and persistence of semi-aquatic breeding sites for larvae and the availability and duration of humid microhabitats in summer/autumn where adults can carry out key activities and shelter from desiccation (40, 49). Whether changes in precipitation act as climatic drivers of transmission is likely to vary geographically according to the habitat preferences of the vectors in that region and the average suitability of breeding sites there. For example, *C. imicola* breeds in wet, organically enriched soil and mud, but the pupae cannot survive flooding of these sites (5, 6). Thus, precipitation increases might favour increased breeding in arid areas, but across the Mediterranean, the presence of this species can be matched statistically to areas that are relatively dry in summer (55). For indigenous European species, however, conditions of high humidity may enable adults to resist desiccation during increasingly warm summers.

Overall, we expect increases in temperature (particularly at night-time and in winter) and precipitation (particularly in summer/autumn and in dry areas) to lead to an increased geographical and seasonal incidence of BTV transmission. This will occur as a result of:

– increases in the range, abundance and seasonal activity of vectors

– increases in the proportion of a vector species that is competent

– increases in the development rates of the virus within vectors

– the extension of transmission ability to additional *Culicoides* species.

Spatio-temporal correspondence between changes in European climate and changes in bluetongue virus

Coincident in time and space with the emergence of BTV in Europe in the late 1990s (56), there have been pronounced increases in night-time and winter temperatures (17, 31), fewer frost days, and changes in moisture conditions (25, 32, 31). As shown in Figure 3, BT incidence in the south of Europe has increased most markedly in areas where temperature has increased by at least 1°C since the 1980s (yellow in Fig. 3c) – in Italy, adjacent west Mediterranean islands and the Balkans. Bluetongue incidence in northern Europe increased in areas that have warmed by almost 1.5°C (orange in Fig. 3c), making these areas as warm as infected areas of Italy, Spain and Greece much further south (Fig. 3a).

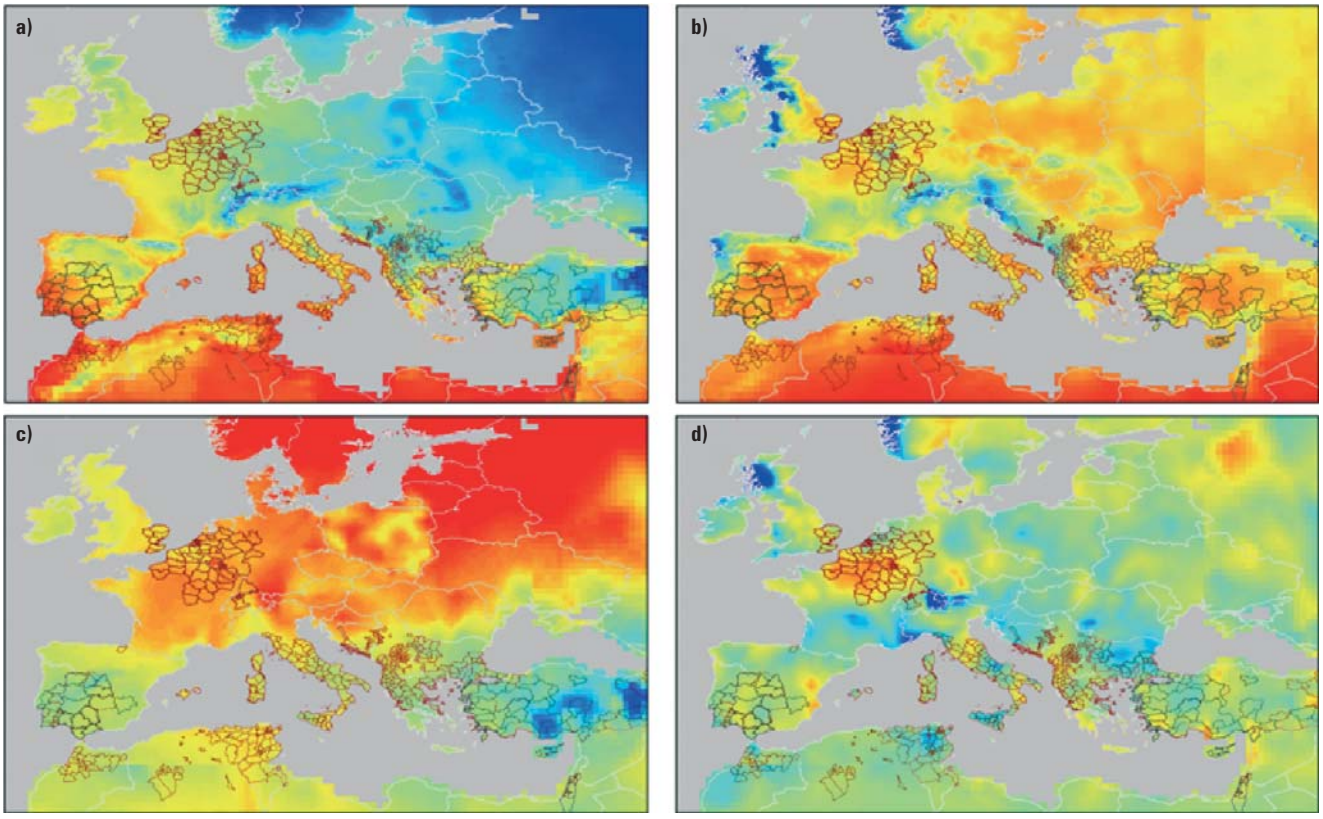

Fig. 3
Spatial variation in recent climate change in Europe
Figs. 3a and 3b show average 1990s conditions of minimum temperature (from –10°C in dark blue to 13°C in dark red) and sum of annual precipitation (from 0 to 2,000 mm) for each sixth of a degree latitude and longitude square in Europe. Figs. 3c and 3d show changes in these conditions between the 1980s and the 1990s. In Fig. 3c temperature changes are shown on a sliding colour scale ranging from a reduction of 2.0°C (dark blue) to an increase of 2.0°C (dark red). Temperature increases are most marked in both central and northern Europe, whilst central Iberia, the border region between Morocco and Algeria, and parts of Turkey have cooled. (Temperature images were produced by temporal Fourier processing the raw time series of data and re-constituting it by summing the annual, bi-annual and tri-annual harmonics – essentially smoothing the data. The minimum values here are the minima of the re-constituted series for the period in question.) In Fig. 3d precipitation changes are shown on a sliding colour scale ranging from an increase of 200 mm a year (dark blue) to a reduction of –200 mm a year (dark red). These are overlaid with the areas historically affected by BT in grey outline and the newly-affected areas in red outline

Areas such as central Iberia, northern Morocco and Algeria – where the distribution of BT has broadly remained stable – have cooled but are still warm on average (Fig. 3a). As expected, the spatial correspondence between changes in BTV and changes in precipitation amounts is less clear, but BTV-affected areas tend to be drier on average (Fig. 3c) and increased BT incidence in northern Europe, north and central bands of mainland Italy, and the Balkans overlaps with areas that have dried since the 1980s.

Biological factors underlying the response of bluetongue virus to climate change

What are the mechanisms that might underlie these responses of BTV to climate change in Europe and what are their consequences for future spread?

Extended distribution of the major African-Asian vector, *C. imicola*

Vector surveillance efforts have been dramatically stepped up in Europe during the recent BT epidemic, such that people are looking for the major African-Asian vector, *C. imicola*, more often and in more places than before. This vastly increased sampling effort might result in an extension of the recorded northern range limit of *C. imicola* regardless of any actual extension on the ground. However, *C. imicola* has now been found in or near sites in which it had been searched for and found to be absent before 1998 (Fig. 1, white stars – see refs listed in [55]), suggesting the actual distribution limit really has extended, at least in places (56).

Have regional climate changes precipitated this extension in *C. imicola*'s northern range limit perhaps by increasing the extent of suitable habitat in Europe? Alternatively, has *C. imicola*, in the absence of climate change, simply been filling suitable habitat in Europe that was always available to it but remained unoccupied due to geographical isolation until recently? Has the environmental envelope of *C. imicola* itself changed over this time period? Without extensive historical and current distribution data for *C. imicola*, these alternatives are extremely difficult to tease apart. However, the most important driver of this species' current distribution across the Mediterranean was recently found to be annual mean temperature, and populations were associated with locations that are warm, not just on average (annual mean 12°C to 20°C), but year-round (55). This leads us to expect that increases in annual mean temperatures and narrowing of daily and annual temperature ranges across Europe (31) would favour range extension in this species. Broadly speaking, the pattern of *C. imicola*'s range extension during the 1990s mirrors the pattern of warming (compare Fig. 1 and Fig. 3c). This species has expanded most into warmed areas – eastern Spain, northern Italy, southern France and north-eastern parts of Greece – whilst areas where temperatures have remained largely unchanged (European Turkey and eastern Bulgaria) have, as yet, seen no such invasion.

Involvement of Palearctic vectors in bluetongue virus transmission in southern Europe

By the early 2000s, several pieces of evidence pointed to a role for indigenous European vector groups, primarily species from the *C. obsoletus* and *C. pulicaris* complexes, in BTV transmission in southern Europe. These included fine-scale overlap of the distributions of these complexes with outbreaks (13, 57, 73), isolation of virus from wild-caught adults of the *C. pulicaris* and *C. obsoletus* complexes in several sites (Fig. 1, dotted circles) (8, 14, 37, 38, 64, 65) and laboratory studies indicating competence levels for BTV comparable to *C. imicola* in some populations (9). These Palearctic complexes are abundant and widespread in northern Europe, but also extend southward into north Africa (4, 67), Turkey (28) and the Middle East (5, 6) and, as such, overlap with both the major vector *C. imicola* and areas of historical BTV incursions across a wide geographical area. Despite this overlap, there is little evidence, either from the timing or fine-scale spatial distribution of historical outbreaks (41, 42), that these complexes played a major role in transmission before the late 1990s (39).

In turn, it has been hypothesised that the recent warming in Europe may have increased the importance of Palearctic vectors – by increasing their population sizes and survival rates to compensate for their low competence levels and by increasing their individual susceptibility through the developmental temperature effects mentioned above (56). In support of this hypothesis, the areas where these Palearctic vectors have been involved in transmission again coincide with those areas of Europe that have warmed the most (Fig. 3c).

It is important to note however, that a similar temporal pattern in their involvement in transmission could have been produced, in the absence of climate change, given geographical variation in these aspects of their vector capacity – if, for example, the populations of the *C. obsoletus* and *C. pulicaris* complexes in the Balkans, Bulgaria, northern Greece, European Turkey have (or had) higher vectorial capacity than do populations in areas historically affected by outbreaks (Spain, Portugal, Morocco and the Greek Islands). In either scenario, the involvement of these complexes in transmission would have been facilitated by their increased spatial (range

extension) and temporal (prolonged seasonal activity) overlap with *C. imicola*, allowing frequent 'hand-over' events of the virus between the traditional and novel vectors. Evidence for the importance of such hand-over events during the current epidemic is substantial. In both Sicily (73) and Lazio and Tuscany provinces, BTV was transmitted initially in lowland areas by *C. imicola*, but was then handed-over and spread inland by species from the *C. pulicaris* and *C. obsoletus* complexes (13).

Consequences of transmission by Palearctic vectors for bluetongue spread

Whether driven by regional warming or not, what were the consequences of the involvement of Palearctic vectors in BTV transmission for the subsequent continental spread of this virus? As expected when any pathogen moves to a new vector, the distribution of the new vector is likely to extend the kinds of environments (or environmental envelope) in which the pathogen can occur. However, despite the wide distribution and high abundances of the Palearctic vectors and their substantial laboratory competence levels for BTV, they were generally assumed to be of relatively minor importance in transmission, whilst BTV remained in southern Europe. Vector surveillance effort (up to 2004), for example, was directed towards defining *C. imicola* free zones (10) between which vaccinated animals could be legally moved for trade or seasonal transhumance – without regard for the abundance of Palearctic vectors in those zones.

For accurate risk assessment it was essential to test this assumption and to determine the following:

– the relative extent to which the Palearctic vectors versus the African-Asian vector, *C. imicola*, were involved in BTV transmission in southern Europe

– the extent to which the environmental envelope of BTV had been extended by the early 2000s (and into which kinds of environment) due to its transmission by Palearctic vectors.

We took an environmental envelope modelling approach to this investigation (55) – analysing the differential degree of overlap between the environmental envelope of BTV and each of its vectors and assuming that the distribution of BTV in environmental space would be closest to that of the vector(s) playing the greatest role in transmission. We first mapped the current, fine-scale, distributions of BTV (501 presence records), *C. imicola* (395 presence and absence records) and the *C. obsoletus* (428 presence records) and *C. pulicaris* (410 presence records) complexes across southern Europe and north Africa. Secondly, we determined (statistically) the key climatic factors that best defined the current envelope of *C. imicola* (this was possible because this species, being the target of historical surveillance efforts, is relatively well-recorded along its northern range edge). Thirdly, we compared the overlap of *C. imicola*'s envelope (defined by these climatic factors) and areas of BTV transmission to the overlap between the Palearctic vectors and BTV transmission.

Locations favoured by *C. imicola* across the Mediterranean were not only warm and thermally stable, but were also relatively dry in summer – consistent with this species' susceptibility to flooding of their breeding sites (see above). The Palearctic *C. obsoletus* and *C. pulicaris* complexes, by contrast, were both found to occur in cooler (down to 7°C annual mean), thermally more variable and wetter (up to 700 mm summer precipitation) locations. The overlap of the distribution of BTV and *C. imicola* in environmental space in southern Europe (and North Africa) is shown on the left-hand panel of Figure 4. On the pairs of environmental axes depicted in Figs. 4a and 4c, around 44% of southern BTV records fall outside of the envelope of *C. imicola* (estimated as the 95% confidence ellipse around presence locations for this species), and do so in the directions of much lower annual mean temperatures (to 6°C or 7°C) and greater ranges of annual temperature (>9°C) on Fig. 4a, and wetter as well as cooler areas on Fig. 4c. Superimposing the sampled distributions of the *C. obsoletus* and *C. pulicaris* complexes on the same pairs of axes (right hand panel Figs. 4b and 4d) we can see they overlap much of the space beyond *C. imicola*'s envelope into which BTV transmission has extended. When measured in multivariate space, their distributions are closer to the overall distribution of BTV transmission than is the distribution of *C. imicola* (even when locations inside *C. imicola*'s range limit were considered separately). This indicates that these Palearctic complexes made a significant contribution to the transmission of BTV in southern Europe – not only northward of the range limit of *C. imicola* but also inside this species' range – and have been instrumental in the spread of the virus into cooler and wetter regions of Europe. Furthermore, the Palearctic complexes overlap extensively with the envelope of *C. imicola* (and with each other) affording ample opportunity for the virus to be 'handed over' between traditional and novel vectors. This study also shed light on the seasonal conditions of temperature and precipitation limiting the distribution of *C. imicola* in the north-east/north-central (wetter in summer and low winter temperatures) and north-west Mediterranean (wetter year-round and cooler) and the environmental changes that would be required for further northward spread in different areas.

Overall, these results for southern Europe suggested that further northward spread of bluetongue from the mid-2000s would most likely depend on the portion of the

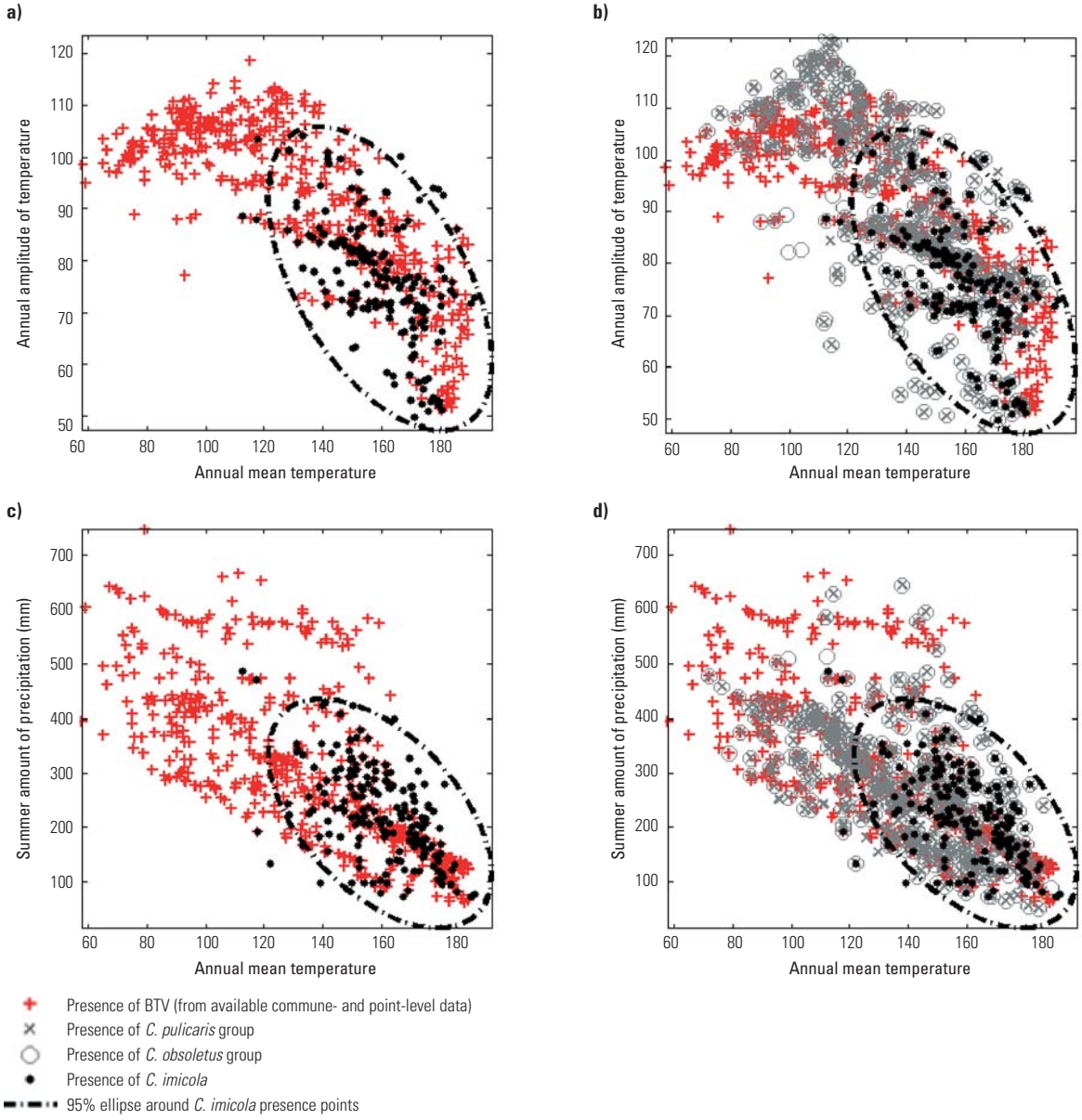

Fig. 4

Climate space occupied by bluetongue virus and its vectors: southern Europe

The axes of bivariate climate space are defined by two pairings of three important determinants of *C. imicola* distribution across Europe: annual mean temperature (in 0.1°C), annual amplitude of temperature (in 0.1°C), and summer amount of precipitation (in mm per summer). In Figs. 4a and 4c the overlap between the presence of BTV and the presence of *C. imicola* in environmental space is shown. In Figs. 4b and 4d their overlap with the presence of other Palearctic *Culicoides* is also shown. Modified with permission from Purse *et al.* (58)

distributions of species within the Palearctic complexes that BTV could occupy (determined by the environmental effects on the viral replication and vectorial capacity of midge populations within these distributions). This was borne out in the summer of 2006, by the appearance of an African strain of BTV in northern Europe (16). The overlap of the distribution of BTV-8 outbreaks in northern Europe in 2006 and 2007 with the distribution of vectors and southern BTV outbreaks in environmental space (defined by the same environmental axes as before) is depicted in Figure 5. Records from the northern portion of these species' ranges (including *C. dewulfi*) have been included in the estimated distributions of the Palearctic complexes (B.V. Purse, unpublished data). It can be seen that the northern BTV outbreaks (in blue on Fig. 5) lie well outside the envelope of *C. imicola* in Europe, occurring in much cooler and wetter environments than those favoured by this species. Compared to previous southern outbreaks (in red on Fig. 5) they are distinct not in mean levels of temperature (annual) or summer precipitation (Fig. 5b) but in the fact that they are also relatively cool year round (Fig. 5a). The extensive overlap with the distribution of the

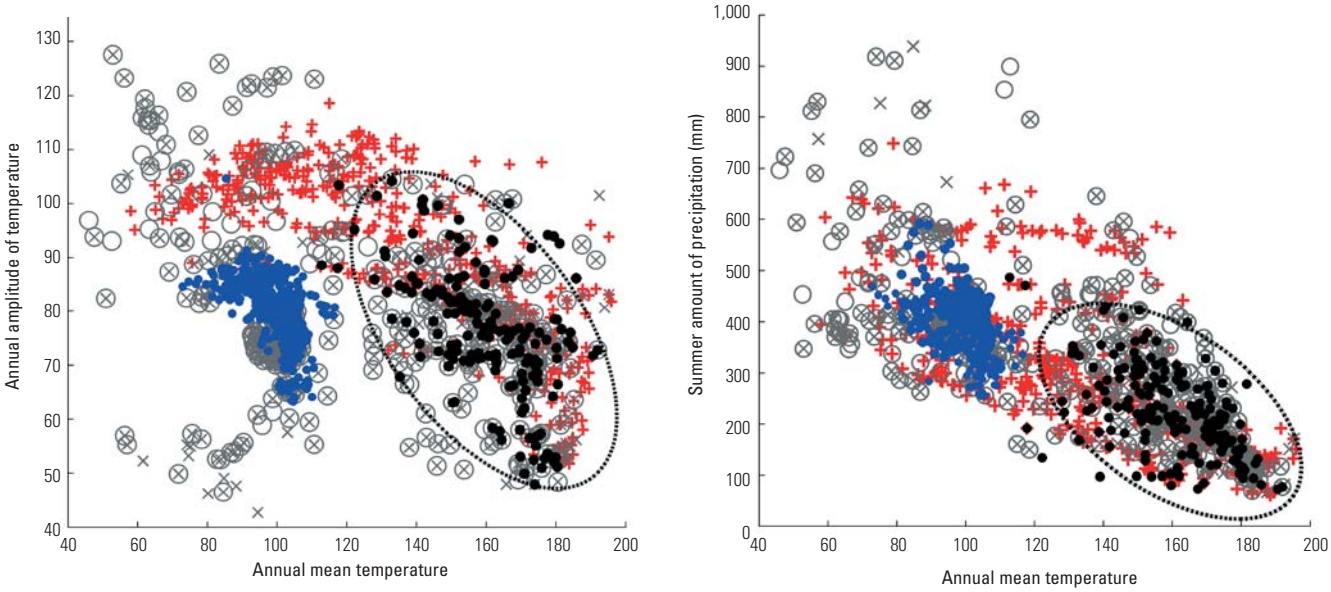

Fig. 5

Climate space occupied by bluetongue virus and its vectors: northern and southern Europe

The axes of bivariate climate space are defined by two pairings of three important determinants of *C. imicola* distribution across Europe: annual mean temperature (in 0.1°C), annual amplitude of temperature (in 0.1°C), and summer amount of precipitation (in mm per summer). The overlap between the presence of BTV (northern in blue circles, southern in red crosses) and the presence of *C. imicola* and Palearctic complexes is shown

Palearctic complexes again indicates a major (if not exclusive) role for these groups as vectors during the northern outbreaks, consistent with virus isolations in the outbreak areas in the field from wild caught adult midges (37, 38). Given that these Palearctic complexes are not widespread in the postulated area of origin of the BTV-8 strain in sub-Saharan Africa, it is unlikely that susceptibility of these vectors and the ability of the virus to replicate inside them gradually evolved in tandem over a long period of co-existence. This is extremely worrying, since it means that some European midge species are capable of transmitting geographically remote strains to which they have not been exposed. In addition, these Palearctic complexes extend far outside the current outbreaks in both geographical (see range limits in Fig. 1) and environmental space (Fig. 5). Further investigation of the distributions, competence and other aspects of vectorial capacity of the species that make up these complexes is essential for prediction of onward spread of bluetongue through Europe.

Vectorial capacity, the number of potentially infectious bites per animal for a particular vector species, is a complex phenomenon for these Palearctic complexes. Geographical variation in vector competence – one aspect of vectorial capacity – has, for example, been noted within the *C. obsoletus* complex in the United Kingdom (UK). Further work is ongoing to pinpoint whether competence is environmentally determined or whether it is heritable – and, if heritable, whether particular species of *C. obsoletus* s.l. and *C. pulicaris* s.l. or genotypes within species are highly competent. Gathering of these kind of data on geographical and temporal variation in the vectorial capacity of these complexes is currently hampered by the difficulties in routinely distinguishing between species by morphological or molecular taxonomic techniques.

A global view of climate change and bluetongue epidemiology

Worldwide, BTV is thought to have evolved within stable, continental 'episystems' where constituent vectors are able to transmit only indigenous viruses despite movements of hosts (due to trade) and vectors between episystems (68). As a consequence, the situation in Europe – of altered vector roles and widespread transmission of 'foreign' strains – is deemed unusual. In fact, on some other continents, long periods of BTV faunal stability have been interrupted by the intermittent introduction and establishment of foreign or novel strains. For example, several southeast Asian strains of bluetongue became established in northern Australia during the 1990s (12, 30) and, between 1999 and 2005, six serotypes that were new to the United States of America were isolated from Florida sheep, cattle or deer (29). For the Europe/Africa/Middle East episystem, a period of instability was precipitated by climate change – probably acting on critical determinants of vectorial capacity (the features of the system that made it responsive to climate change [56] are summarised in

Table I
Features of the European bluetongue virus-*Culicoides* episystem precipitating its response to climate change

Feature	Explanation
Wide host preferences	Bluetongue virus (BTV) uses a wide range of wild and domestic ruminant hosts such that susceptible host populations are likely to be spatially continuous across agricultural systems
Wide range of susceptible vectors with different ecologies	Vector populations of *Culicoides* are widespread and abundant and occupy a wide range of moist soil/dung microhabitats found all across agricultural systems. They are catholic in their biting habits, feeding upon any available large mammal. In Europe, indigenous *Culicoides* vector complexes (specifically *C. pulicaris* and *C. obsoletus*) have a wide zone of overlap with the major Old world vector *Culicoides imicola.*, providing the opportunity for frequent and widespread 'hand-over' events of the virus between major and novel vector groups. The indigenous vector complexes also extend much further northwards in Europe than *C. imicola*
Temperature-mediation of vectorial capacity	The temperature effects on the vectorial capacity of both traditional vectors and extension of competence to 'non-vectors'
Sub-clinical infection and 'silent' circulation	Most hosts are sub-clinically infected, most infections are neither identified nor removed rapidly from the population, and so persist as sources of infection for biting vectors
Over-wintering ability	BTV can persist at low titres inside adult vectors for up to 35 days and is later able to replicate and be transmitted when the temperature increases (as would occur in spring). Viral RNA has also recently been recovered from *Culicoides* larvae (77), calling into question whether trans-ovarial transmission may indeed be possible in some species. In addition, in areas where adult vectors are unable to persist in substantial numbers over winter (such as Bulgaria [57]) it has been hypothesised that the virus can instead persist covertly in the ruminant host itself inside the γδ T-cells of the immune system (69)

Table I). The major lesson to be drawn from this emergence is that vectorial capacity should be considered as temporally and geographically variable, within and between *Culicoides* vectors, and that the genetic and environmental factors underpinning this variation should be thoroughly investigated. Just because a *Culicoides* species or genotype has been historically refractory to a BTV strain in one area, does not mean that it will remain so in other places or at other times, or even to other strains of the virus. Given the global nature of climate change and the fact that short-term changes in climate can produce short-term changes in vector or virus distributions across most continental bluetongue episystems (54, 75, 81), it should be considered within a suite of other potential abiotic and biotic factors when investigating the mechanisms for dynamism in BT epidemiology worldwide.

Considering future changes in BT epidemiology in Europe, it is conceivable that European livestock may quickly develop immunity to extant strains of BTV in Europe. However, molecular epidemiological evidence indicates that novel strains (to which the population will be naïve) are still entering Europe on an annual basis (47, 48) and, in turn, BTV and related orbiviruses that share the same or similar vectors (e.g. African horse sickness virus and epizootic haemorrhagic disease virus) represent a continuing threat, at least in the short term. Molecular work also indicates that some European strains have re-assorted (i.e. have swapped their genome segments) to create new strains that may potentially have novel biological properties. Thus, even in the absence of entry of further BTV strains into Europe, it is conceivable that the mixture and re-assortment of eastern and western field and vaccine strains could lead to the emergence of new strains that are better suited to transmission under local conditions. How is knowledge of the environmental and biological factors involved in invasion, establishment and spread of BTV being integrated into risk assessment tools for bluetongue in Europe?

Risk management tools for bluetongue in Europe

Tools for predicting the arrival, establishment and spread of BTV into Europe have developed apace during the recent emergence. Considering the process of arrival, the development and analysis of large nucleotide and amino acid sequence databases (containing geographically and temporally referenced isolates) for BTV have enhanced our ability to identify the origins of new viral strains entering Europe or circulating on the fringes (47, 48). The probability of arrival of new BTV strains via the movements of animals and animal products is often hard to predict, as epitomised by the unknowable entry of BTV-8 into Europe in 2006 (16). Hoar *et al.* (26) have developed Monte-Carlo simulation models to predict the probability of importing a viraemic animal based on the frequency of importation and the countrywide seroprevalence across a range of different exporting countries.

Wind-borne dispersal of *Culicoides* is an important process governing both the arrival of BTV into new land masses and the subsequent spread (66). A range of authors have developed models to better quantify the importance of this process in past incursions (1) and to predict and monitor the likelihood of such movements, their timing and direction – over land as well as over the sea. Ducheyne *et al.* (15) successfully matched wind trajectories (at a pressure level or height thought to permit *Culicoides* survival during movement) with movement patterns inferred from recorded outbreaks in Greece and Bulgaria. Others have taken a more mechanistic approach, borrowing the plume models of atmospheric physicists that predict spread from a point source (20, 21). These incorporate, for example, information on the suitability of local climatic conditions for take-off and landing of adult *Culicoides*, as well as their particle size.

Geographical and temporal variation in the probability of establishment of BTV should ideally be investigated within the framework of the basic reproduction number – R_0, i.e. the number of new cases that arises when a case is introduced into a naïve population. If climate changes affect vector-borne diseases, they will do so through the parameters and variables of the R_0 equation (63). This approach requires a great deal of detailed knowledge on the rates of biological processes in the host and the vector (and their dependencies on environmental variables) and the ratio of vectors to hosts; this information is often unavailable – necessitating short-cuts. Wilson *et al.* (78), for example, have predicted when and where in the UK BTV might be transmitted under different temperature regimes on the basis of one of the key parameters of the R_0 equation, such as the extrinsic incubation period in the vector, and its relationship with temperature. It is indeed conceivable that the maximum extent of spread across northern Europe, where vectors are ubiquitous and abundant, might eventually be limited by thermal limits on viral replication. Gubbins *et al.* (22), formulated a two host one vector model for R_0 and incorporated information on the temperature dependence of biting rates, the extrinsic incubation period and vector mortality. They found that R_0 was highest between 20°C and 25°C. Below 10°C, vectors were unable to complete the extrinsic incubation period whilst at high temperatures (30°C to 35°C), high vector mortality limits R_0.

The vector-host ratio is an essential ingredient of the R_0 equation and it is probable that spatial and seasonal variation in vector numbers drive spatial and seasonal patterns in many vector-borne diseases. Many studies have attempted to map the habitat suitability for different midge vectors across Europe. These distribution models have variously incorporated both landscape factors (23) and average seasonal climatic conditions (using both meteorological and remotely-sensed data) as predictors (2, 3, 4, 10, 55, 58, 70). These have been quite successful in predicting the range limits and regional presence of *C. imicola*, the major African-Asian vector and for teasing apart broad-scale interactions of BTV with different vector groups in Europe (55). Their lack of predictive ability at the farm scale, however, indicates the importance of local-scale factors (farm husbandry, hosts and microclimate) in determining population levels. For the Palearctic complexes, development of models is particularly restricted by current lack of species-specific distributional (and other ecological) data. Scaling up between local and regional-scale predictions of establishment requires that future vector surveillance systems for bluetongue can deliver species-specific information (on breeding habitats and the seasonality of adult *Culicoides* populations) that enables us to relate their demographic rates and carry capacities to environmental variables.

Conclusions

Changes in BT incidence in Europe have been matched by spatio-temporal changes in regional climates, including the specific climatic drivers of BTV infection. However, biotic processes, of changing vector roles and distributions, have been as important as climatic processes in driving the invasion of Europe by multiple bluetongue strains. Enhanced transmission of the virus by indigenous European vectors has been instrumental in the spread and persistence of infection in cooler and wetter areas of both southern and northern Europe following invasion. Vectorial capacity of *Culicoides* species is dynamic and climate-mediated, making it difficult to state unequivocally that particular species cannot or will not be involved in transmission – even of strains that enter Europe unexpectedly from geographically remote regions. There is an urgent need to collect detailed ecological information on indigenous European vector species to support the development and validation of risk tools for bluetongue.

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Les invasions de fièvre catarrhale du mouton et d'autres infections à orbivirus en Europe : le rôle des processus biologiques et climatiques

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Résumé
L'offensive, en Europe méridionale, de nombreuses souches du virus de la fièvre catarrhale du mouton (maladie transmise par les moucherons) depuis la fin des années 1990 est un exemple précieux de l'impact du changement climatique sur les maladies à transmission vectorielle. Toutefois, la propagation de la maladie dans tout le continent et sa gravité s'expliquent surtout par des modifications des interactions biotiques entre les populations des vecteurs et celles des hôtes dans les zones récemment envahies. La transmission par des vecteurs paléarctiques permet à la fièvre catarrhale du mouton de s'établir facilement dans les régions plus fraîches et plus humides d'Europe du Nord et du Sud. Les auteurs examinent les principaux processus biologiques et climatiques associés à ces invasions ainsi que les enseignements que l'on peut en tirer pour gérer plus efficacement les risques liés à la fièvre catarrhale du mouton et à d'autres virus transmis par les moucherons en Europe.

Mots-clés
Changement climatique – Culicoides – Enveloppe écologique – Europe – Interaction virus-vecteur – Invasion – Virus de la fièvre catarrhale du mouton.

Las invasiones de lengua azul y otras infecciones por orbivirus en Europa: papel de los procesos biológicos y climáticos

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Resumen
Desde los años noventa el sur de Europa asiste a la penetración de múltiples cepas del virus de la lengua azul, transmitido por moscas enanas, fenómeno éste que brinda un raro y elocuente ejemplo de la influencia del cambio climático sobre una enfermedad mediada por vectores. No obstante, la subsiguiente y espectacular propagación de esta enfermedad y de la carga que trae consigo ha obedecido sobre todo a la alteración de las relaciones bióticas entre el vector y las comunidades de hospedadores en las zonas recién invadidas. La transmisión por vectores paleárticos facilita el asentamiento de la enfermedad en regiones más frescas y húmedas tanto del sur como del norte de Europa. Los autores examinan los importantes procesos biológicos y climáticos que intervienen en

estas invasiones y exponen las enseñanzas que cabe extraer para afrontar eficazmente en Europa el riesgo de infección por el virus de la lengua azul u otros virus transmitidos por la mosca enana.

Palabras clave
Cambio climático – Culicoides – Envoltura ambiental – Europa – Interacción entre virus y vector – Invasión – Virus de la lengua azul.

References

1. Alba A., Casal J. & Domingo M. (2004). – Possible introduction of bluetongue into the Balearic Islands, Spain, in 2000, via air streams. *Vet. Rec.*, **155**, 460-461.

2. Baylis M., Meiswinkel R. & Venter G.J. (1999). – A preliminary attempt to use climate data and satellite imagery to model the abundance and distribution of *Culicoides imicola* (Diptera: Ceratopogonidae) in southern Africa. *J. S. Afr. vet. Assoc.*, **70** (2), 80-89.

3. Baylis M., Mellor P.S., Wittmann E.J. & Rogers D.J. (2001). – Prediction of areas around the Mediterranean at risk of bluetongue by modelling the distribution of its vector using satellite imaging. *Vet. Rec.*, **149** (21), 639-643. Erratum: *Vet. Rec.*, **150** (13), 404.

4. Baylis M. & Rawlings P. (1998). – Modelling the distribution and abundance of *Culicoides imicola* in Morocco and Iberia using climatic data and satellite imagery. *Arch. Virol. Suppl.*, **14**, 137-153.

5. Braverman Y. (1978). – Characteristics of *Culicoides* (Diptera, Ceratopogonidae) breeding places near Salisbury, Rhodesia. *Ecol. Entomol.*, **3**, 163-170.

6. Braverman Y., Galun R. & Ziv M. (1974). – Breeding sites of some *Culicoides* species (Diptera, Ceratopogonidae) in Israel. *Mosq. News*, **34**, 303-308.

7. Calistri P. & Caporale V. (2003). – Bluetongue in Italy: a brief description of the epidemiological situation and the control measures applied. *Bull. OIE*, **2**, 15-17.

8. Caracappa S., Torina A., Guercio A., Vitale F., Calabro A., Pupari G., Ferrantelli V., Vitale M. & Mellor P.S. (2003). – Identification of a novel bluetongue virus vector species of *Culicoides* in Sicily. *Vet. Rec.*, **153**, 71-74.

9. Carpenter S.T., Lunt H.L., Arav D., Venter G.J. & Mellor P.S. (2006). – The oral susceptibility of UK *Culicoides* species complexes to bluetongue virus infection. *J. med. Entomol.*, **43**, 73-78.

10. Conte A., Giovannini A., Savini L., Goffredo M., Calistri P. & Meiswinkel R. (2003). – The effect of climate on the presence of *Culicoides imicola* in Italy. *J. vet. Med., B*, **50**, 139-147.

11. Cook G. (1992). – Effect of global warming on the distribution of parasitic and other infectious diseases: a review. *J. roy. Soc. Med.*, **85**, 688-691.

12. Daniels P.W., Sendow I., Pritchard L.I., Sukarsih I. & Eaton B.T. (2003). – Regional overview of bluetongue viruses in South East Asia: viruses, vectors and surveillance. *Vet. ital.*, **40**, 94-100.

13. De Liberato C., Purse B.V., Goffredo M., Scholl F. & Scaramozzino P. (2003). – Geographical and seasonal distribution of the bluetongue virus vector, *Culicoides imicola*, in central Italy. *Med. vet. Entomol.*, **17**, 388-394.

14. De Liberato C., Scavia G., Lorenzetti R., Scaramozzino P., Amaddeo D., Cardeti G., Scicluna G., Ferrari G., Autorini G.L. (2005). – Identification of *Culicoides obsoletus* (Diptera: Ceratopogonidae) as a vector of bluetongue virus in central Italy. *Vet. Rec.*, **156**, 301-304.

15. Ducheyne E., Codina B., Bécu S., De Deken R., Nomikou K., Mangana-Vougaki O., Georgiev G., Purse B.V. & Hendrickx G. (2007). – Quantifying the dispersal of *Culicoides* species in Greece and Bulgaria by wind. *Geospatial Hlth*, **2**, 177-189.

16. European Food Standards Agency (EFSA) (2007). – Epidemiological analysis of the 2006 bluetongue virus serotype 8 epidemic in north-western Europe, EFSA bluetongue epidemiology working group, February 2007. EFSA, Parma.

17. Fomby T.B. & Vogelsang T.J. (2003). – Test of common deterministic trend slopes applied to quarterly global temperature data. *In* Maximum likelihood estimation of misspecified models: twenty years later. Advances in Econometrics, Vol. 17. JAI Press, Greenwich, Connecticut, USA.

18. Food and Agriculture Organization of the United Nations (FAO) (2001). – Livestock geography: an introductory atlas of resources. FAO, Rome.

19. Giangaspero M., Vanopdenbosche E., Clercq K.D., Tabbaa D. & Nishikawa H. (1995). – Sero-epidemiological survey for bluetongue virus, orf virus, caprine herpes virus type 1 and foot and mouth disease virus type A, O and C in Awassi sheep in Syria. *Vet. ital.*, **31**, 24-29.

20. Gloster J., Mellor P.S., Burgin L., Sanders C. & Carpenter S. (2007). – Will bluetongue come on the wind to the United Kingdom in 2007? *Vet. Rec.*, **160**, 422-426.

21. Gloster J., Mellor P.S., Manning A.J., Webster H.N. & Hort M.C. (2007). – Assessing the risk of windborne spread of bluetongue in the 2006 outbreak of disease in northern Europe. *Vet. Rec.*, **160**, 54-56.

22. Gubbins S., Carpenter S., Baylis M., Wood J.L.N. & Mellor P.S. (2007). – Assessing the risk of bluetongue to UK livestock: uncertainty and sensitivity analysis of a temperature-dependent model for the basic reproduction number. *J. Roy. Soc. Interface*, **10**, 1098-1110.

23. Guis H., Tran A., de La Rocque S., Baldet T., Gerbier G., Barrague B., Biteau-Coroller F., Roger F., Viel J.F. & Mauny F. (2007). – Use of high spatial resolution satellite imagery to characterize landscapes at risk for bluetongue. *Vet. Res.*, **38** (5), 669-683.

24. Hassan A. (1992). – Status of bluetongue in the Middle East and Africa. *In* Bluetongue, African horse sickness and related orbiviruses. Proc. 2nd International Symposium (T.E. Walton & B.I. Osburn, eds), 17-21 June 1991, Paris. CRC Press, Boca Raton, Florida, 38-41.

25. Haylock M.R. & Goodess C.M. (2004). – Interannual variability of European extreme winter rainfall and links with mean large-scale circulation. *Int. J. Climatol.* **24**, 759-776.

26. Hoar B.R., Carpenter T.E., Singer R.S. & Gardner I.A. (2004). – Probability of introduction of exotic strains of bluetongue virus into the US and into California through importation of infected cattle. *Prev. vet. Med.*, **66**, 79-91.

27. Institute for Animal Health (2007). – Bluetongue outbreaks. Available at: http://www.iah.ac.uk/disease/bt_aw.shtml (accessed in October 2007).

28. Jennings D.M. (1983). – *Culicoides* from Western Turkey in relation to bluetongue disease of sheep and cattle. *Rev. Elev. Méd. vét. Pays trop.*, **36**, 67-70.

29. Johnson D.J., Mertens P.P.C., Maan S. & Ostlund E.N. (2007). – Exotic bluetongue viruses identified from ruminants in the southeastern US from 1999-2006. *In* Proc. of the American Association of Veterinary Laboratory Diagnosticians 50th Annual Conference, October 2007, Reno, Nevada, 118 pp. Available at: http://data.memberclicks.com/site/aavld/2007%20AAVLD%20Procdngs%20book%20(2).pdf (accessed in January 2008).

30. Kirkland P.D. (2003). – Bluetongue viruses, vectors and surveillance in Australia: the current situation and unique features. *In* Proc. Third International OIE Bluetongue Symposium. Taormina, Italy.

31. Klein Tank A.M.G. & Konnen G.P. (2003). – Trends in indices of daily temperature and precipitation extremes in Europe, 1946-1999. *J. Clim.*, **16**, 3665-3680.

32. Klein Tank A.M.G., Wijngaard J.B. & Van Engelen A.F.V. (2002). – Climate of Europe: assessment of observed daily temperature and precipitation extremes. KNMI (Royal Netherlands Meteorological Society), De Bilt, the Netherlands. Available at: http://knmi.nl/samenw/eca.

33. Kovats R.S., Campbell-Lendrum D.H., McMichael A.J., Woodward A. & Cox J.S. (2001). – Early effects of climate change: do they include changes in vector-borne disease? *Philos. Trans. roy. Soc. Lond., B, biol. Sci.*, **356**, 1057-1068.

34. Kuhn K.G., Campbell-Lendrum D.H., Armstrong B. & Davies C.R. (2003). – Malaria in Britain: past, present and future. *Proc. natl Acad. Sci. USA*, **100**, 9997-10001.

35. Kuhn K.G., Campbell-Lendrum D.H. & Davies C.R. (2004). – Tropical diseases in Europe? How we can learn from the past to predict the future. *EpiNorth J.*, **5** (1), 6-13.

36. Martens P. & Moser S.C. (2001). – Health impacts of climate change. *Science*, **292**, 1065-1066.

37. Mehlhorn H., Walldorf V., Klimpel S., Jahn B., Jaeger F., Eschweiler J., Hoffman B. & Beer M. (2007). – First occurrence of *Culicoides obsoletus*-transmitted bluetongue virus epidemic in Central Europe. *Parasitol. Res.*, **101** (1), 219-228.

38. Meiswinkel R., van Rijn P., Leus P. & Goffredo M. (2007). – Potential new *Culicoides* vector of bluetongue virus in Northern Europe. *Vet. Rec.*, **161**, 564-565.

39. Mellor P.S. & Boorman J. (1995). – The transmission and geographical spread of African horse sickness and bluetongue viruses. *Ann. trop. Med. Parasitol.*, **89** (1), 1-15.

40. Mellor P.S., Boorman J. & Baylis M. (2000). – *Culicoides* biting midges: their role as arbovirus vectors. *Annu. Rev. Entomol.*, **45**, 307-340.

41. Mellor P.S., Jennings M. & Boorman J.P.T. (1984). – *Culicoides* from Greece in relation to the spread of bluetongue virus. *Rev. Elev. Méd. vét. Pays trop.*, **37**, 286-289.

42. Mellor P.S., Jennings D.M., Wilkinson P.J. & Boorman J.P.T. (1985). – *Culicoides imicola*: a bluetongue virus vector in Spain and Portugal. *Vet. Rec.*, **116**, 589-590.

43. Mellor P.S. & Leake C.J. (2000). – Climatic and geographic influences on arboviral infections and vectors. *In* An update on zoonoses (P.-P. Pastoret, ed.). *Rev. sci. tech. Off. int. Epiz.*, **19** (1), 41-54.

44. Mellor P.S. & Pitzolis G. (1979). – Observations of breeding sites and light-trap collections of *Culicoides* during an outbreak of bluetongue in Cyprus. *Bull. entomol. Res.*, **69**, 229-234.

45. Mellor P.S., Rawlings P., Baylis M. & Wellby M.P. (1998). – Effect of temperature on African horse sickness virus infection in *Culicoides*. *In* African horse sickness (P.S. Mellor, M. Baylis, C. Hamblin, C. Calisher & P.P.C. Mertens, eds). Springer, Vienna.

46. Mellor P.S. & Wittmann E.J. (2002). – Bluetongue virus in the Mediterranean Basin 1998-2001. *Vet. J.*, **164** (1), 20-37.

47. Mertens P.P.C. & Attoui H. (eds) (2007). – Summary of the molecular epidemiology of bluetongue virus in Europe since 1998: introductions, origins and movements of individual virus strains. Available at http://www.iah.bbsrc.ac.uk/dsRNA_virusproteins/ReoID/BTV-mol-epidme.htm (accessed in October 2007).

48. Mertens P.P.C., Maan N.S., Prasad G., Samuel A.R., Shaw A.E., Potgeiter A.C., Anthony S.J. & Maan S. (2007). – Design of primers and use of RT-PCR assays for typing European bluetongue virus isolates: differentiation of field and vaccine strains. *J. gen. Virol.*, **88**, 2811-2823.

49. Murray M.D. (1991). – The seasonal abundance of female biting midges, *Culicoides brevitarsis* Kieffer (Diptera: Ceratopogonidae), in coastal south-eastern Australia. *Aust. J. Zool.*, **39**, 333-342.

50. Parmesan C. & Yohe G. (2003). – A globally coherent fingerprint of climate change impacts across natural systems. *Nature*, **421**, 37-42.

51. Patz J.A. (2002). – A human disease indicator for the effects of recent global climate change. *Proc. Natl Acad. Sci. USA*, **99**, 12506-12508.

52. Patz J.A., Campbell-lendrum D., Holloway T. & Foley J.A. (2005). – Impact of regional climate change on human health. *Nature*, **438**, 310-317.

53. Paweska J.T., Venter G.J. & Mellor P.S. (2002). – Vector competence of South African *Culicoides* species for bluetongue virus serotype 1 (BTV-1) with special reference to the effect of temperature on the rate of virus replication in *C. imicola* and *C. bolitinos*. *Med. vet. Entomol.*, **16**, 10-21.

54. Purse B.V., Baylis M., Tatem A.J., Rogers D.J., Mellor P.S., Van Ham M., Chizov-Ginzburg A. & Braverman Y. (2004). – Predicting the risk of bluetongue through time: climate models of temporal patterns of outbreaks in Israel. *Rev. sci. tech. Off. int. Epiz.*, **23** (3), 761-775.

55. Purse B.V., McCormick B.J.J., Baylis M., Boorman J.P.T., Borras D., Burgu I., Capela R., Caracappa S., Collantes F., De Liberato C., Delgado J.A., Denison E., Georgiev G., El Harak M., de La Rocque S., Lhor Y., Lucientes J., Mangana O., Mellor P.S., Miranda M.A., Nedelchev N., Nomikou K., Ozkul A., Patakakis M., Pena I., Scaramozzino P., Torina A. & Rogers D.J. (2007). – Incriminating bluetongue virus vectors with climate envelope models. *J. appl. Ecol.*, **44** (6), 1231-1242.

56. Purse B.V., Mellor P.S., Rogers D.J., Samuel A.R., Mertens P.P.C. & Baylis M. (2005). – Climate change and the recent emergence of bluetongue in Europe. *Nat. Rev. Microbiol.*, **3** (2), 171-181.

57. Purse B.V., Nedelchev N., Georgiev G., Veleva E., Boorman J., Denison E., Veronesi E., Carpenter S., Baylis M. & Mellor P.S. (2006). – Spatial and temporal distribution of bluetongue and its *Culicoides* vectors in Bulgaria. *Med. vet. Entomol.*, **20**, 335-344.

58. Purse B.V., Tatem A.J., Caracappa S., Rogers D.J., Mellor P.S., Baylis M. & Torina A. (2004). – Modelling the distributions of *Culicoides* bluetongue virus vectors in Sicily in relation to satellite-derived climate variables. *Med. vet. Entomol.*, **18**, 90-101.

59. Randolph S.E. (2004). – Evidence that climate change has caused 'emergence' of tick-borne diseases in Europe. *Int. J. Med. Microbiol.*, **37**, 5-15.

60. Reda I.M., Bastawisi I.M., Ismail I.M. & Agag A.E. (1992). – Is bluetongue virus still circulating in the blood of ruminants in Egypt? *Egypt. J. agric. Res.*, **70** (4), 1333-1339.

61. Reiter P. (1998). – Global warming and vector-borne disease in temperate regions and at high altitudes. *Lancet*, **351**, 839-840.

62. Rogers D.J. & Randolph S.E. (2003). – Studying the global distribution of infectious diseases using GIS and RS. *Nat. Rev. Microbiol.*, **1**, 231-236.

63. Rogers D.J. & Randolph S.E. (2006). – Climate change and vector-borne diseases. *Adv. Parasitol.*, **62**, 345-381.

64. Savini G., Goffredo M., Monaco F., de Santis P. & Meiswinkel R. (2003). – Transmission of bluetongue virus in Italy. *Vet. Rec.*, **152**, 119.

65. Savini G., Goffredo M., Monaco F., Di Gennaro A., Cafiero M.A., Baldi L., de Santis P., Meiswinkel R. & Caporale V. (2005). – Bluetongue virus isolations from midges belonging to the *Obsoletus* complex (*Culicoides*, Diptera: Ceratopoginidae) in Italy. *Vet. Rec.*, **157**, 133-139.

66. Sellers R.F. (1992). – Weather, *Culicoides* and the distribution and spread of African horse sickness viruses. *In* Bluetongue, African horse sickness and related viruses: Proceedings of the 2nd International Symposium (T.E.Walton & B.I. Osburn, eds) CRC Press, Boca Raton, 284-290.

67. Szadziewski R. (1984). – Ceratopogonidae from Algeria VI *Culicoides* LATR. *Polskie Pismo Entomol.*, **54**, 163-182.

68. Tabachnick W.J. (2003). – The history of bluetongue and a current global overview. *In* Proc. Third International OIE Bluetongue Symposium, Taormina, Italy.

69. Takamatsu H., Mellor P.S., Mertens P.P.C., Kirkham P.A., Burroughs J.N. & Parkhouse R.M.E. (2003). – A possible overwintering mechanism for bluetongue virus in the absence of the insect vector. *J. gen. Virol.*, **84**, 227-235.

70. Tatem A.J., Baylis M., Mellor P.S., Purse B.V., Capela R., Pena I. & Rogers D.J. (2003). – Prediction of bluetongue vector distribution in Europe and north Africa using satellite imagery. *Vet. Microbiol.*, **97** (1-2), 13-29.

71. Taylor W.P. (1986). – The epidemiology of bluetongue. *In* Animal Virus Research Institute Pirbright, UK, 60th Anniversary Celebration. *Rev. sci. tech. Off. int. Epiz.*, **5** (2), 351-356.

72. Taylor W.P., Sellers R.F., Gumm I.D., Herniman K.A.J. & Owen L. (1985). – Bluetongue epidemiology in the Middle East. *In* Bluetongue and related orbiviruses. Proc. 1st International Symposium (T.L. Barbert & M.M. Jochim, eds), 16-20 January 1984, Monterey. Alan R. Liss Inc., New York.

73. Torina A., Caracappa S., Mellor P.S., Baylis M. & Purse B.V. (2004). – Spatial distribution of bluetongue virus and its *Culicoides* vectors in Sicily. *Med. vet. Entomol.*, **18**, 81-89.

74. Van Dijk A.A. & Huismans H. (1982). – The effect of temperature on the in vitro transcriptase reaction of bluetongue virus, epizootic haemorrhagic disease virus and African horse sickness virus. *Onderstepoort J. vet. Res.*, **49**, 227-232.

75. Walker A.R. (1977). – Seasonal fluctuations of *Culicoides* species (Diptera: Ceratopogonidae) in Kenya. *Bull. entomol. Res.*, **67**, 217-233.

76. Walther G.R., Post E., Convey P., Menzel A., Parmesan C., Beebee T.J.C., Fromentin J.M., Hoegh-guldberg O. & Bairlein F. (2002). – Ecological responses to recent climate change. *Nature*, **416**, 389-395.

77. White D.M., Wilson W.C., Blair C.D. & Beaty B.J. (2005). – Studies on overwintering of bluetongue viruses in insects. *J. gen. Virol.*, **86**, 453-462.

78. Wilson A.J., Carpenter S., Gloster J. & Mellor P.S. (2007). – Re-emergence of BTV-8 in northern Europe in 2007. *Vet. Rec.*, **161**, 487-489.

79. Wittman E.J. & Baylis M. (2000). – Climate change: effects on *Culicoides*-transmitted viruses and implications for the UK. *Vet. J.*, **160**, 107-117.

80. Wittmann E.J., Mellor P.S. & Baylis M. (2002). – Effect of temperature on the transmission of orbiviruses by the biting midge, *Culicoides sonorensis*. *Med. vet. Entomol.*, **16**, 147-156.

81. Wright J.C., Getz R.R., Powe T.A., Nusbaum K.E., Stringfellow D.A., Mullen G.R. & Lauerman L.H. (1993). – Model based on weather variables to predict seroconversion to bluetongue virus in Alabama cattle. *Prev. vet. Med.*, **16** (4), 271-278.

Appendix F

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Bluetongue in Europe and the Mediterranean Basin: History of occurrence prior to 2006

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Abstract

Bluetongue virus (BTV) exists around the world in a broad band covering much of the Americas, Africa, southern Asia and northern Australia. Historically, it also occasionally occurred in the southern fringes of Europe. It is considered to be one of the most important diseases of domestic livestock. Recently BTV has extended its range northwards into areas of Europe never before affected and has persisted in many of these locations causing the greatest epizootic of bluetongue (BT), the disease caused by BTV, on record. Indeed, the most recent outbreaks of BT in Europe are further north than this virus has ever previously occurred anywhere in the world. The reasons for this dramatic change in BT epidemiology are complex but are linked to recent extensions in the distribution of its major vector, *Culicoides imicola*, to the involvement of novel *Culicoides* vector(s) and to on-going climate-change. This paper investigates these recent outbreaks in the European theatre, up to the beginning of 2006, highlights prospects for the future and sets the scene for the following papers in this special issue.

Keywords: Bluetongue; *Culicoides*; Vectors; Europe; Incursions; Climate-change

1. Introduction

Bluetongue virus (BTV) is a dsRNA virus within the genus *Orbivirus* of the family Reoviridae and exists as 24 distinct serotypes. The virus causes an insect-borne, infectious, non-contagious disease of ruminants (i.e. bluetongue: BT) and is transmitted between its hosts by *Culicoides* biting midges (Diptera: Ceratopogonidae). Venereal transmission between ruminants has been recorded but occurs very rarely and is not considered to be of

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epidemiological significance (Parsonson, 1990). Worldwide BTV has been estimated to cause direct (disease) and indirect (trade, vaccines, etc.) losses of over $3 billion per year (Tabachnick et al., 1996).

2. The hosts and the disease

BTV is probably able to infect all species of ruminant but severe disease usually occurs only in the fine-wool and mutton breeds of sheep and some species of deer (see: Mellor and Boorman, 1995). Clinical signs may include fever, depression, excessive salivation, nasal discharge, facial oedema, hyperaemia and ulceration of the oral mucosa, coronitis, lameness, and death. Older animals tend to be more susceptible to disease than younger ones and the severity of clinical signs seems to vary with the breed of the animal, the serotype and strain of the infecting virus and, with certain rather ill-defined interactions with the environment (see: Mellor and Boorman, 1995). Howell (1963) reports that in completely naïve populations of cattle clinical signs and even occasional deaths may be seen but generally speaking unimproved sheep, goats, cattle and wild ruminants are usually highly resistant to the clinical effects of infection. Consequently, the vast majority of BTV episodes throughout the world are completely silent. This covert presence of the virus, alternating with occasional outbreaks of severe disease has had a considerable and adverse effect upon international trade in bovines and ovines and their germ plasms as countries free from BTV strive to maintain that status (Mellor, 1994).

3. Transmission

In the field BTV is transmitted between its vertebrate hosts almost entirely via the bites of various species of *Culicoides* biting midge (Du Toit, 1944; Walker and Davies, 1971; Braverman and Galun, 1973; Cybinski et al., 1980; Mellor et al., 1984a; St George and Muller, 1984; Blackburn et al., 1985; Standfast et al., 1985; Mellor, 1990; Venter et al., 1991). The distribution of BTV is therefore limited to those regions where competent vector species of *Culicoides* are present and its transmission to those times of the year when the climatic conditions are favourable for adult vector activity and for virus replication in, and transmission by, the vectors (Sellers and Mellor, 1993; Mellor, 1994; Mellor et al., 2000; Mellor and Leake, 2000; Wittmann and Baylis, 2000; Purse et al., 2005).

In most epidemic zones, peak populations of adult vector *Culicoides* occur in the late summer and autumn and this is therefore when BT is usually seen (Mellor and Boorman, 1995). In such situations, the annual bouts of disease may reflect new virus introductions or may be the visible evidence of low-level persistence. Annual re-introduction is possible if endemic foci of the virus are close by, as infected *Culicoides* may be transported on the wind over distances of over 100 km (Sellers et al., 1977, 1979a,b; Sellers and Pedgley, 1985; Alba et al., 2004). Since BTV has not been proven to be transmitted transovarially through its vectors (Mellor, 1990) and is rarely transmitted directly from vertebrate to vertebrate, long-term persistence (i.e. an endemic zone) is currently thought to be possible only in areas where active adult vectors are present virtually throughout the year. In such situations if vector-free periods do occur they must be of shorter duration than the maximum period of viraemia in the local ruminant population (up to 54 days in sheep and 60–100 days in cattle), otherwise the last infected host will have died or recovered before new adult vectors arrive on the scene.

4. World distribution of BTV

BTV is traditionally understood as occurring around the world in a broad band stretching from about 35°S to 40°N although in certain areas (i.e. western North America, China and Kazakhstan) it may extend up to around 50°N (Dulac et al., 1989; Zhang et al., 1999, 2004; Lundervold et al., 2003). By and large this distribution is a reflection of the distribution of its *Culicoides* vectors and the temperature required for BTV replication in and transmission by the vectors.

In Europe, until recently, the above conditions were apparently fulfilled only in parts of Portugal, SW Spain, a few Greek Islands adjacent to the Anatolian Turkey and Cyprus, since prior to the 1990s these were the only areas to have experienced BTV. In the period 1956–1960, BTV-10 entered Portugal and SW Spain from North Africa and caused the deaths of almost 180,000 sheep (Manso-Ribeiro and Noronha, 1958; Campano Lopez and Sanchez Botija, 1958; Gorman, 1990), while during the years 1979–1980, BTV-4 briefly entered the Greek Islands of Rhodes and Lesbos (Vassalos, 1980; Dragonas, 1981). Cyprus seemed to be the only area of Europe where BTV occurred regularly (Sellers, 1975). In the late 1990s, however, Europe has witnessed a dramatic change in the epidemiology of BT.

5. BTV in the Mediterranean Basin 1998–2005

Between 1998 and the end of 2005 incursions of BTV occurred into many countries around the Mediterranean Basin that had previously never recorded the virus. At least five serotypes of BTV were involved and in all, virus activity was detected in at least 12 European countries three N. Africa countries and Israel. The total number of sheep dying from disease or culled has been estimated at well over 1 million which makes this by far the most severe outbreak of BT on record (Purse et al., 2005). The Proceedings of the 3rd International Symposium on Bluetongue held in Taormina, Italy (26–29 October 2003) and published in two volumes as a special edition of Veterinaria Italiana in 2004 provides a wealth of information on the early stages of the 1998–2005 BTV incursions into Europe and should be consulted by the interested reader.

6. Bluetongue virus in Greece, Turkey, the Balkans, Cyprus and Israel

6.1. Greece

BTV was first recorded in Greece in October 1979 when an outbreak of BTV-4 was confirmed on the island of Lesbos adjacent to Anatolian Turkey and from whence the infection was thought to have originated, as virus activity had already been reported there during 1978 and early 1979 (Vassalos, 1980; Yonguç et al., 1982). The outbreak was extensive within the half of the island closest to Turkey affecting 17 Communes with a sheep population of some 29,300. Sixty-eight flocks with about 5000 sheep were involved and the morbidity rate in individual flocks varied from 10 to 90% with a mortality rate of 29% (Vassalos, 1980; Mastroyanni et al., 1981; Nomikou et al., 2004). Clinical signs were not seen in any of the 5500 cattle or 18,000 goats on the island, neither were they observed in sheep after the end of 1979 (Nomikou et al., 2004). In the following year serological evidence of BTV but not disease was also recorded in sheep on island of Rhodes (Dragonas, 1981). Entry of the virus into the Greek islands, in the absence of official imports of ruminants was assumed to have been via movement of infected, wind-borne *Culicoides* from Turkey (Boorman and Wilkinson, 1983). Control on the Greek islands was effected by movement of animals' restrictions, prohibition of the export of live ruminants and, on Lesbos, by slaughter

of all infected, recovered and seropositive animals. Following implementation of these measures and in the absence of disease or seroconversion Greece was declared BT-free in 1991 (Nomikou et al., 2004).

In October 1998 BTV was again confirmed in Greece, on four Islands (Rhodes, Leros, Kos and Samos) close to the Anatolian Turkish coast (Anon, 1998a, b). The virus was identified as BTV-9 (Anon, 2000h) and this was the first occasion that this serotype had been recorded in Europe, though it had previously been identified in western Anatolian Turkey (Taylor and Mellor, 1994).

Outbreaks on the islands continued until December 1998 (Anon, 1999a), and then ended, probably because activity of the vector midge, *Culicoides imicola*, is at a minimum during this period and for the remainder of the winter (Anon, 2000f; M. Patakakis, personal communication). No other outbreaks of BT were reported anywhere in eastern Europe or adjacent regions over the next 5 months.

In August 1999, however, BTV was reported from mainland Greece for the first time ever, initially in the NE adjacent to the Turkish and Bulgarian borders (Anon, 1999b) and, just subsequent to the announcement of its presence in these areas. During September and October the virus spread across northern Greece as far west as Thessaloniki and Larissa, and as far south as Magnisia and Evia, thus involving approximately one third of Greek territory (Anon, 1999d). New cases of BT continued to be reported in some areas of Greece, into December 1999 (Anon, 2000a). Also, during September 1999 a separate incursion of BTV was reported into the Greek island of Lesbos and further outbreaks were reported on several Dodecanese islands (Anon, 1999c). By the end of 1999, serological evidence of the virus and/or clinical signs of BT had been reported from 13 Aegean islands and 10 mainland Prefectures (Anon, 2000f; Mellor and Wittmann, 2002). During 2000 low-level BTV transmission continued to occur during the summer months and was also detected in the NW of Greece, a previously unaffected area (Mellor and Wittmann, 2002). Then in late August 2001 a more active BTV focus was once more detected on Greek territory, initially in the NW of the country. The virus was first detected close to the Albanian border (Anon, 2001a) and advanced into the country in a south-westerly direction, along the routes of rivers and canyons, and in the direction of the prevailing winds. This was interpreted as being a new incursion into Greece rather than a recrudescence. By November 2001 the incursion included the Prefectures of Grevena, Ioannina, Kastoria, Aitoloakarnania, Evritania, Larissa, Preveza, Thesprotia and Trikala, thus encompassing a broad swath stretching south-west from the Albanian border to Larissa on the Aegean coast. The island of Lesbos close to the Anatolian coast of Turkey also experienced 19 new outbreaks in what was presumably a separate incursion. Since autumn 2001, however, all Greek territories have been free of reported BTV activity.

During this series of Greek BTV incursions in addition to BTV-9, three further serotypes of the virus were identified: 1, 4 and 16 (Anon, 2000h). BTV-4 and -9 had both been reported previously, from regions immediately to the east of Europe i.e. BTV-4 from Lesbos, Anatolian Turkey, Syria, Jordan, Cyprus and Israel, and BTV-9 from Lesbos, Anatolian Turkey, Syria and Jordan (Taylor and Mellor, 1994; Taylor, 1987; Vassalos, 1980; Mellor and Pitzolis, 1979; Shimshony, 1987). BTV-16 occurs regularly in Israel (Taylor and Mellor, 1994; Shimshony, 1987) and, during the present series of outbreaks it was also reported from Anatolian Turkey. BTV-1, however, had not previously been reported from any country in or adjacent to the Mediterranean Basin.

Control in Greece was through, surveillance, animal movement restrictions, slaughter and, insecticide treatment of infected and ‘‘at risk’’ premises, at no time was vaccination practiced.

6.2. Turkey

BTV was first reported from Turkey in 1977 in Aydin Province in the western part of Anatolia (Yonguç et al., 1982). The outbreak, due to BTV-4, persisted into 1979 and spread to include the western Anatolian Provinces of Çanakkale, Balikesir, Manisa, Denizli and Antalya, plus Kocaeli on the Black Sea coast (Ertűrk et al., 2004). Although the outbreak was considered to have ended by the end of 1980, controlled mainly by the use of a locally produced attenuated vaccine serological evidence of BTV activity in cattle, which were not vaccinated, continued until at least 1982 and possibly 1988 (Burgu et al., 1992). At no time during these outbreaks in Anatolian Turkey was BTV reported from the European part of the country.

In July 1999 a BTV incursion was reported into European Turkey involving animals in two Provinces (Edirne and Kirklareli) bordering Bulgaria and mainland Greece (Anon, 1999b). Then in October and November 1999 BT was reported for the first time in the current epidemic from four Provinces in the western part of Anatolia (Izmir, Aydin, Manisa and Denizli: Anon, 1999d). In August 2000, BT was again reported from western Anatolia (Izmir) but this time serotype 16 of the virus was isolated (Anon, 2000c; Hamblin, personal communication). In 2003 serological surveys in cattle were undertaken in five provinces in European Turkey and 23 Provinces in Anatolia (six in the west, eight in the NE, and nine in the SE) (see: Final Report EU project QLK2-CT-2000-00611). Seroprevalence rates of up to 90% were detected in some regions. Serum neutralization tests against BTV-4, -9 and -16, showed that all three serotypes were active during 2003 in Anatolia, and BTV-9 and -16 were also active in European Turkey (see: Final Report EU project QLK2-CT-2000-00611). Control was implemented via clinical surveillance, animal movement restrictions and the use of a locally produced live attenuated virus vaccine against BTV-4 (Mellor and Wittmann, 2002).

6.3. Bulgaria

In June 1999, BTV entered Bulgaria for the first time ever and serotype 9 was confirmed in Burgas in the south-eastern part of the country bordering the Black Sea (Anon, 1999e). The virus spread rapidly, across southern Bulgaria, eventually involving ruminants in four Districts: Burgas, Yambol, Haskovo and Kardjaly (Anon, 1999b). In total, 85 villages were affected and clinical signs were recorded at a low rate of prevalence (average 1.7%) in every flock examined (Purse et al., 2006). At the same time, nine out of 19 sentinel herds situated in Yambol, Haskovo and Kardjaly Districts also sero-converted, confirming BTV transmission at least 30 km from disease outbreak areas and up to 70 km from the Bulgarian border, which suggests that the virus was much more widespread than the reported outbreaks of disease (Purse et al., 2006). Outbreaks persisted until late October 1999, when ambient temperatures fell sharply and transmission apparently ceased. For almost 2 years no further evidence of BTV transmission was recorded, however, in September 2001 a second incursion of BTV-9 was detected in the extreme western part of Bulgaria involving 76 villages in seven Districts (Vidin, Montana, Vratsa, Sofia, Pernik, Kiustendil and Blagoevgrad) close to the borders with the FYR of Macedonia, Yugoslavia and Romania (Purse et al., 2006). A characteristic of these new outbreaks was that clinical signs were rarely observed (i.e. in only 27 of 25,929 susceptible sheep in the affected areas) and almost all of the outbreaks were within 40 km of the international borders. Operation of a sentinel herd system during 2001 indicated that, just as in 1999, transmission ceased in October with the arrival of cooler weather. The sentinel system continued to operate during 2002 when seroconversion to BTV but no clinical disease was recorded in the Smolyan and Blagoevgrad Districts in SW

Bulgaria, close to the Greek border. Subsequent to 2002 and up to the end of 2005 no further BTV activity was detected in Bulgaria.

Control in Bulgaria was implemented via clinical and serological surveillance, animal movement restrictions, insecticide treatment and, in 1999–2000, restricted use of a South African attenuated, pentavalent vaccine against BTV serotypes 3, 8, 9, 10 and 11.

6.4. Other Balkan countries (FYR Macedonia, Albania, Yugoslavia, Croatia, Bosnia)

After the reports of BTV in Greece and Bulgaria during 2001, the FYR of Macedonia authorities introduced clinical surveillance for BT. In September 2001 typical clinical signs were identified in sheep in the Kriva Palanka Region in the north of the country close to the border with Yugoslavia (Anon, 2001b). The affected animals were slaughtered, zoosanitary control measures implemented and clinical surveillance extended throughout the whole country with serological surveillance in some areas. Further, low-level BTV activity was identified in October in an additional seven regions ranged across the country from east to west (Anon, 2001c). The number of animals affected was low and the clinical signs mild. The last clinical case was identified on 16th October 2001, subsequent to which time the ambient temperature decreased. No further BTV activity has been reported in the country.

At about the same time as in Macedonia, BTV was identified for the first time in Yugoslavia, in the south, south east and south west of Serbia, the south of Montenegro, and in the autonomous, UN administered, Province of Kosovo. Thirty-seven outbreaks in 16 municipalities were recorded in Serbia proper and the virus was identified as BTV-9 (Anon, 2001d, e). In 2002, serological surveys indicated that the virus had extended into more northerly areas of Serbia and Montenegro reaching the River Sava which at 44°50′N was the furthest north that BTV had reached in Europe at the time (Djuricic et al., 2004). In September 2003, BTV activity was again reported from four municipalities in Serbia. In Kosovo, subsequent to 2001, BTV has not been reported officially but serological evidence from cattle and sheep shows that the virus has continued to circulate during 2002–2004 (Osmani et al., 2006).

Clinical and serological evidence of BTV activity was first reported in Croatia in late 2001 in sheep in the Dubrovnik Region (Anon, 2001f). In 2002, a sentinel system of bovines in southern Croatia recorded seroconversions to BTV-9 during October–November of that year, with the areas affected being rather more northerly than in 2001 (Labrovic et al., 2004; Listes et al., 2004). In 2004, serological evidence of BTV activity was again reported in the Dubrovnik Region of Croatia, and the presence of BTV-9 and -16 identified—this was and is the only report of BTV-16 from any part of the Balkans. Subsequent to October 2004 BTV activity has not been reported in the country.

In Bosnia and Herzegovina, BTV activity was detected for the first time ever in August 2002 in 15 municipalities in the south and south east of the country close to the borders with Montenegro and Serbia, and the virus was identified as BTV-9. Further information has not been forthcoming.

Albania also reported the presence of BTV for the first time in 2002. In a survey of cattle, sheep and goats in October–November of that year, seroconversions to BTV-9 were detected in all of 15 districts surveyed, with cattle showing the highest prevalence (Di Ventura et al., 2004). The areas surveyed were mainly along the borders with Greece, Macedonia, Kosovo and Montenegro, although two Districts on the Adriatic coast were also covered plus Tirane in the centre of the country which exhibited the highest prevalence of infection (61%). Subsequent to 2002 no further BTV activity has been reported.

6.5. Cyprus

Cyprus is the one country in Europe that historically can be considered to fall within the BTV endemic zone. Evidence of BTV infection in the country stretches back as far as 1924 (Gambles, 1949; Howell, 1963) and virus activity has been recorded in 27/83 years since then (Neophytou personal communication). Initially, BTV-3 was identified but subsequently most outbreaks seem to have been due to BTV-4 (Gambles, 1949; Sellers, 1975). However, in December 2003, BTV-16 was identified in sentinel cattle in Larnaca District in the south of the country (Anon, 2004i). The extent and severity of BTV outbreaks in Cyprus have apparently varied from year to year with the most virulent being in 1924, 1939, 1943, 1951, 1965 and 1977. Vaccination of sheep with a monovalent live virus vaccine against BTV-4 was carried out between 1946 and 1957 but then was discontinued owing to widespread abortions following its use (Pitzolis, 1987). Because of the frequent incursions of BTV into Cyprus the authorities tend to adopt a pragmatic approach and have learned to live with the virus (Neophytou, personal communication).

6.6. Israel

Israel is situated in a region where BTV has been reported regularly (Braverman et al., 2004; Hassan, 1992). The first evidence of clinical disease was in sheep in 1943–1944 in northern parts of the country and since then the virus has appeared in most years (Shimshony, 2004). Outbreaks usually commence in July and continue until December with individual cases sometimes being detected at other times. Occasionally clinical signs are also seen in cattle. Five serotypes of BTV have been identified (2, 4, 6, 10 and 16) and control involves vaccination with a pentavalent live virus vaccine that includes all of these types (Shimshony, 2004). During the course of the current series of outbreaks in Europe and up to end of 2005, activity only by BTV-16 has been reported from Israel, in September 2003 (Anon, 2003a, b).

7. Bluetongue virus in North Africa, Italy, France, Spain and Portugal

7.1. North Africa—Tunisia

In January 2000 BT was reported for the first time ever from Tunisia, in areas in the NE of the country. The time of the incursion was estimated as early December 1999 (Anon, 2000a; Hammami, 2004) and the virus was typed as BTV-2 (Anon, 2000g). The origin of the incursion is uncertain, however, as foot and mouth disease virus had also entered Tunisia (and Algeria) during 1999, probably via cattle from Côte d'Ivoire and Guinea (Knowles and Davies, 2000), it is possible that BTV could have used the same or a similar route. Cattle in Africa often experience sub-clinical infections with BTV, and BTV-2 is common in sub-Saharan West Africa (Herniman et al., 1980, 1983).

No new cases of BT were detected during February–May 2000 but from June further outbreaks of BTV-2 were reported from 10 Districts in the eastern and central parts of the country (Anon, 2000d,i; Hammami, 2004). These persisted in some areas until October 2000 (Anon, 2000d). Control measures implemented in 2000 included isolation of infected flocks, spraying animal holdings with insecticide and mass vaccination (in 2000, 2001 and 2002) with a monovalent, live BTV-2 vaccine (Hammami, 2004).

In December 2002, a limited number of new outbreaks caused by BTV-2 occurred in central Tunisia in unvaccinated flocks but subsequently and up to early 2006 no further evidence of virus circulation was reported.

7.2. North Africa—Algeria

In July 2000 BTV-2 was reported from eastern Algeria close to the Tunisian border and outbreaks continued into September (Anon, 2000b,e; Hammami, 2004), including areas up to 250 km to the west of Tunisia (Anon, 2000c). In addition, samples taken from animals near Algiers, over 400 km to the west of Tunisia also recorded positive for BTV antibodies (Mellor and Wittmann, 2002). Control was based upon the use of insecticides and clinical surveillance (Hammami, 2004). Further BTV activity in the country has not been reported up to early 2006.

7.3. North Africa—Morocco

In late 2000, BTV antibodies were recorded in animals from a number of provinces across northern Morocco but there was no evidence of clinical disease (Abaddi, personal communication). Despite serological monitoring no further evidence of BTV activity in Morocco was detected in the succeeding 3 years (Hammami, 2004).

However, in 2004 the situation changed and starting in August and continuing to the end of the year a further incursion of BTV was recorded, this time clinically affecting sheep in 14 provinces in the north-west (Anon, 2004a–g). Surprisingly, this incursion was caused by BTV-4 (Anon, 2004b), and is therefore separate from the 2000–2002 incursions of BTV-2 into other areas of North Africa. So far the origin of this incursion of BTV 4 is unknown.

7.4. Italy

In August 2000 BTV-2 was confirmed for the first time ever on Italian territory. Sardinia was affected first (Anon, 2000c) but the virus spread rapidly to Sicily and southern mainland Italy (Anon, 2000k). The incursion into Sardinia was very severe with more than 6000 flocks affected and over 260,000 sheep dying (Anon, 2000k; Calistri et al., 2004). Outbreaks continued in the three regions through to the autumn, and then at a low level during the winter, until May 2001 by which time over 260,000 animals in 6869 flocks had been affected (Calistri et al., 2004). There then followed, what is termed the second epidemic, which lasted until April 2002. Once again Sardinia, Sicily and Calabria were involved, plus Basilicata, Campania, Latium and Tuscany with over 6800 outbreaks and the loss of approximately 250,000 sheep and goats (Calistri et al., 2004).

Vaccination was not employed until January 2002 when a campaign to vaccinate all susceptible domestic species, with a monovalent live BTV-2 vaccine, commenced in the affected regions (Calistri et al., 2004).

In April 2002, the third epidemic began which extended until April 2003. During this period in addition to BTV-2; BTV-4, BTV-9 and BTV-16 were also detected and clinical disease was observed in eight provinces (Basilicata, Calabria, Campania, Lazio, Molise, Puglia, Sardinia, and Sicily). BTV-4, -9 and -16 had previously been reported from the eastern Mediterranean area (Turkey, Greece and the Balkans) but the route of incursion into Italy remains uncertain, though illegal animal movement is suspected (Giovannini et al., 2004a,b). In areas where BTV-2 and -9 occurred together a live bivalent vaccine was employed, otherwise a monovalent vaccine against

BTV-2 was used. BTV-4 and -16 were not included in the vaccination programme at this stage (Giovannini et al., 2004a,b). The results of the vaccination campaigns suggest that where cover reached 90% clinical disease either disappeared or was much reduced and virus spread greatly diminished. However, in regions with lower levels of cover, no such reductions occurred (Giovannini et al., 2004a,b).

During the summer of 2003 a fourth epidemic commenced. Seroconversions were detected in many southern and central Italian Provinces but clinical disease was seen only in Sardinia where BTV-4 was isolated. Outbreaks continued into 2004 and by the end of the year circulation of one or more of BTV-2, -4, -9 and -16 had been recorded in 12 regions extending from Sicily in the south to Tuscany in the north, and from Puglia in the east to Sardinia in the west (see: http://www.te.izs.it/). Vaccination was carried out in all affected regions using the appropriate combinations of BTV-2, -4, -9 and -16 live virus vaccines.

During 2005 for the sixth consecutive year BTV circulation was recorded, in at least 10 provinces: Lazio (BTV-2 and -16), Liguria (BTV-16), Marche (BTV-16), Molise (BTV-2 and -9), Campania (BTV-2 and -9), Puglia (BTV-2, -9 and -16), Basilicata (BTV-2, -4 and -16), Calabria (BTV-2 and -16), Sicily (BTV-2, -4 and -16) and Sardinia (BTV-2, -4 and -16), and activity continued until at least until November (see: http://www.te.izs.it/). Again vaccination was carried in each of the affected areas.

7.5. France (Corsica)

BTV was recorded for the first time on the French island of Corsica, in October 2000 and the virus was identified as BTV-2 (Anon, 2000j). A vaccination campaign was quickly organized using a monovalent live vaccine and commenced during the winter of 2000–2001. However, full cover was not achieved as a second epidemic of BTV-2 occurred in July 2001 affecting animals, mainly in unvaccinated flocks, in the north and south of the island. Three hundred and thirty five flocks were affected, a sevenfold increase on 2000 (Breard et al., 2004). Accordingly, a second vaccination campaign was mounted during the winter and spring of 2001–2002 which seems to have been successful as no cases of BT were reported during 2002. In October 2003 a further BTV incursion was recorded into Corsica affecting vaccinated sheep in the south part of the island. The virus was identified as BTV-4 and a third vaccination campaign mounted from early November using a bivalent live, vaccine. In August 2004 and for the fourth year out of five BT was reported in Corsica, BTV-4 occurring in the north and west of the island, and BTV-16 in the south west (Anon, 2004h). A further vaccination campaign was mounted using live vaccines against BTV-2, -4 and -16, and further BTV transmissions were not detected after September 2004.

7.6. Spain

The first outbreaks of BT in Spain commenced on two Balearic Islands (Menorca and Majorca) in September–October 2000 (Anon, 2000d; Miranda et al., 2003; Gomez-Tejedor, 2004). Three hundred and five outbreaks occurred and the virus was identified as BTV-2. A vaccination campaign in sheep using a monovalent live BTV-2 vaccine was mounted on both islands from October 2000. BTV transmission was not recorded beyond November 2000 but a further vaccination campaign in sheep was mounted in the spring of 2001 and was extended to Ibiza even though no cases of BT were detected there (Gomez-Tejedor, 2004).

However, in October 2003 a second incursion of BTV was reported, into the eastern part of Menorca. The virus was identified as BTV-4 and a vaccination campaign based upon a live monovalent vaccine against that serotype mounted (Anon, 2003a,b; Gomez-Tejedor, 2004).

In October 2004 a further incursion of BTV-4, this time into mainland Spain, was identified (Gomez-Tejedor, 2004). The outbreak commenced in the southern Province of Cadiz in Andalucia and spread rapidly through Andalucia and part of Extramadura; the Spanish enclave of Ceuta in north Morocco was also affected (Gomez-Tejedor, 2004). Once again a vaccination campaign was launched in sheep using a live, monovalent BTV-4 vaccine. After a pause during the winter period the outbreak of BTV-4 in mainland Spain continued to spread during the summer of 2005, reaching further north than ever before, until by November 2005 it involved provinces in Andalucia, Castilla la Mancha, Castilla y Leon, Extramadura and Madrid (see: http://rasve.mapya.es/). Also in 2005, a single isolate of BTV-2 was made from a herd of sentinel bovines in mainland Spain (Elliott, personal communication). The origin of the 2004–2005 outbreaks of BTV-4 in mainland Spain was undoubtedly northern Morocco where the same serotype of virus was detected just prior to and during the Spanish outbreaks. Molecular studies also confirm the close similarity between the Spanish and Moroccan isolates of BTV-4 (Mertens, unpublished observations). The origin of the single isolate of BTV-2 is difficult to determine. However, molecular analysis has confirmed that it is virtually indistinguishable from the BTV-2 vaccine virus previously used in several countries and zones in southern Europe including the Spanish Balearic islands.

7.7. Portugal

BTV was first detected in sheep in Portugal in November 2004 in the Evora District of Alentejo, close to the border with Spain, and by December had spread to the Castelo Branco District of Beira Interior (Anon, 2004a,b; Boinas et al., 2005). The virus was identified as BTV-4 (Fevereiro et al., 2005) and a vaccination campaign in sheep mounted using a live BTV-4 vaccine (Boinas et al., 2005). At the same time a countrywide serological surveillance system was initiated. In 2005, seroconversion to BTV but no disease occurred in sentinel bovines until at least October (Boinas, personal communication). Further information from Portugal is not available. The Portuguese outbreaks are clearly an extension of the Spanish mainland incursions which originated in Morocco.

8. Origins of the 1998–2005 BTV incursions into Europe

The incursions of BTV-1, -4, -9 and -16 into Greece, European Turkey and the Balkans originated to the east or south east of Europe as shown by Mertens and his colleagues (see: http://www.iah.bbsrc.ac.uk/dsRNA_virus_proteins/ReoID/BTV-mol-epidem.htm). In this context it should be remembered that BTV-2, -3, -4, -6, -9, -10, -13, and -16 have all previously been reported from Anatolian Turkey, Syria, Jordan and/or Israel and, the westward movement of some of these through Turkey is well documented (Gambles, 1949; Urman et al., 1980; Vassalos, 1980; Yonguç et al., 1982; Burgu et al., 1992; Taylor and Mellor, 1994). BTV-1, however, has not previously been reported from these regions but Maan et al. (2004) have shown that the European isolates of this virus have a close genetic lineage to isolates of the serotype from India. It is therefore likely that, even though not recorded, BTV-1 has been introduced into the regions just to the east of Europe by the intense movements of ruminants from southern Asia along the Eurasian ruminant street. Consequently, it is likely that the area to the east of Europe contains a

large pool of assorted BTV serotypes some of which have previously been recorded and some, like BTV-1, which have not. Under the right environmental or animal movement conditions (trade) these BTV serotypes may be expected to extend into Europe and their presence thereby constitutes a significant threat to European livestock.

The European incursion of BTV-2 has a different origin. Prior to entry into Europe this virus was reported from Tunisia in 1999 and Algeria in 2000 but the virus was not endemic in this region as neither country had previously experienced an incursion by any serotype of BTV, and Morocco had not recorded the presence of BTV since 1956 (Placidi, 1957; Hammami, 2004). Consequently, the origin of this virus is uncertain but as foot and mouth disease virus had also entered Tunisia and Algeria during 1999, probably via cattle smuggled from Cote d'Ivoire and Guinea into Algeria (Knowles and Davies, 2000), it is possible that BTV could have followed a similar route. Many cattle in Africa experience sub-clinical infections with BTV, and BTV-2 is common in several areas of sub-Saharan West Africa (Herniman et al., 1983). Having reached North Africa, BTV-2 then expanded rapidly northwards into Sardinia, Corsica, the Balearics, Sicily and southern mainland Italy (Calabria). The importation of infected animals and their products has, in many cases, been ruled out (Miranda et al., 2003; Calistri et al., 2004) so the most likely mode of incursion is via the passive transport of infected vectors on the wind as ''aerial plankton''. Over the years many authors have written extensively on this subject providing both circumstantial and real evidence to suggest that *Culicoides* can travel hundreds of kilometres by such means (Sellers et al., 1979a,b; Hayashi et al., 1979; Murray and Nix, 1987; Sellers and Maarouf, 1991; Sellers and Mellor, 1993; Alba et al., 2004). Specifically, in the case of the BTV-2 outbreaks in Sardinia, Calistri et al. (2004) have shown that the first cases occurred in the southernmost part of the island which, due to its geographic proximity with North Africa, is where landfall of wind-borne *Culicoides* would be expected. These authors also reported that the initial incursion was, preceded by and coincided with, unusual climatic conditions in which dust storms originating in the infected areas of North Africa moved northwards across southern Italy and Sardinia, suggesting that such wind movements could have also transported infected midges.

Similarly, in 2004 an incursion of BTV-4 into the western Mediterranean Basin occurred. The outbreak was first detected in NE Morocco and then spread across the Straits of Gibraltar into the southernmost province of mainland Spain (Cadiz). Further spread of the virus within Spain, and then into Portugal was connected with the movement of animals but the initial incursion may have been due to windborne *Culicoides*. This is supported by the fact that unpublished analyses have shown that southerly winds blowing over NW Morocco and then the Cadiz area of Spain were prevalent at the time of the incursion and occurred during the time of the evening when *Culicoides* activity is maximal (Gloster, unpublished observations).

All of this information provides strong circumstantial evidence that infected *Culicoides* can be dispersed on the wind, distances of several hundred kilometres, thereby introducing virus into regions remote from the source of infection. It also suggests that movement of *Culicoides* vectors over such long distances is not a rare event. From earlier observations taken in Turkey, Greece, Spain and the USA, Sellers and Mellor (1993) have suggested that such flights by BTV-infected *Culicoides* are associated with temperatures from sea level to 1,500 m of 12.6–30 °C and, from a more recent study, can involve transit times of up to at least 10 h (Sanders et al., unpublished observations).

9. Vector species of *Culicoides*

C. imicola has long been known as a vector of BTV in those parts of the Mediterranean region affected by BTV prior to 1998 (i.e. Israel, Cyprus, Anatolian Turkey, the Greek islands of Rhodes

and Lesbos, Morocco, SW Spain and Portugal, see: Mellor and Wittmann, 2002). Indeed, the northernmost locations from which *C. imicola* had been identified in the region prior to 1998 (Portugal, Spain, Morocco, Algeria, Israel, Anatolian Turkey, Rhodes and Lesbos) is virtually identical to the list of the places affected, at one time or another by BTV prior to 1998. Furthermore, although *C. imicola* had been sought in many adjacent areas that had not been affected by BTV (northern and eastern Spain, Tunisia, Sicily, parts of mainland Italy, Bulgaria, mainland Greece) it had never been recorded from any of them (Callot et al., 1964; Callot and Kremer, 1969; Kremer et al., 1971; Chaker, 1981; Mellor et al., 1984b; Gloukova et al., 1991; Boorman et al., 1996; Scaramozzino et al., 1996; Dilovski et al., 1992; Gallo et al., 1984; Patakakis, personal communication; Georgiev and Nedelchev, unpublished observations; Wilkinson, personal communication). This suggested, that *C. imicola* was the only important BTV vector in the region otherwise the virus would presumably, on some occasions, have occurred in its absence. Consequently, the 1998–2005 BTV incursions into many parts of the Mediterranean Basin previously unaffected by the virus, including areas where *C. imicola* had been sought for but not found, was a new and totally unexpected turn of events. The entry of BTV into such areas, suggested, either that the range of *C. imicola* had recently expanded or that other vector species of *Culicoides* were, for the first time, transmitting BTV in the region. In the event, the answer seems to include both of these possibilities. Vector surveys in the BTV-affected areas from 2000 have recorded the presence of *C. imicola* at many locations where it was previously looked for in the 1970s and 80s but not found i.e. mainland Greece, mainland Italy, Sicily, Sardinia, Corsica, the Balearics, much of eastern Spain and parts of southern mainland France (Giovannini et al., 2004a,b; Goffredo et al., 2001, 2004; Miranda et al., 2003; Monteys and Saiz-Ardanaz, 2003; Purse et al., 2005; Monteys et al., 2004; Monteys et al., 2005). The range of this species, therefore, seems to have expanded northwards in recent times to include much of the northern coast of the Mediterranean Sea and most of the leg of Italy as far north as 44°N (Goffredo et al., 2001). The work of Purse et al. (2005) suggests that this sudden expansion is being driven by recent and on-going changes in the European climate.

However, even though *C. imicola* has extended northwards so dramatically, the range of BTV has spread beyond the range of this vector in certain parts of Europe, particularly the Balkans (Mellor, 2004a, b), and even within the overall distribution of *C. imicola*, in some areas, BTV transmission has taken place in locations where *imicola* is either very rare or absent (e.g. parts of Sicily, Lazio and Tuscany in Italy) (Purse et al., 2005; De Liberato et al., 2005). This means that in these areas novel vector species of *Culicoides* must be transmitting the virus. Recent studies have shown that two widespread and abundant Palearctic *Culicoides* species complexes (*Culicoides obsoletus* plus *Culicoides dewulfi* and, *Culicoides pulicaris*) comprise very large proportions of the *Culicoides* populations in the non-*imicola* BT areas and these species also show fine-scale spatial and temporal correlation with BTV outbreaks (Torina et al., 2004; De Liberato et al., 2003, 2005). Also, during the current outbreaks, multiple isolations of BTV have been made from wild-caught individuals of both species complexes (Caracappa et al., 2003; Savini et al., 2003, 2004; De Liberato et al., 2005). Earlier vector competence studies with these species suggested that they had very low oral susceptibility rates for BTV (Mellor and Jennings, 1988; Jennings and Mellor, 1988). However, the recent climate warming in Europe is likely to have increased their importance as vectors both by increasing their population sizes (as insects are poikilothermic, warmer weather leads to more frequent blood feeding and thereby increases the number of eggs produced) and by increasing their levels of oral susceptibility, through the temperature-controlled virus developmental effects described by Mellor (2004b). So climate warming may be the underlying reason why these novel vectors are, for the first time, playing an

important part in BTV transmission. The future role of these novel vectors in the transmission of BTV is a matter of much speculation as they are both common and widespread across the whole of central and northern Europe (Purse et al., 2005). What this means in terms of increasing risk from BTV to livestock of the more northerly countries of Europe may be deduced from the contents of the following papers in this special issue.

Conflict of interest

None.

References

Alba, A., Casal, J., Domingo, M., 2004. Possible introduction of bluetongue into the Balearic Islands, Spain, in 2000, via air streams. Vet. Rec. 155, 460–461.
Anon, 1998a. Disease outbreaks reported to the OIE in November–December 1998. Bull. Off. Int. Epizoot. 110, 506.
Anon, 1998b. Bluetongue in Greece: confirmation of diagnosis. Off. Int. Epizoot. Dis. Info. 11, 166–167.
Anon, 1999a. Disease outbreaks reported to the OIE in January–February 1999. Bull. Off Int. Epizoot. 111, 18.
Anon, 1999b. Disease outbreaks reported to the OIE in July–August 1999. Bull. Off. Int. Epizoot. 111, 379.
Anon, 1999c. Disease outbreaks reported to the OIE in September–October 1999. Bull. Off. Int. Epizoot. 111, 449.
Anon, 1999d. Disease outbreaks reported to the OIE in November–December 1999. Bull. Off. Int. Epizoot. 111, 543.
Anon, 1999e. Bluetongue in Bulgaria: additional information. Off. Int. Epizoot. Dis. Info. 12, 122.
Anon, 2000a. Disease outbreaks reported to the OIE in January–February 2000. Bull. Off. Int. Epizoot. 112, 16.
Anon, 2000b. Disease outbreaks reported to the OIE in July–August 2000. Bull. Off. Int. Epizoot. 112, 449.
Anon, 2000c. Disease outbreaks reported to the OIE in September–October 2000. Bull. Off. Int. Epizoot., 112, 552.
Anon. 2000d. Disease outbreaks reported to the OIE in November-December 2000. Bull. Off. Int. Epizoot. 112, 687–688.
Anon, 2000e. Bluetongue in Algeria. EMPRES Transbound. Anim. Dis. Bull. 14, 20–1.
Anon, 2000f. Bluetongue in Greece: follow-up report. Off. Int. Epizoot. Dis. Info. 13, 9–13.
Anon, 2000g. Bluetongue in Tunisia: additional information. Off. Int. Epizoot. Dis. Info. 13, 21.
Anon, 2000h. Bluetongue in Greece: typing of virus strains isolated in 1998–1999. Off. Int. Epizoot. Dis. Info. 13, 33–34.
Anon, 2000i. Bluetongue in Tunisia: follow-up report No. 2. Off. Int. Epizoot. Dis. Info. 13, 176.
Anon, 2000j. Bluetongue in France: in the island of Corsica. Off. Int. Epizoot. Dis. Info. 13, 195–197.
Anon, 2000k. Bluetongue in Italy: follow-up report No. 1. Off. Int. Epizoot. Dis. Info. 13, 209–210.
Anon, 2001a. Bluetongue in Greece and Italy. Off. Int. Epizoot. Dis. Info. 14, 215–218.
Anon, 2001b. Bluetongue in the Former Yugoslav Republic of Macedonia. Off. Int. Epizoot. Dis. Info. 14, 234.
Anon, 2001c. Bluetongue in the Former Yugoslav Republic of Macedonia: follow-up report No. 1. Off. Int. Epizoot. Dis. Info. 14, 265–268.
Anon, 2001d. Bluetongue in Kosovo (FRY), territory under United Nations interim administration. Off. Int. Epizoot. Dis. Info. 14, 242–243.
Anon, 2001e. Bluetongue in Yugoslavia. Off. Int. Epizoot. Dis. Info. 14, 252.
Anon, 2001f. Bluetongue in Croatia: Suspected outbreak. Off. Int. Epizoot. Dis. Info. 14, 291.
Anon, 2003a. Bluetongue in Israel. Off. Int. Epizoot. Dis. Info. 16, 247.
Anon, 2003b. Bluetongue in Israel (additional Information). Off. Int. Epizoot. Dis. Info. 16, 258.
Anon, 2004a. Bluetongue in Morocco. Off. Int. Epizoot. Dis. Info. 17, 273.
Anon, 2004b. Bluetongue in Morocco (Follow up Report no. 1). Off. Int. Epizoot. Dis. Info. 17, 297.
Anon, 2004c. Bluetongue in Morocco (Follow-up Report no. 2). Off. Int. Epizoot. Dis. Info. 17, 318.
Anon, 2004d. Bluetongue in Morocco (Follow-up Report no. 3). Off. Int. Epizoot. Dis. Info. 17, 329.
Anon, 2004e. Bluetongue in Morocco (Follow-up Report no. 4). Off. Int. Epizoot. Dis. Info. 17, 346.
Anon, 2004f. Bluetongue in Morocco (Follow-up Report no. 5). Off. Int. Epizoot. Dis. Info. 17, 368.
Anon, 2004g. Bluetongue in Morocco (Follow-up Report no. 6). Off. Int. Epizoot. Dis. Info. 17, 383.
Anon, 2004h. Bluetongue in France in the Island of Corsica. Off. Int. Epizoot. Dis. Info. 17, 266.
Anon, 2004i. Bluetongue in Cyprus. Off. Int. Epizoot. Dis. Info. 17, 82–83.
Blackburn, N.K., Searle, L., Phelps, R.J., 1985. Viruses isolated from *Culicoides* (Dipt. Cerat.) caught at the veterinary research farm, Mazoure, Zimbabwe. J. Entomol. Soc. S. Afr. 48, 331–336.

Boinas, F., Amador, R., Pereira da Fonseca, I., Nunes, T., Fevereiro, M., Agrela Pinheiro, C., 2005. Perspectives of bluetongue control in Portugal. In: Proceedings od the 3rd Vet. Sci. Cong. Santarém, Sociedade Portuguesa de Ciências Veterinárias, Lisbon, Portugal, October 13–15, p. 18.

Boorman, J.P.T., Wilkinson, P.J., 1983. Potential vectors of bluetongue in Lesbos, Greece. Vet. Rec. 113, 395–396.

Boorman, J., Mellor, P.S., Scaramozzino, P., 1996. A new species of *Culicoides* (Diptera: Ceratopogonidae) from southern Italy. Parassitologia 38, 501–503.

Braverman, Y., Galun, R., 1973. The occurrence of *Culicoides* in Israel with reference to the incidence of bluetongue. Ref. Vet. 30, 121–127.

Braverman, Y., Baylis, M., Baylis, M., Tatem, A.J., Rogers, D.J., Mellor, P.S., Purse, B.V., 2004. What factors determine when epidemics occur in the Mediterranean? Prediction of disease risk through time by climate-driven models of the temporal distribution of outbreaks in Israel. Vet. Ital. 40, 235–242.

Breard, E., Hamblin, C., Hammami, S., Sailleau, C., Dauphin, G., Zientara, S., 2004. The epidemiology and diagnosis of bluetongue with particular reference to Corsica. Res. Vet. Sci. 77, 1–8.

Burgu, I., Urman, H.K., Akca, Y., Yonguc, A.D., Mellor, P.S., Hamblin, C., 1992. Serological survey and vector surveillance for bluetongue in southern Turkey. In: Walton, T.E., Osburn, B.I. (Eds.), Proceedings of the 2nd International Symposium Bluetongue, African Horse Sickness and Related Orbiviruses. CRC Press, Boca Raton, FL, pp. 168–174.

Calistri, P., Giovannini, A., Conte, A., Nannini, D., Santucci, U., Patta, C., Rolesu, S., Caporale, V., 2004. Bluetongue in Italy. Part 1. Vet. Ital. 40, 243–251.

Callot, J., Kremer, M., 1969. Description d'un *Culicoides* nouveau: *C. jumineri* (Diptera, Ceratopogonidae) trouve en Tunisie. Bull. Soc. Exo. Pathol. 62, 1112–1118.

Callot, J., Kremer, M., Juminer, B., 1964. Contribution a l'etude des *Culicoides* (Diptera, Ceratopogonidae) de Tunisie. Arch. Inst. Pasteur Tunis 41, 357–364.

Campano Lopez, A., Sanchez Botija, C., 1958. L'epizootie de fievre catarrhale ovine en Espagne (Blue Tongue). Bull. Off. Int. Epizoot. 50, 65–93.

Caracappa, S., Torina, A., Guercio, A., Vitale, F., Calabro, A., Purpari, G., Ferrantelli, V., Vitale, M., Mellor, P.S., 2003. Identification of a novel bluetongue virus vector species of *Culicoides* in Sicily. Vet. Rec. 153, 71–74.

Chaker, E., 1981. Memoire presente en vue de l'obtention du diplome d'etudes et de recherches en biologie humaine. Universite Louis Pasteur de Strasbourg, pp. 196.

Cybinski, D.H., Muller, M.J., Squire, K.R.E., Standfast, H.A., St George, T.D., Uren, M.F., Dyce, A.L., 1980. Bluetongue vector studies. CSIRO Div. Anim. Hlth. Res Reports, 1979–1980. 19.

De Liberato, C., Purse, B.V., Goffredo, M., Scholl, F., Scaramozzino, P., 2003. Geographical and seasonal distribution of the bluetongue virus vector, *Culicoides imicola*, in central Italy. Med. Vet. Entomol. 17, 388–394.

De Liberato, C., Scavia, G., Lorenzetti, R., Scaramozzino, P., Amaddeo, D., Cardeti, G., Scicluna, M., Ferrari, G., Autorino, G.L., 2005. Identification of *Culicoides obsoletus* (Diptera: Ceratopogonidae) as a vector of bluetongue virus in central Italy. Vet. Rec. 156, 301–304.

Di Ventura, M., Tittarelli, M., Semproni, G., Bonfini, B., Savini, G., Conte, A., Lika, A., 2004. Serological surveillance of bluetongue virus in cattle, sheep and goats in Albania. Vet. Ital. 40, 101–104.

Dilovski, M., Nedelchev, N., Petkova, K., 1992. Studies on the species composition of *Culicoides*—potential vectors of the virus of the bluetongue in Bulgaria. Vet. Sci. 26, 52–56.

Djuricic, B., Nedic, D., Lausevic, D., Pavlovic, M., 2004. The epizootiological occurrence of bluetongue in the central Balkans. Vet. Ital. 40, 105–107.

Dragonas, P.N., 1981. Evolution of bluetongue in Greece. OIE Mon. Epizoot. Circ. 9, 10–11.

Du Toit, R.M., 1944. Transmission of bluetongue and African horse sickness by *Culicoides*. Onderstepoort J. Vet. Sci. Anim. Ind. 19, 7–16.

Dulac, G.C., Dubuc, C., Myers, D.J., Afshar, A., Taylor, E.A., 1989. Incursion of bluetongue virus type 11 and epizootic haemorrhagic disease of deer type 2 for two consecutive years in the Okanagan Valley. Can. Vet. J. 30, 351.

Ertűrk, A., Tartar, N., Kabakali, O., Incoglu, S., Cizmeci, S.G., Barut, F.M., 2004. The current situation of bluetongue in Turkey. Vet. Ital. 40, 137–140.

Fevereiro, M., Luis, T., Vaz, A., Ramos, F., Fagulha, M.T., Barros, S., Duarte, M.M., Cruz, M.B. S, 2005. Bluetongue virus serotype 4 in Portugal. In: Proceedings of the 3rd Vet. Sci. Cong. Santarém, Sociedade Portuguesa de Ciências Veterinárias, Lisbon, Portugal, October 13–15, p. 107.

Gallo, C., Guercio, V., Caracappa, S., Boorman, J.P.T., Wilkinson, P.J., 1984. Indagine siero-entomologica sulla possible presenza del virus bluetongue nei bovini in Sicilia. Atti della Societa Italiana di Buiatria 16, 393–398.

Gambles, R.M., 1949. Bluetongue in sheep in Cyprus. J. Comp. Path. 59, 176–190.

Giovannini, A., Calistri, P., Conte, A., Savini, L., Nannini, D., Patta, C., Santucci, U., Caporale, V., 2004a. Bluetongue virus surveillance in a newly infected area. Vet. Ital. 40, 188–197.

Giovannini, A., Calistri, P., Nannini, D., Paladini, C., Santucci, U., Patta, C., Caporale, V., 2004b. Bluetongue in Italy. Part II. Vet. Ital. 40, 252–259.

Gloukova, V.M., Nedelchev, N.K., Rousev, I., Tanchev, T., 1991. On the fauna of blood-sucking midges of the genus *Culicoides* (Diptera: Ceratopogonidae) in Bulgaria. Vet. Sci. 25, 63–66.

Goffredo, M., Satta, G., Torina, A., Federico, G., Scaramozzino, P., Cafiero, M.A., Lelli, R., Meiswinkel, R., 2001. The 2000 bluetongue virus outbreak in Italy: distribution and abundance of the principle vector *Culicoides imicola* Kieffer. In: Proceedings of the 10th International Symposium of American Association of Veterinary Laboratory Diagnosticians, Salsomaggiore, Parma, AAVLD, Ames, July 4–7, pp. 308–309.

Goffredo, M., Conte, A., Meiswinkel, R., 2004. Distribution and abundance of *Culicoides imicola*, Obsoletus Complex and Pulicaris Complex (Diptera: Ceratopogonidae) in Italy. Part I. Vet Ital. 40, 270–273.

Gomez-Tejedor, C., 2004. Brief overview of the bluetongue situation in Mediterranean Europe, 1998–2004. Vet. Ital. 40, 57–60.

Gorman, B.M., 1990. The bluetongue viruses. Curr. Top. Microbiol. Immunol. 162, 1–19.

Hammami, S., 2004. North Africa: a regional overview of bluetongue virus, vectors, surveillance and unique features. Vet. Ital. 40, 43–46.

Hassan, A., 1992. Status of bluetongue in the Middle East and Africa. In: Walton, T.E., Osburn, B.I. (Eds.), Proceedings of the 2nd International Symposium on Bluetongue, African Horse Sickness and Related Orbiviruses, Paris. CRC Press, Boca Raton, FL. pp. 38–41.

Hayashi, K., Suzuki, H., Asahina, S., 1979. Notes on the transoceanic insects-captured on East China Sea in 1976, 1977, and 1978. Trop. Med. 21, 1–10.

Herniman, K.A.J., Gumm, I.D., Owen, L., Taylor, W.P., Sellers, R.F., 1980. Distribution of bluetongue virus and antibodies in some countries of the eastern hemisphere. OIE Bull. 92, 581–586.

Herniman, K.A.J., Boorman, J.P.T., Taylor, W.P., 1983. Bluetongue virus in a Nigerian dairy cattle herd. 1. Serological studies and correlation of virus activity to vector population. J. Hyg. Camb. 90, 177–193.

Howell, P.G., 1963. Bluetongue. In: Emerging Diseases of Animals, FAO, Rome, pp. 111–153.

Jennings, D.M., Mellor, P.S., 1988. The vector potential of British *Culicoides* species for bluetongue virus. Vet. Microbiol. 17, 1–10.

Knowles, N.J.D., Davies, P.R., 2000. Origin of recent outbreaks of foot-and-mouth disease in North Africa, the Middle East and Europe. In: Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, 3rd ed. Borovets, Bulgaria. FAO, Rome, pp. 39–45.

Kremer, M., Leberre, G., Beaucournu-Saguez, F., 1971. Notes sur le *Culicoides* (Diptera, Ceratopogonidae) de Corse. Description de C. corsicus n. sp. Ann. Parasitol. Humaine Comp. 46, 635–660.

Labrovic, A., Poljak, Z., Separovic, S., Jukic, B., Lukman, D., Listes, E., Bosnic, S., 2004. Spatial distribution of bluetongue in cattle in southern Croatia in the last quarter of 2002. Vet. Ital. 40, 217–220.

Listes, E., Bosnic, S., Benic, M., Lojkic, M., Cac, Z., Cvetnic, Z., Madic, J., Separovic, S., Labrovic, A., Savini, G., Goffredo, M., 2004. Serological evidence of bluetongue and a preliminary entomological study in southern Croatia. Vet. Ital. 40, 221–225.

Lundervold, M., Milner-Gulland, E.J., O'Callaghan, C.J., Hamblin, C., 2003. First evidence of bluetongue virus in Kazakhstan. Vet. Microbiol. 92, 281–287.

Maan, S., Samuel, A.R., Maan, N.S., Attoui, H., Rao, S., Mertens, P.P.C., 2004. Molecular epidemiology of bluetongue viruses from disease outbreaks in the Mediterranean Basin. Vet. Ital. 40, 489–496.

Manso-Ribeiro, J., Noronha, F.M.O., 1958. Fievre catarrhale du mouton au Portugal (Blue Tongue). Bull. Off. Int. Epizoot. 50, 46–64.

Mastroyanni, M., Axiotis, I., Stoforos, E., 1981. Study of the first outbreak of bluetongue disease in sheep in Greece. Bull. Hell. Vet. Ass. 32, 138–144.

Mellor, P.S., 1990. The replication of bluetongue virus in *Culicoides* vectors. Curr. Top. Microbiol. Immunol. 162, 143–161.

Mellor, P.S., 1994. Bluetongue. State Vet. J. 4, 7–10.

Mellor, P.S., 2004a. Infection of the vectors and bluetongue epidemiology in Europe. Vet. Ital. 40, 167–174.

Mellor, P.S., 2004b. Environmental influences on arbovirus infections and vectors. In: Gillespie, S.H., Smith, G.L., Osbourn, A. (Eds.), SGM Symposium, 63. Microbe–Vector Interactions in Vector-Borne Diseases. Cambridge University Press, Cambridge, pp. 181–197.

Mellor, P.S., Boorman, J., 1995. The transmission and geographical spread of African horse sickness and bluetongue viruses. Ann. Trop. Med. Parasitol. 89, 1–15.

Mellor, P.S., Jennings, D.M., 1988. The vector potential of British Culicoides species for bluetongue virus. In: Roy, P., Osburn, B. (Eds.), Proceedings of dsRNA Virus Symposium on Orbiviruses and Birnaviruses. Oxford, September 9–13, 1986, pp. 12–21.

Mellor, P.S., Leake, C.J., 2000. Climatic and geographical influences on arboviral infections and vectors. OIE Sci. Tech. Rev. 19, 41–54.

Mellor, P.S., Pitzolis, G., 1979. Observations on breeding sites and light trap collections of *Culicoides* during an outbreak of bluetongue in Cyprus. Bull. Entomol. Res. 69, 229–234.

Mellor, P.S., Wittmann, E.J., 2002. Bluetongue virus in the Mediterranean Basin 1998–2001. Vet. J. 164, 20–37.

Mellor, P.S., Osborne, R., Jennings, M., 1984a. Isolation of bluetongue and related viruses from *Culicoides* in the Sudan. J. Hyg. Camb. 93, 621–628.

Mellor, P.S., Jennings, M., Boorman, J.P.T., 1984b. *Culicoides* from Greece in relation to the spread of bluetongue virus. Rev. Elev. Med. Vet. Pays. Trop. 37, 286–289.

Mellor, P.S., Boorman, J., Baylis, M., 2000. *Culicoides* biting midges: their role as arbovirus vectors. Ann. Rev. Entomol. 45, 307–340.

Miranda, M.A., Borras, D., Rincon, C., Alemany, A., 2003. Presence in the Balearic Islands (Spain) of the midges *Culicoides imicola* and *Culicoides obsoletus* group. Med. Vet. Entomol. 17, 52–54.

Monteys, V.S.I., Saiz-Ardanaz, M., 2003. *Culicoides* midges in Catalonia (Spain), with special reference to likely bluetongue virus vectors. Med. Vet. Entomol. 17, 288–293.

Monteys, V.S., Aranda, C., Escosa, R., Pages, N., Ventura, D., 2004. Results of current surveillance of likely bluetongue virus vectors of the genus *Culicoides* in Catalonia. Spain. Vet. Ital. 40, 130–132.

Monteys, V.S.I., Ventura, D., Pages, N., Aranda, C., Escosa, R., 2005. Expansion of *Culicoides imicola*, the main bluetongue virus vector in Europe, into Catalonia, Spain. Vet. Rec. 156, 415–417.

Murray, M.D., Nix, H.A., 1987. Southern limits of distribution and abundance of the biting-midge *Culicoides brevitarsis* Kieffer (Diptera, Ceratopogonidae) in Southeastern Australia—an application of the growest model. Aust. J. Zool. 35, 575–585.

Nomikou, K., Mangana-Vougiouka, O., Panagiotatos, D.E., 2004. Overview of bluetongue in Greece. Vet. Ital. 40, 108–115.

Osmani, A., Murati, B., Kabashi, Q., Goga, I., Berisha, B., Wilsmore, A.J., Hamblin, C., 2006. Evidence for the presence of bluetongue virus in Kosovo between 2001 and 2004. Vet. Rec. 158, 393–396.

Parsonson, I.M., 1990. Pathology and pathogenesis of bluetongue infections. Curr. Top. Microbiol. Immunol. 162, 119–141.

Pitzolis, G., 1987. A review of bluetongue in Cyprus with evidence of the windborne spread of vectors. In: Taylor, W.P. (Ed.), Proceedings of the Meeting in the Community Programme for Coordination of Agricultural Research on Bluetongue in the Mediterranean Region. IZS dell' Abruzzo e del Molise, October 3–4, 1985, Teramo, Italy. CEC, Brussels, Luxembourg, pp. 27–38.

Placidi, L., 1957. La bluetongue au Maroc. Bull. Acad. Vét. 30, 79–84.

Purse, B.V., Mellor, P.S., Rogers, D.J., Samuel, A.R., Mertens, P.P.C., Baylis, M., 2005. Climate change and the recent emergence of bluetongue in Europe. Nat. Rev. Micro. 3, 171–181.

Purse, B.V., Nedelchev, N., Georgiev, G., Veleva, E., Boorman, J., Veronesi, E., Carpenter, S., Baylis, M., Mellor, P.S., 2006. Spatial and temporal distribution of bluetongue and its *Culicoides* vectors in Bulgaria. Med. Vet. Entomol. 20, 335–344.

Savini, G., Goffredo, M., Monaco, F., de Santis, P., Meiswinkel, R., 2003. Transmission of bluetongue virus in Italy. Vet. Rec. 152, 119.

Savini, G., Goffredo, M., Monaco, F., Di Gennaro, A., de Santis, P., Meiswinkel, R., Caporale, V., 2004. The isolation of bluetongue virus from field populations of the Obsoletus Complex in central Italy. Vet. Ital. 40, 286–291.

Scaramozzino, P., Boorman, J.P.T., Vitale, F., Semproni, G., Mellor, P.S., 1996. Entomological survey on Ceratopogonidae in central-southern Italy. Parassitologia 38, 1–2.

Sellers, R.F., 1975. Bluetongue in Cyprus. Aust. Vet. J. 51, 198–203.

Sellers, R.F., Maarouf, A.R., 1991. Possible introduction of epizootic haemorrhagic-disease of deer virus (Serotype-2) and bluetongue virus (serotype-11) into British-Columbia in 1987 and 1988 by infected *Culicoides* carried on the wind. Can. J. Vet. Res. 55, 367–370.

Sellers, R.F., Mellor, P.S., 1993. Temperature and the persistence of viruses in *Culicoides* during adverse conditions. OIE Sci. Tech. Rev. 12, 733–755.

Sellers, R.F., Pedgley, D.E., 1985. Possible windborne spread to western Turkey of bluetongue virus in 1977 and of Akabane virus in 1979. J. Hyg. Camb. 95, 149–158.

Sellers, R.F., Pedgley, D.E., Tucker, M.R., 1977. Possible spread of African horse sickness in the wind. J. Hyg. Camb. 79, 279–298.

Sellers, R.F., Pedgley, D.E., Tucker, M.R., 1979a. Possible windborne spread of bluetongue to Portugal, June–July 1956. J. Hyg. Camb. 81, 189–196.

Sellers, R.F., Gibbs, E.P., Herniman, K.A., Pedgley, D.E., Tucker, M.R., 1979b. Possible origin of the bluetongue epidemic in Cyprus, August 1977. J. Hyg. Camb. 83, 547–555.

Shimshony, A., 1987. Bluetongue activity in Israel, 1950–1985: the disease, virus prevalence, control methods. In: Taylor, W.P. (Ed.), Bluetongue in the Mediterranean Region. Proceedings of the Meeting in the Community Programme for Coordination of Agricultural Research. IZS dell' Abruzzo e del Molise, October 3–4, 1985, Teramo, Italy. CEC, Brussels, Luxembourg, pp. 1–22.

Shimshony, A., 2004. Bluetongue in Israel—a brief historical overview. Vet. Ital. 40, 116–118.

St George, T.D., Muller, M.J., 1984. The isolation of bluetongue virus from *Culicoides brevitarsis*. Aust. Vet. J. 61, 95.

Standfast, H.A., Dyce, A.L., Muller, M.J., 1985. Vectors of bluetongue virus in Australia. In: Barber, T.L., Jochim, M.M. (Eds.), Bluetongue and Related Orbiviruses. Alan R. Liss, New York, pp. 177–186.

Tabachnick, W.J., Robertson, M.A., Murphy, K.E., 1996. *Culicoides variipennis* and bluetongue disease. Ann. N. Y. Acad. Sci. 791, 219–226.

Taylor, W.P., 1987. Bluetongue in Syria and Jordan. In: Taylor, W.P. (Ed.), Proceedings of the Meeting in the Community Programme for Coordination of Agricultural Research. IZS dell' Abruzzo e del Molise, October 3–4, 1985, Teramo, Italy. CEC, Brussels, Luxembourg, pp. 39–47.

Taylor, W.P., Mellor, P.S., 1994. Bluetongue virus distribution in Turkey 1978–1981. Epidemiol. Infect. 112, 623–633.

Torina, A., Caracappa, S., Mellor, P.S., Baylis, M., Purse, B.V., 2004. Spatial distribution of bluetongue virus and its *Culicoides* vectors in Sicily. Med. Vet. Entomol. 18, 81–89.

Urman, H.K., Mille, U.È., Mert, N., Berkin, S.Ë., Kahramen, M.M., Yue, H., Avvurran, H., 1980. TuÈrkiye'de buzagilarda konjenital epizootik arthrogryposis ve hydranencephalie olaylari. Ankara UÈ niversitesi. Veteriner Fakgltesi Dergisi 26, 287–295.

Vassalos, M., 1980. Cas de fièvre catarrhale du mouton dans l'Ile de Lesbos (Grèce). Bull. Off. Int. Epizoot. 92, 547–555.

Venter, G.J., Hill, E., Pajor, I.T.P., Nevill, E.M., 1991. The use of a membrane feeding technique to determine the infection rate of *Culicoides imicola* (Dipt. Cerat.) for two bluetongue virus serotypes in South Africa. Onderstepoort J. Vet. Res. 58, 5–9.

Walker, A., Davies, F.C., 1971. A preliminary survey of the epidemiology of bluetongue in Kenya. J. Hyg. Camb. 69, 47–61.

Wittmann, E.J., Baylis, M., 2000. Climate-change: effects on *Culicoides*-transmitted viruses and implications for the UK. Vet. J. 107–117.

Yonguç, A.D., Taylor, W.P., Csontos, L., Worrall, E., 1982. Bluetongue in western Turkey. Vet. Rec. 111, 144–146.

Zhang, N., MacLachlan, N.J., Bonneau, K.R., Zhu, J., Li, Z., Zhang, K., Zhang, F., Xia, L., Xiang, W., 1999. Identification of seven serotypes of bluetongue virus from the People's Republic of China. Vet. Rec. 145, 427–429.

Zhang, N., Li, Z., Zhang, F., Zhu, J., 2004. Studies on bluetongue disease in the Peoples' Republic of China. Vet. Ital. 40, 51–56.

Appendix G

Bluetongue in Europe and the Mediterranean Basin

11

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Introduction

Bluetongue virus (BTV) is traditionally understood as occurring around the world in a broad band stretching from about 35°S to 40°N although in certain areas (i.e. western North America and northern China), it may extend up to around 50°N (Mellor *et al*., 2000; Mellor and Wittmann, 2002). As BTV is transmitted between its vertebrate hosts almost entirely via the bites of *Culicoides* biting midges, its world distribution is limited to geographical areas where competent vector species of *Culicoides* are present and its transmission to those times of the year when climatic conditions are (1) favourable for adult vector activity and (2) sufficiently warm for long enough to allow virus replication in, and transmission by, the vectors.

In Europe, until recently, the above conditions were apparently fulfilled only in parts of Portugal, SW Spain, certain Greek Islands adjacent to the Anatolian Turkish coast and Cyprus since, prior to the 1990s, these were the only areas to have experienced BTV incursions. During the period 1956–1960, BTV-10 entered Portugal and SW Spain from North Africa and caused the deaths of almost 180 000 sheep (Manso-Ribeiro et al., 1957; Campano Lopez and Sanchez Botija, 1958; Manso-Ribeiro and Noronha, 1958; Gorman, 1990), while during the years 1979–1980, BTV-4 entered the Greek Islands of Rhodes and Lesbos (Vassalos, 1980; Dragonas, 1981). Cyprus seemed to be the only area of Europe where BTV

occurred regularly, and virus activity had been detected there in at least 21 of the 50 years between 1924 and 1973 (Sellers, 1975).

In the late 1990s, however, Europe has witnessed a dramatic change in the epidemiology of bluetongue (BT).

Bluetongue virus in the Mediterranean Basin, 1998–2005

Between 1998 and 2005, incursions of BTV occurred into many countries around the Mediterranean Basin that are usually free from the virus. Indeed, most of the countries involved had no previous record of BTV on their territory. At least five serotypes of virus were involved, originating from several sources to the east and south of the Mediterranean Basin. In all, virus activity was detected in at least 12 European countries, three countries in N. Africa and Israel, in the Near East. The total number of sheep dying from disease or culled for control and welfare purposes has been estimated at well over one million, which makes this by far the most severe outbreak of BT on record (Purse *et al*., 2005).

Bluetongue virus in Greece, Turkey, the Balkans, Cyprus and Israel

Greece

In October 1998, after an absence of almost 20 years from Europe, BTV was confirmed in four Greek Islands (Rhodes, Leros, Kos and Samos) close to the Anatolian Turkish coast (Figures 11.1 and 11.2; Anon, 1998a, b; Anon,

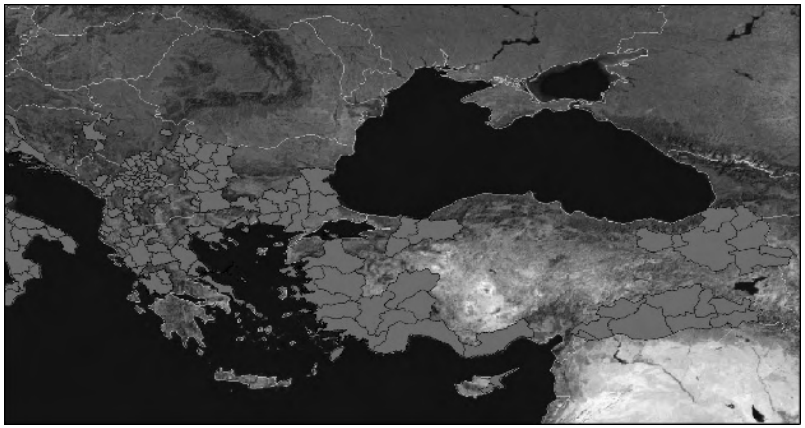

Figure 11.1 Bluetongue virus (BTV) activity in the Eastern Mediterranean Basin, 1998–2005, overall area affected (base maps: NASA and Earthsat, 2004).

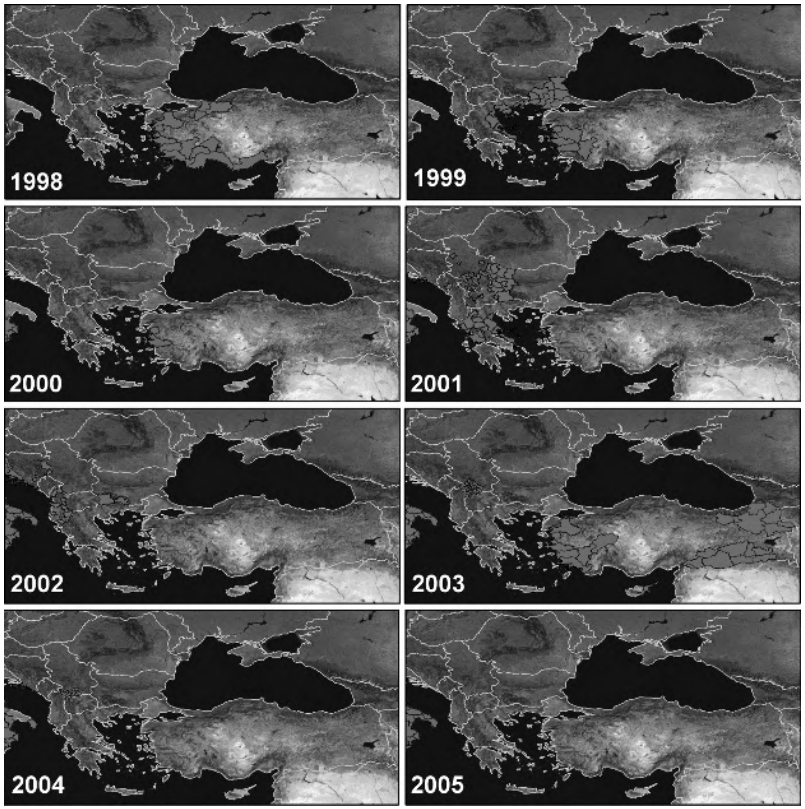

Figure 11.2 Bluetongue virus (BTV) activity in the Eastern Mediterranean Basin by year, from 1998 to 2005 (base maps: NASA and Earthsat, 2004).

1999a). The virus was identified as being BTV serotype 9 (Anon, 2000h), and this was the first occasion that this serotype had been recorded anywhere in Europe, though it had been identified serologically in western Anatolian Turkey during the period 1979–1980 (Taylor and Mellor, 1994a).

Outbreaks in the four Greek islands were recorded until late December 1998 (Anon, 1999a, 2000d), and then ended, probably because activity of the major vector in the area, *Culicoides imicola* Kieffer, is at a minimum during this period and for the remainder of the winter (Anon, 2000f; M. Patakakis, Personal Communication). No further outbreaks of BT anywhere in eastern Europe or Asia Minor were reported over the next 5 months.

In August 1999, however, the Greek authorities reported the presence of BTV in mainland Greece for the first time, initially in the north-eastern Prefecture adjacent to the Turkish and Bulgarian borders (see Figures 11.1

and 11.2; Anon, 1999b) and just subsequent to the announcement of BTV in adjacent areas of Bulgaria (in June) and European Turkey (in July) (see below). During September and October, the virus spread across northern Greece travelling in a westerly direction as far west as Thessaloniki and Larissa and as far south as Magnisia and Evia, thus rendering approximately one-third of Greek territory 'infected' (see Figures 11.1 and 11.2; Anon, 1999d). New cases of BT continued to be reported in some areas of Greece into December 1999 (Anon, 2000a). Also, during September 1999, a separate incursion of BTV into the Greek island of Lesbos was reported, and further outbreaks were reported in several Dodecanese islands (Anon, 1999c). By the end of 1999, serological evidence of the virus and/or clinical signs of BT in Greece had been reported from 13 Aegean islands and also from 10 mainland prefectures (see Figures 11.1 and 11.2; Anon, 2000f; Mellor and Wittmann, 2002). During 2000, low-level BTV transmission in Greece continued to occur, sporadically, during the summer months and BTV-4 was detected in the Prefecture of Arta in the NW of the country, a previously unaffected area (Anon, 2000b, 2001a; Mellor and Wittmann, 2002; Nomikou, 2006, Personal Communication), but in late August 2001, evidence of a rather more active BTV focus was once more detected in Greek territory, initially in the NW of the country. The virus was first detected in the Prefectures of Grevena, Ioannina and Kastoria close to the Albanian border (Anon, 2001c), and the disease was reported to advance into the country in a south/south-westerly direction, along the routes of rivers and canyons, in the direction of the prevailing winds (see Figures 11.1 and 11.2). This information was interpreted as suggesting that the outbreak represented a new incursion into Greece (presumably from areas to the north) rather than resurgence. Eventually by November 2001, the incursion had extended to include the Prefectures of Aitoloakarnania, Evritania, Larissa, Preveza, Thesprotia and Trikala also, thus encompassing a broad swath of the country stretching south-west from the Albanian border to Larissa on the Aegean coast (see Figures 11.1 and 11.2). The island of Lesbos close to the Anatolian coast of Turkey also experienced 19 new outbreaks in what, because of the distances involved, was presumably a separate incursion. Since autumn 2001, however, the Greek territories have been free of reported BTV activity.

A complicating factor that emerged during this series of Greek BTV incursions was that in addition to BTV-9, three further serotypes were identified: 1, 4 and 16 (Anon, 2000h). Both BTV-4 and -9 had been reported previously from regions immediately to the east of Europe, i.e. BTV-4 and -9 from Lesbos, Anatolian Turkey, Syria and Jordan (Taylor and Mellor 1994a; Taylor, 1987; Mellor and Pitzolis, 1979; Vassalos, 1980;) and BTV-4 from Cyprus and Israel (Mellor and Pitzolis, 1979; Shimshony, 1987) at various times during the period 1978–1980. BTV-16 occurs regularly in Israel (Shimshony, 1987; Taylor and Mellor, 1994a), and during the present series of outbreaks, it was also reported, in August 2000, from Anatolian Turkey (see below). BTV-1, however, had not been previously reported from any country in or adjacent to the Mediterranean Basin.

During the course of the Greek outbreaks, control was implemented through surveillance, animal movement restrictions, slaughter and insecticide treatment of infected and 'at-risk' premises; at no time was vaccination practiced anywhere in Greek territory.

Turkey

In July 1999, the Turkish authorities reported a BTV incursion into European Turkey involving animals in two provinces bordering Bulgaria and mainland Greece (see Figures 11.1 and 11.2; Anon, 1999b). Then in October and November 1999, outbreaks of BT were reported, for the first time in the current epizootic, from western Anatolian Turkey involving animals in four provinces (see Figures 11.1 and 11.2; Anon, 1999d). Retrospectively, unofficial sources from Turkey also suggested that BTV had been active throughout western and southern Anatolian Turkey, from early 1998 (see Figures 11.1 and 11.2; A. Ozkul, Personal Communication). In August 2000, BT was again reported (from Izmir Province) but this time serotype 16 of the virus was isolated (see Figures 11.1 and 11.2; Anon, 2000c; C. Hamblin, Personal Communication). In 2003, serological surveys in cattle were carried out in five provinces in European Turkey and 23 provinces in Anatolian Turkey (six in the west, eight in the NE and nine in the SE) (see Figures 11.1 and 11.2; Final Report EU project QLK2-CT-2000-00611; A. Ozkul, Personal Communication). Seroprevalence rates of up to 90% were detected in some regions. Serum neutralization tests against BTV-4, -9 and -16 showed that all three serotypes were active during 2003 in Anatolian Turkey and that BTV-9 and -16 were also active in European Turkey (Final Report EU project QLK2-CT-2000-00611; A. Ozkul, Personal Communication). Control has been implemented in Turkey via clinical surveillance, animal movement restrictions and also by the use of a locally produced live attenuated virus vaccine against BTV-4 (Mellor and Wittmann, 2002).

Bulgaria

In June 1999, BTV invaded Bulgaria for the first time ever and serotype 9 was confirmed in south-east part of the country (see Figures 11.1 and 11.2; Anon, 1999e). The virus spread rapidly in a westerly direction across southern Bulgaria, eventually involving ruminant animals in four districts: Bourgas, Yambol, Haskovo and Kardjaly (see Figures 11.1 and 11.2; Anon, 1999b). In total, 85 villages were affected, and clinical signs were recorded in every flock examined in these villages, albeit at a low prevalence rate of 1.7% within flocks (Purse *et al*., 2006). At the same time, nine out of 19 sentinel herds of mixed ovine and bovines situated in Yambol, Haskovo and Kardjaly districts also seroconverted confirming BTV transmission at least 30 km from disease outbreak areas and up to 70 km from the Bulgarian border, which suggests that the areas of BTV transmission were considerably more widespread than the reported outbreaks of disease (Purse *et al*., 2006). Disease outbreaks persisted until late October 1999, at which time daily temperatures fell sharply

and transmission apparently ceased. Subsequent to this time and for almost two years, no serological or clinical evidence of BTV transmission was recorded; however, in September 2001, a second incursion, also of BTV-9, was detected in the extreme western part of the country involving 76 villages in seven districts (Vidin, Montana, Vratsa, Sofia, Pernik, Kiustendil and Blagoevgrad) on or close to the borders with the FYR of Macedonia, Yugoslavia and Romania (see Figures 11.1 and 11.2; Purse *et al*., 2006). Unlike the first incursions, a characteristic of these new outbreaks was that clinical signs were rarely observed (i.e. in only 27 of 25 929 susceptible sheep in affected areas) and almost all of the outbreaks were within 40 km of the international borders. Operation of a sentinel herd system in the region during 2001 indicated that, just as in 1999, transmission ceased in October with the advent of cooler weather. The sentinel system continued to operate in western and southern Bulgaria during 2002 when seroconversion to BTV, but no clinical disease was recorded in the south of the Smolyan and Blagoevgrad Districts in SW Bulgaria, close to the Greek border (see Figures 11.1 and 11.2). Subsequent to 2002 and up to mid-2006, BTV activity has not been detected anywhere within the territory of Bulgaria.

Control has been implemented in Bulgaria via clinical and serological surveillance, animal movement restrictions, insecticide treatment and, in 1999–2000, restricted use of an imported, live attenuated, pentavalent vaccine against BTV serotypes 3, 8, 9, 10 and 11.

Other Balkan countries (Macedonia, Albania, Yugoslavia, Kosovo, Croatia and Bosnia)

Following on from the reports of BTV recrudescence in Greece and Bulgaria during 2001, the Macedonian veterinary authorities introduced compulsory clinical surveillance for BT. In September 2001, typical signs of BT were identified in sheep in the Kriva Palanka Region in the north of the country close to the border with Yugoslavia (see Figures 11.1 and 11.2; Anon, 2001h). The affected animals were slaughtered, zoosanitary control measures implemented and clinical surveillance extended throughout the whole country with serological surveillance in some areas. During early October, further areas of sporadic BTV activity were identified in an additional seven regions scattered across the country from east to west, with most cases being in the regions of Kriva Palanka, Berovo and Krusevo (see Figures 11.1 and 11.2; Anon, 2001j). The number of animals affected was reported to be low and the clinical signs mild. The last clinical case was identified on 16 October 2001, subsequent to which time the ambient temperature decreased. No further BTV activity has been reported from the country.

At more or less the same time as in the FYR of Macedonia, BT was also identified for the first time in 2001 in the Federal Republic of Yugoslavia, in the south, south-east and south-west of Serbia, the south of Montenegro, and also in the autonomous province of Kosovo (see Figures 11.1 and 11.2; Anon,

2001k; Anon, 2000l). Thirty-seven outbreaks in 16 municipalities were recorded in Serbia proper, and the virus was identified as BTV-9 (Anon, 2001f). In 2002, serological surveys indicated that the virus had extended into more northerly areas of Serbia and Montenegro reaching the River Sava, which at about 44°50′N is the furthest north that BTV has ever reached in Europe (see Figures 11.1 and 11.2; Djuricic *et al*., 2004). In September 2003, BTV activity was again reported from Yugoslavia, from four municipalities in Serbia. In Kosovo, subsequent to 2001, BTV has not been reported officially, but serological evidence from cattle and sheep in the province clearly show that the virus has continued to circulate during 2002, 2003 and 2004 (see Figures 11.1 and 11.2; Osmani *et al*., 2006).

The presence of BTV in Croatia was first reported in late 2001 on the basis of serological and clinical findings in sheep in the Dubrovnik Region (see Figures 11.1 and 11.2; Anon, 2001m). In late 2002, a sentinel system of bovines was operated in parts of southern Croatia and seroconversions to BTV-9 were detected during October–November of that year, with the areas affected being rather more northerly than in 2001 (see Figures 11.1 and 11.2; Labrovic *et al*., 2004; Listes *et al*., 2004). In 2004, serological evidence of BTV transmission in Croatia was again reported from sentinel bovines also in the Dubrovnik Region and the presence of BTV-9 and -16 identified – this was and is the only report of BTV-16 from any part of the Balkans. Subsequent to October 2004, BTV activity has not been reported in any part of Croatia.

In Bosnia and Herzegovina, BTV activity was detected for the first time ever in August 2002 in 15 municipalities in the south and south-east of the country close to the borders with Montenegro and Serbia (see Figures 11.1 and 11.2), and the virus was identified as BTV-9 (Anon, 2002). Further information on BTV in the country has not been forthcoming.

Albania also reported the presence of BTV for the first time in 2002, and in a serological survey of cattle, sheep and goats carried out between October and November of that year, seroconversions to BTV-9 were detected in all of 15 districts surveyed, cattle showing the highest prevalence of infection (see Figures 11.1 and 11.2; Di Ventura *et al*., 2004). The areas surveyed were arrayed mainly along the borders with Greece, Macedonia, Kosovo and Montenegro, although two districts on the Adriatic coast were also covered and Tirane District in the centre of the country exhibited the highest prevalence of BTV infection (i.e. 61%). No further information of BTV infection in Albania, subsequent to 2002, has been forthcoming.

Cyprus

Cyprus is the one country in Europe that could be historically considered to fall within the BTV endemic zone. However, since 1998 and up to mid-2006, BTV activity has been detected only during October–December 2003 in sentinel herds of cattle in the Larnaca District (see Figures 11.1 and 11.2). The virus was identified as BTV-16.

Israel

Israel is situated in a region where BTV occurs and has been reported regularly since 1924 (Sellers, 1975; Taylor *et al*., 1985; Hassan, 1992; Braverman *et al*., 2004). Control involves vaccination with a pentavalent, live attenuated virus vaccine, which includes types 2, 4, 6, 10 and 16 (Shimshony, 2004). During the course of the current series of outbreaks in Europe and up to mid-2006, activity only by BTV type 16 has been reported from Israel and only in the month of September 2003 (Anon, 2003a, b).

Bluetongue virus in North Africa, Italy, France, Spain and Portugal

North Africa – Tunisia

In January 2000, BT was reported for the first time in Tunisia, in areas in the NE of the country (Figures 11.3 and 11.4). The time of incursion was estimated as early December 1999 and clinical cases continued to be detected in January 2000 (Anon, 2000a; Hammami, 2004). The virus was typed as BTV-2 in late January 2000 (Anon, 2000g). The origin of this incursion is uncertain but is likely to be separate from that involving Turkey, Greece and the Balkan countries in eastern Europe. Since foot and mouth disease virus had also entered Tunisia (and Algeria) during 1999, probably via cattle smuggled from Côte d'Ivoire and Guinea into Algeria (Knowles and Davies, 2000), it is possible that BTV could have followed a similar route. Cattle in Africa often experience subclinical infections with BTV, and BTV-2 is common in several areas of sub-Saharan West Africa (Herniman *et al*., 1980, 1983).

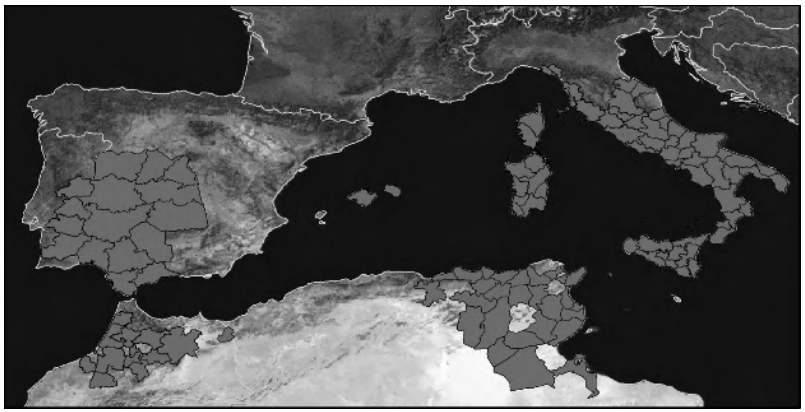

Figure 11.3 Bluetongue virus (BTV) activity in the Central and Western Mediterranean Basin, 1999–2005, overall area affected (base maps: NASA and Earthsat, 2004).

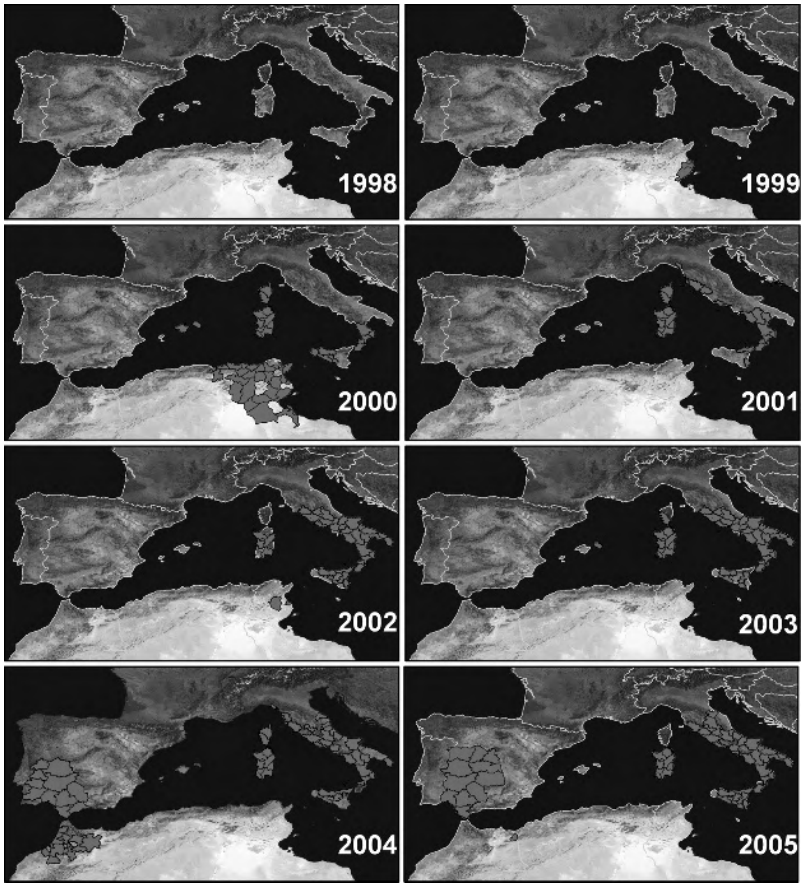

Figure 11.4 Bluetongue virus (BTV) activity in the Central and Western Mediterranean Basin by year, from 1998 to 2005 (base maps: NASA and Earthsat, 2004).

New cases of BT were not detected in Tunisia during February–May 2000, but from June, further outbreaks of BTV-2 were reported from 10 districts mostly distributed in the eastern and central parts of the country (see Figures 11.3 and 11.4; Anon, 2000d, i; Hammami, 2004). These persisted in some areas until October 2000 (Anon, 2000d). Control measures implemented in 2000 included the isolation of infected flocks, spraying of animal holdings with insecticide and mass vaccination (in 2000, 2001 and 2002) with a monovalent, attenuated BTV-2 vaccine (Hammami, 2004).

In December 2002, a limited number of new outbreaks caused by BTV-2 occurred in the central part of Tunisia in unvaccinated flocks, and subsequent to that time, no evidence of virus circulation has been reported.

North Africa – Algeria

In July 2000, BTV-2 was reported from eastern Algeria (see Figures 11.3 and 11.4) and outbreaks continued into September (Anon, 2000b, e; Hammami, 2004). All of the areas initially affected were close to the Tunisian border. However, in September 2000, BTV was also confirmed in areas ranging up to 250 km to the west of Tunisia (see Figures 11.3 and 11.4; Anon, 2000c). Samples taken from animals near Algiers, over 400 km to the west of Tunisia, were also positive for BTV-specific antibodies (Mellor and Wittmann, 2002). The control strategy adopted by the Algerian veterinary authorities was based upon the use of insecticides to control the vectors and clinical surveillance (Hammami, 2004). Further, BTV activity in the country has not been reported till mid-2006.

North Africa – Morocco

In late 2000, BTV-specific antibodies were apparently recorded in animals from a number of provinces stretching across northern Morocco, but there was no evidence of clinical disease, and details of the locations of the affected areas are not available (M. Abaddi, Personal Communication; Y. Lhor, unpublished observations). Despite serological monitoring, no further evidence of BTV activity in Morocco was detected in the succeeding three years (Hammami, 2004).

However, in 2004, the situation changed and starting in August and continuing to the end of the year, a further incursion of BTV into Morocco was recorded, this time clinically affecting sheep in 14 provinces in the north-west of the country (see Figures 11.3 and 11.4; Anon, 2004a–g). Surprisingly, this incursion was caused by BTV-4 (Anon, 2004b) and is therefore totally separate from the 2000–2002 incursions of BTV-2 into other areas of North Africa. So far the origin of this incursion of BTV-4 into Morocco is unknown.

Italy

In August 2000, BTV (serotype 2) was confirmed for the first time ever in Italian territory. The island of Sardinia was affected first (Anon, 2000c) but by October, the virus had spread to Sicily and southern mainland Italy (see Figures 11.3 and 11.4; Calabria; Anon, 2000k). The incursion into Sardinia was particularly severe with more than 6000 flocks affected and over 260 000 sheep dying, either directly from BT or in the course of implementing control measures (i.e. slaughter of animals suspected of being infected) (Anon, 2000k; Calistri *et al*., 2004). Outbreaks continued in the three regions through the summer and autumn, and also at a low level through the winter, until May

2001 by which time over 260 000 animals in 6869 flocks had been affected (Calistri *et al*., 2004). There then followed what is termed the second epidemic, which lasted until April 2002. Once again Sardinia, Sicily and Calabria were affected but the virus spread into Basilicata, Campania, Latium and Tuscany also causing over 6800 outbreaks and the loss of approximately 250 000 sheep and goats (see Figures 11.3 and 11.4; Calistri *et al*., 2004).

During the first epidemic and for much of the second, vaccination was not employed, but in January 2002, vaccination of all susceptible domestic species with a monovalent, attenuated BTV-2 vaccine commenced in affected regions (Calistri *et al*., 2004).

In Italy, the third epidemic began in April 2002 and extended until April 2003. During this period, BTV-9 and BTV-16, in addition to BTV-2, were detected for the first time in Italy and clinical disease occurred in eight provinces (Basilicata, Calabria, Campania, Lazio, Molise, Puglia, Sardinia and Sicily) (see Figures 11.3 and 11.4). Both BTV-9 and -16 had previously been reported from the eastern Mediterranean area (Turkey, Greece and the Balkans), but the route of incursion into Italy is uncertain, though illegal trade in animals is suspected (Giovannini *et al*., 2004a). In those areas where BTV-2 and -9 occurred together (southern mainland Italy and Sicily), an attenuated, bivalent vaccine was employed in place of the monovalent BTV-2 vaccine (Patta *et al*., 2004; Giovannini *et al*., 2004b). The results of the vaccination campaigns suggest that in regions where vaccine cover reached approximately 80%, clinical disease either disappeared or was much reduced. In such regions, the spread of infection was also significantly reduced. However, in regions with lower levels of vaccine cover, no such significant reduction in the spread of disease and infection occurred (Patta *et al*., 2004).

During the summer of 2003, the fourth epidemic commenced. Seroconversions were detected in many provinces in south and central Italy but clinical disease was apparently seen only in Sardinia, where, for the first time in Italy, BTV-4 was isolated. Sadly, this was not the end of the Italian outbreaks and by the end of 2004, circulation of various combinations of BTV-2, -4, -9 and -16 had been recorded in 12 regions extending from Sicily in the south to Tuscany in the north and from Puglia in the east to Sardinia in the west (see Figures 11.3 and 11.4; see http://www.te.izs.it/). Vaccination was carried out in all of the affected regions using the appropriate combinations of BTV-2, -4, -9 and -16 live virus vaccines.

During 2005 for the seventh consecutive year, BTV circulation continued in Italy, in at least 10 provinces: Lazio (BTV-2 and -16), Liguria (BTV-16), Marche (BTV-16), Molise (BTV-2 and -9), Campania (BTV-2 and -9), Puglia (BTV-2, -9 and -16), Basilicata (BTV-2, -4 and -16), Calabria (BTV-2 and -16), Sicily (BTV-2, -4 and -16) and Sardinia (BTV-2, -4 and -16), and at least until November (see Figures 11.3 and 11.4; see http://www.te.izs.it/). Again vaccination was carried out using the appropriate combinations of serotypes in each of the affected areas.

France (Corsica)

In October 2000, BTV was recorded for the first time ever in the French island of Corsica, the virus being identified as BTV-2 (see Figures 11.3 and 11.4; Anon, 2000j). The French authorities speedily authorised a vaccination campaign, using a monovalent, attenuated virus vaccine, which commenced during the winter of 2000–2001. However, apparently full protection was not attained as a second epidemic of BTV-2 commenced in July 2001, affecting animals, mainly in unvaccinated flocks, in both the north and south of the island. In all, in 2001, 335 flocks were affected, a seven-fold increase in 2000 (Breard *et al*., 2004). Accordingly, a second vaccination campaign was mounted during the winter and spring of 2001–2002, which seems to have been successful as no cases of BT were reported during 2002. However, in October 2003, a further BTV incursion into Corsica was recorded affecting vaccinated sheep in the south part of the island (see Figures 11.3 and 11.4). The virus was identified as BTV-4 on the 31st of October and a third vaccination campaign was mounted from early November using a bivalent, live attenuated vaccine against BTV-2 and -4. In August 2004 and for the fourth year, BT was reported in Corsica, this time two virus serotypes being identified: BTV-4 in the north and west of the island and BTV-16 in the south-west (Anon, 2004h). In response, a further vaccination campaign was mounted using live attenuated vaccines against BTV 2, 4 and 16, and no further evidence of BTV transmission was detected after September 2004.

Spain

The first outbreaks of BT in Spain commenced on two of the Balearic Islands (Menorca and Majorca) in September–October 2000 (see Figures 11.3 and 11.4; Anon, 2000l; Miranda *et al*., 2003; Gomez-Tejedor, 2004). Three hundred and five outbreaks occurred and the virus was identified as BTV-2. A vaccination campaign in sheep based upon a monovalent, attenuated BTV-2 vaccine was mounted on both islands commencing in October 2000 and was completed before the end of the year. Evidence of BTV transmission, as assessed in sheep and sentinel herds of bovines, did not extend beyond November 2000, but a further vaccination campaign in sheep was mounted in the spring of 2001 and was extended to cover the island of Ibiza, even though no cases of BT were detected there (Gomez-Tejedor, 2004).

However, in October 2003 and extending until the end of the year, a second incursion of BTV into the eastern part of the island of Menorca was reported (see Figures 11.3 and 11.4). The virus was identified as BTV-4, and a vaccination campaign based upon an attenuated monovalent vaccine against that serotype was rapidly mounted (Gomez-Tejedor, 2004).

In October 2004, a further incursion of BTV-4 into Spain was identified by seroconversions in sentinel bovines, this time into mainland Spain (see Figures 11.3 and 11.4; Gomez-Tejedor, 2004). The outbreak apparently commenced in the southern part of mainland Spain in the province of Cadiz in Andalucia and

spread rapidly through four provinces in Andalucia and two in Extramadura; the Spanish enclave of Ceuta on the north coast of Morocco was also affected (Gomez-Tejedor, 2004). Once again a vaccination campaign was launched using an attenuated, monovalent BTV-4 vaccine in sheep. However, commencing in July 2005 and running through until the time of writing, the outbreak of BTV-4 in mainland Spain continued to spread, reaching further north than ever before, until by November 2005 it involved provinces in Andalucia (4), Castilla la Mancha (2), Castilla y Leon (2), Extramadura (2) and Madrid (1) (see Figures 11.3 and 11.4; see: http://rasve.mapya.es/). Interestingly, in 2005, all but one of the Spanish outbreaks occurred in bovines, whereas in 2004, 43 were in bovines, 200 in sheep and 77 in goats. Also in 2005, a single isolate of BTV-2 was made in July 2005 from a herd of sentinel bovines in mainland Spain (Elliott, Personal Communication). The origin of the 2004–2005 outbreaks of BTV-4 in mainland Spain was undoubtedly north-west Morocco, where the same serotype of virus was detected just prior to and during the Spanish outbreaks. Molecular studies also confirm the close similarity between the Spanish and Moroccan isolates of BTV-4 (Mertens, unpublished observations). The origin of the single isolate of BTV-2 from mainland Spain in 2005 is more difficult to determine. However, molecular analysis has confirmed that it is virtually indistinguishable from the BTV-2 vaccine virus previously used in several countries and zones in southern Europe including the Spanish Balearic islands.

Portugal

Bluetongue virus was first confirmed in sheep in Portugal in November 2004 in the Evora District of Alentejo, close to the border with Spain, and by December had spread to the Castelo Branco District of Beira Interior (see Figures 11.3 and 11.4; Anon, 2004a, b; Boinas *et al*., 2005). The virus was identified as BTV-4 (Fevereiro *et al*., 2005) and a vaccination campaign in sheep was mounted in the affected areas using a live attenuated BTV-4 vaccine (Boinas *et al*., 2005). At the same time a country-wide serological surveillance system in bovines was initiated followed by an entomological surveillance system. In 2005, seroconversion to BTV but no disease was detected in sentinel herds of bovines until at least October (Boinas, Personal Communication). Further information from Portugal is not yet available. The Portuguese outbreaks, in view of the serotype of virus involved, the areas infected and the times of infection are clearly an extension of the Spanish mainland incursions that originated in Morocco.

Origins of the outbreaks

There are a number of sources of the 1998–2005 BTV incursions into the countries of southern and eastern Europe. The incursions of BTV serotypes 1, 4, 9 and 16 into the eastern part of the Mediterranean Basin (Greece, European

Turkey and the Balkans) some of which then spread further west (4, 9 and 16) clearly originated from the east of Europe. In this context it should be borne in mind that BTV serotypes 2, 4, 6, 9, 10, 13, and 16 have all been reported over a number of years from Anatolian Turkey, Syria, Jordan and/or Israel. Indeed, the westward movement of some of these and of other viruses (e.g. Akabane) through Turkey is well documented (Urman *et al*., 1980; Vassalos, 1980; Yonguc *et al*., 1982; Burgu *et al*., 1992; Taylor and Mellor, 1994a, b). BTV-1, however, has not previously been reported from these areas but the work of Maan *et al*. (2004) has shown that the European isolates of this virus have a genetic lineage closely related to isolates of the serotype from India. It is therefore likely that BTV-1 is also present in Turkey and/or the Middle East but, by chance, has not yet been isolated from these regions, probably because of the limited amount of work on BTV that has been carried out there. These findings strongly suggest that the area to the east of Europe contains a pool of assorted BTV serotypes some of which have previously been identified from Europe and some, like BTV-1, which have not. Under the right environmental conditions, these BTV serotypes, clearly, can extend into and expand within Europe and as we have seen in the period 1998–2005 constitute a significant threat to European livestock (see Figures 11.1 and 11.3). The trick is to understand what these 'right conditions' are so as to be able to predict such incursions and thereby minimise their impact. How this can be achieved is dealt with elsewhere in this book.

The European incursion of BTV-2 has a different origin from the eastern incursions. Prior to entry into Europe this virus was first reported from Tunisia in late 1999 and Algeria in 2000 but the virus (see Figures 11.3 and 11.4), at least until recently, has clearly not been endemic to this region as neither country had previously experienced a BTV incursion of any serotype, and Morocco to the west had not recorded the presence of BTV since 1956 (Placidi, 1957; Hammami, 2004). So from where might this incursion into North Africa and Europe have come? The origin is uncertain but as foot and mouth disease virus had also entered Tunisia (and Algeria) during 1999, probably via cattle smuggled from Cote d'Ivoire and Guinea into Algeria (Knowles and Davies 2000), it is very possible that BTV could have followed a similar route. Cattle in Africa often experience sub-clinical infections with BTV, and BTV-2 is common in several areas of sub-Saharan West Africa (Herniman *et al*., 1983). It is also possible, though less likely, that BTV-2 could have expanded westwards across North Africa through Egypt and Libya from the Middle East where it also occurs; however, no evidence of such an expansion has entered the public domain. Sequence comparisons of BTV-2 isolates from Tunisia and Algeria, with recent isolates from Israel and other locations in the Middle East, and also from locations in West Africa south of the Sahara might help to resolve this uncertainty. Having reached North Africa, BTV-2 then expanded rapidly northwards during the summer and autumn of 2000 into Sardinia, Corsica, two of the Spanish Balearics, Sicily

and southern mainland Italy (Calabria) – in that order (see Figures 11.3 and 11.4). The movement of infected animals and their products has, in many instances, been ruled out as the cause of these incursions (Miranda *et al*., 2003; Calistri *et al*., 2004), but the question – how else might it have been achieved? – remains. The most likely mode is via the passive transportation of infected vectors on the wind as aerial plankton. Over the years, many authors have written extensively on this subject providing both circumstantial and real evidence to suggest that *Culicoides* can travel hundreds of kilometres by such means (Sellers *et al*., 1979a, b; Hayashi *et al*., 1979; Murray, 1987; Sellers and Maarouf, 1991; Sellers and Mellor, 1993). In the case of the current BTV-2 outbreaks in Sardinia, Calistri *et al*. (2004) have shown the following:

- The first cases of BT occurred in the southernmost part of the island, which, due to its geographic proximity with North Africa, is where landfall of wind-borne *Culicoides* would be expected.
- During the first 15 days after notification of the first outbreak, 262 infected flocks were detected along 200 km of the south-west coast of the island.
- The BTV incubation period is compatible with the hypothesis of a common source for these infections.
- In the same time frame, BTV-2 was also reported in the Balearic Islands, over 200 km to the west.
- The period of the initial outbreak was preceded by and coincided with unusual climatic conditions in which dust storms originating in the infected areas of North Africa moved northwards across southern Italy and Sardinia.

Similarly, an incursion of BTV-4 into the Balearics occurred in October 2003 following outbreaks of the same serotype during the summer and autumn in Sardinia and Corsica, islands situated over 300 km to the east of the Balearics (see Figures 11.3 and 11.4).

In 2004, a further incursion of BTV-4 into the Mediterranean Basin occurred (see Figures 11.3 and 11.4). The outbreak was first detected on 28 August 2004 in NE Morocco but had spread across the Straits of Gibraltar into the southernmost province of mainland Spain (Cadiz) by 30 September (see Figures 11.3 and 11.4). Further spread of the virus both northwards and westwards within Spain and then into Portugal was connected with the movement of infected animals, but the initial incursion was not linked to animal movements and may, therefore, have been due to windborne *Culicoides*. Unpublished analyses have shown that southerly winds blowing over NW Morocco and then the Cadiz area of Spain were prevalent at the time of the incursion and occurred during the time of the diel (evening) when *Culicoides* activity is maximal (Gloster, unpublished observations).

All of this information provides strong circumstantial evidence that infected *Culicoides* can be dispersed on the wind distances of several 100 km, thereby

introducing disease into regions remote from the source of infection. It also suggests that movement of *Culicoides* vectors on the wind over such long distances is not a rare event. This is a matter of some concern since elsewhere in this book (Chapter 15) Baylis and his co-authors show that transmission of BTV from infected vectors to susceptible ruminants is an exceptionally efficient process, which suggests that the introduction of BTV via wind-borne vector *Culicoides* as described above may be a regular and very successful survival strategy of the virus.

Vector species of *Culicoides* in Europe and the Mediterranean Basin

Culicoides imicola has long been known as a vector of BTV in those parts of the region affected or suspected of being affected by the virus prior to 1998 (i.e. Israel, Cyprus, Anatolian Turkey, the Greek islands of Rhodes and Lesbos, Morocco, SW Spain and Portugal) (see Mellor and Wittmann, 2002). Indeed, the northernmost locations from which *C. imicola* had been identified in the Mediterranean Basin prior to 1998 (Portugal, SW Spain, Morocco, Algeria, Israel, Anatolian Turkey, Rhodes and Lesbos) are virtually identical to the list of the places affected, at one time or another by BTV prior to 1998. Furthermore, although *C. imicola* had been sought in many rather more northerly but adjacent areas in the region that had not been affected by BTV (northern and eastern Spain, Tunisia, Sicily, parts of mainland Italy, Bulgaria and mainland Greece), it had never been recorded from any of them (Callot *et al*., 1964; Callot and Kremer, 1969; Kremer *et al*., 1971; Chaker 1981; Mellor *et al*., 1984; Gloukova *et al*., 1991; Boorman *et al*., 1996; Scaramozzino *et al*., 1996; Dilovski *et al*., 1992; Patakakis, Personal Communication; Georgiev and Nedelchev, unpublished observations; Wilkinson, Personal Communication). This suggested that *C. imicola* was not only the most important BTV vector in the region but was also the *only* important vector species, otherwise the virus would, presumably, on some occasions have occurred in its absence. Consequently, the 1998–2005 BTV incursions into many areas of the Mediterranean Basin previously unaffected by the virus, including areas where *C. imicola* had been sought for but not found, was a new and totally unexpected turn of events. The entry of BTV into such areas, therefore, suggested that either the range of *C. imicola* had recently expanded or novel vector species of *Culicoides* were, for the first time, mediating BTV incursions in the region. In the event, the answer seems to include both of these possibilities. Vector surveys carried out in the BTV-affected areas during 2000 and subsequently have recorded the presence of populations of *C. imicola* at many locations in mainland Greece, mainland Italy, Sicily, Sardinia, Corsica, the Balearics, much of eastern Spain and parts of southern mainland France

(Goffredo *et al*., 2001, 2004; Miranda *et al*., 2003; Monteys and Saiz-Ardanaz, 2003; Giovannini *et al*., 2004a; Monteys *et al*., 2004; Monteys *et al*., 2005; Purse *et al*., 2005). Interestingly, and in context with the above findings, Baylis *et al*. (2001) had already used a series of remotely sensed variables to attempt to predict areas of the Mediterranean Basin that were climatically suitable for populations of *C. imicola* and hence at risk to BT, and this early work correctly identified almost all of the above locations as being suitable for *C. imicola*. In essence, therefore, the range of *C. imicola* does seem to have expanded northwards to include much of the northern coast of the Mediterranean Sea and most of the leg of Italy as far north as 44°N (Goffredo *et al*., 2001). The work of Purse *et al*. (2005) suggests that this sudden expansion is being driven by recent and ongoing changes in the European climate.

However, even though *C. imicola* has extended northwards so dramatically, the range of BTV has extended over 400 km beyond the distribution of this vector in certain parts of Europe, particularly the Balkans (Mellor, 2004), and even within the overall distribution of *C. imicola*, in some areas, BTV transmission has taken place in locations where *C. imicola* is either very rare or absent (e.g. parts of Sicily, Lazio and Tuscany in Italy) (De Liberato *et al*., 2005; Purse *et al*., 2004, 2005). This clearly means that in these areas, novel vector species of *Culicoides* are transmitting the virus – but what is the identity of these new vectors and why have they not previously been involved in BTV transmission in the region? Recent studies have shown that two widespread and abundant Palearctic *Culicoides* species complexes (*Culicoides obsoletus* and *Culicoides pulicaris*) comprise very large proportions of the *Culicoides* populations in the non-*C. imicola* BT areas and these species also show fine-scale spatial and temporal correlation with BT outbreaks (De Liberato *et al*., 2003, 2005; Torina *et al*., 2004). Also, during the current outbreaks, BTV has been regularly isolated in the field from wild-caught individuals of both species complexes (Caracappa *et al*., 2003; Savini *et al*., 2003, 2004, 2005; De Liberato *et al*., 2005). Earlier vector competence studies with these species indicated that they had a very low oral susceptibility to BTV (Mellor and Jennings, 1988; Jennings and Mellor, 1988), but the recent climate warming in Europe may well have increased their importance as vectors – by increasing their population sizes and survival rates to compensate for their low competence levels and by increasing levels of susceptibility through the temperature-controlled virus developmental effects as described elsewhere in this book (see Chapter 14). So climate warming may be the underlying reason why these novel vectors seem, for the first time, to be playing an important part in BTV transmission. The future role of the *C. obsoletus* and *C. pulicaris* species complexes in the transmission of BTV is a matter of real concern as these species are common and widespread across the whole of central and northern Europe (Purse *et al*., 2005). What, in a time of climate change, this means in terms of increasing risk from BT to livestock of the more northerly countries in Europe may become apparent in future years.

Bluetongue virus in Europe in 2006 and 2007

During the period from late 2006 to 2007, BTV appears to have fulfilled or exceeded all of the concerns previously raised about its northerly extension in range due to climate change and the involvement of Palearctic vector species of *Culicoides* in the more northerly areas (Mellor and Boorman, 1995). During 2006 a series of incursions of BTV into various parts of the European region occurred, the most significant by far being the introduction of BTV-8, a serotype new to Europe, into locations far beyond the usual range of any BTV and indeed further north than BTV has ever been recorded anywhere in the world (e.g. up to 53°N). This outbreak was first detected in the Maastricht area of the Netherlands on 14 August 2006 (International Society for Infectious Diseases, 2006a; World Organisation for Animal health, 2006) and spread rapidly along a broadly east–west axis to involve most of the country, virtually the whole of Belgium, much of NW Germany, Luxembourg and the northern borders of France. By the end of 2006, over 2000 outbreaks had been declared (EFSA, 2007a). The virus was reported to cause significant disease not only in sheep but also in cattle, which is indicative of a totally naïve population (Howell, 1963). At the time of writing, the origin of the incursion and its mode of entry are unknown, though there has been much speculation (see EFSA, 2007b), but molecular epidemiological investigations conducted at the Community Reference Laboratory (CRL) for bluetongue, based at IAH-Pirbright in the UK, have shown that the strains of BTV-8 isolated in NW Europe are most similar to BTV-8 isolates from sub-Saharan West Africa (International Society for Infectious Diseases, 2006b). However, this preliminary information is not conclusive as very few isolates of this serotype are available for comparison, and work is therefore ongoing. In the locations where BTV-8 has occurred in NW Europe, the traditional vector, *C. imicola*, is totally absent but members of the *Obsoletus* complex are extremely common. Indeed, in early October 2006, BTV nucleic acids were detected in a pool of non-blood–engorged, parous *C. dewulfi* in the Netherlands (Meiswinkel *et al*., 2007), a species that most workers have traditionally placed in the *Obsoletus* complex because of their close morphological similarity (Campbell and Pelham-Clinton, 1960). Recently, however, this species has been separated into a group on its own (Meiswinkel *et al*., 2004; Gomulski *et al*., 2005). This outbreak therefore provides a further example to those cited above in the Balkans and parts of Italy, showing that BTV can be efficiently transmitted by native Palearctic *Culicoides* species in the absence of *C. imicola.* The summer of 2006 was exceptionally warm, and July, when the incursion was first detected, was the warmest in northern Europe since records began in 1850. Temperatures across Belgium, the Netherlands, northern France and NW Germany during July were more than 5°C warmer than the 1961–1990 average. These are precisely the conditions that would be expected to enhance the competence levels of vector *Culicoides* in the region (see Chapter 14, this volume).

Towards the end of 2006 and with the advent of cooler weather, the number of BTV-8 outbreaks in northern Europe decreased and ended. Subsequently, there was no evidence of BTV transmission in northern Europe during the first few months of 2007. However, sadly in late April 2007, a sentinel bovine seroconverted in NW Germany (International Society for Infectious Diseases, 2007). This was the first indication that BTV-8 had successfully overwintered in northern Europe, and the seroconversion occurred close to the earliest date at which vector–host transmission would be predicted to be capable of resuming (Wilson *et al*., 2007).

During 2007, the outbreak of BTV-8 spread far beyond its greatest extent during 2006, reaching as far north as Denmark (Anon, 2007a) and as far east as the Czech Republic (Anon, 2007b) (Figure 11.5). Towards the end of 2007, the southern extension of the BTV-8 outbreak reached southern France and the first cases of BTV-8 in northern Spain were reported in January 2008 (Anon, 2008) (see Figure 11.5). The first UK case was reported in early August (Defra, 2007), although retrospective climatological analysis suggests that introduction probably took place in early August (Anon, 2007c). By the end of 2007, cases had been reported on 66 holdings in the UK, although over 40 more infected holdings were subsequently identified as a result of pre-movement testing requirements.

Figure 11.5 Bluetongue virus (BTV) restriction zones in the European Union at the time of going to press, showing the current BTV-8 epizootic in Northern Europe and the recent incursion of BTV-1 into Northern Spain and SW France (Adapted from: http://ec.europa.eu/food/animal/diseases/controlmeasures/bluetongue_en.htm) (See colour plate 21).

The 2007 epizootic of BTV-8 in northern Europe was far more aggressive than in 2006. Between July and October 2007, around 25 000 more sheep died in Belgium than during the same period in 2006, representing approximately one-sixth of the national flock (Wilson and Mellor, 2008). By the end of 2007, well over 30 000 farms in northern Europe had been infected, causing over €150 million damage through direct costs and many times that in lost trade (Hoogendam, 2007). The 2006 present epizootic of BTV-8 in northern Europe is believed to have caused greater economic damage than any previous single-serotype BT outbreak (Wilson and Mellor, 2008).

In addition to the above, fresh incursions of BTV-1 into Algeria and Morocco occurred during summer 2006 (Anon, 2006a, b) and extended into Sardinia (Anon, 2006c). Incursions of BTV-16 into Cyprus and Israel also occurred during October and November of the year and of BTV-4 and -15 into Israel in November and December (Anon, 2006d; http://www.reoviridae.org/dsrna_virus_proteins/ReoID/BTV-isolates.htm). The incursion of BTV-15 into Israel in late 2006 was the first occasion on which this serotype has been reported in the Mediterranean Basin. Serological evidence of BTV-8 was also detected in SE Bulgaria in late 2006 but no virus was isolated (International Society for Infectious Diseases, 2006c). Following the BTV-1 activity in Algeria, Morocco and Sardinia in autumn 2006, BTV-1 was detected in Spain for the first time in July 2007 (Anon, 2007d). This incursion established in southern Spain during August spread to Portugal in September (Anon, 2007e) before jumping to the Basque region in northern Spain in October, from which it spread to SW France in November (Anon, 2007f) (see Figure 11.5).

The precise origins of all of these incursions are uncertain but previous work has confirmed the presence of a number of BTV serotypes in the Middle and Near East and in sub-Saharan West Africa (Taylor and Mellor, 1994a, Herniman *et al*., 1983), and some of these have now been recorded in Europe. However, the list of serotypes from such areas is at best an out of date snapshot, and it is certain that others remain to be discovered. It is also certain that the recent unprecedented incursions of BTV-8 into NW Europe mean that as a result of changing climate, virtually the whole of Europe must now be considered to be at risk to this disease and probably to other *Culicoides*-transmitted diseases such as epizootic haemorrhagic disease, African horse sickness and Akabane.

Mechanisms of overwintering

In temperate regions, the activity of the *Culicoides* vectors of BTV generally drops to low levels or zero during the winter. Reports of BTV activity in temperate areas may therefore be separated by many months during which no new cases are detected. However, the same virus strains sometimes reappear in

the following year, strongly suggesting that the virus is capable of persisting in such regions without detectable activity (Osmani *et al.*, 2006). This phenomenon, termed 'overwintering', has been recognised for many years (Nevill, 1971) and various potential mechanisms for it have been suggested, most of which fall into one of two categories: virus persistence in the host or virus persistence in the vector.

Vertical transmission of BTV in vector midges has long been considered as a possible mechanism for the persistence of the virus over the vector-free winter period, as it is known that in temperate countries and zones, most *Culicoides* survive the winter as fourth instar larvae. Until recently, experiments designed to detect such vertical transmission in *Culicoides* species have consistently reported negative results (Jones and Foster, 1971; Nunamaker *et al.*, 1990; Mellor, 2000) but a recent American study detected fragments of BTV RNA in *Culicoides* larvae and pupae, suggesting that this mechanism may deserve further attention (see Chapter 14, this volume and White *et al.*, 2006). In addition, although midges are short-lived during the summer, several studies have suggested that they may live longer at cold temperatures than previously believed (Gerry and Mullens, 2000; Lysyk, 2007), raising the possibility that a small number of BTV-infected *Culicoides* adults may survive to carry BTV from one season to the next. Such a mechanism would facilitate the overwintering of BTV in regions where the winter period is relatively mild and short.

In the vertebrate hosts of BTV, cattle and sheep are considered to be infectious for up to 60 days following infection (Anon, 2007g), although most infections last for 2–4 weeks (Singer *et al.*, 2001; Gubbins *et al.*, 2008). However, one experimental study has suggested that BTV may be capable of persistently infecting certain inflammatory cells in sheep for substantially longer periods, the virus reappearing in the skin when an inflammatory response is provoked (Takamatsu *et al.*, 2003). The authors suggest that such a response may be triggered by the springtime resumption of biting, by midges, providing an elegant mechanism for virus to persist over the winter period. However, this laboratory-based study has not yet been shown to occur in the field nor has it been supported by similar studies carried out in the United States and Australia (Melville *et al.*, 2004; White and Mecham, 2004).

An alternative mechanism for persistence in the ruminant host is via transplacental infection. Such a mechanism has been described and demonstrated (Gibbs *et al.*, 1979) but is believed to be either a laboratory artefact induced by the use of tissue culture-passaged viruses (Kirkland and Hawkes, 2004) or extremely rare and so not of relevance in the field (MacLachlan *et al.*, 1992; Melville and Gard 1992). However, recent findings in England where PCR-positive and infectious virus-positive calves were born to dams infected in 2007 with BTV-8 during pregnancy suggest that transplacental transmission may be occurring in the field. Such a mechanism, if common, would help explain the widespread overwintering of BTV-8 in NW Europe from 2006 to

2007, which anecdotal information suggests, occurred particularly frequently in areas where transmission had been very intense and so presumably in locations where there were many infected animals. At the time of writing, further evidence supporting the occurrence of overwintering of BTV-8 via transplacental transmission in the field has been provided by a recent report from Northern Ireland where a number of calves born to seropositive cattle during the vector-free period were viraemic (Gildernew, 2008). Clearly, there is an urgent need to confirm that BTV-8 is being transmitted in this way and if it is, to elucidate the basis of the mechanism and determine the duration and titre of infectious viraemia in the newborn animals. It will also be important to determine whether such a mechanism is a characteristic of the particular strain of BTV-8 present in northern Europe or whether it occurs with other field strains and serotypes of BTV also. Confirmation of the existence of transplacental transmission of BTV in the field is likely to have a significant impact upon regulations governing the international movement of animals.

Summary

Bluetongue exists around the world in a broad band covering much of the America, Africa, southern Asia, northern Australia and, occasionally, the southern fringe of Europe. It is considered to be one of the most important diseases of domestic livestock. Recently the virus causing this disease has extended its range northwards into areas of Europe never before affected and has persisted in many of these locations causing the greatest epizootic of the disease on record. Indeed, the most recent outbreaks of BT in Europe are further north than this virus has ever previously occurred anywhere in the world. The reasons for this dramatic change in BT epidemiology are complex but are linked to recent extensions in the distribution of its major vector, *C. imicola*, to the involvement of novel *Culicoides* vector(s) and to ongoing climate change. This paper explores these areas, highlights prospects for the future and discusses recent findings relating to the ability of BTV-8 to overwinter in northern Europe.

References

Anon (1998a) Disease outbreaks reported to the OIE in November–December 1998. *Bull. Off. Int. Epizoot.* **110**, 506.

Anon (1998b) Bluetongue in Greece: confirmation of diagnosis. *Off. Int. Epizoot. Dis. Info* **11**, 166–7.

Anon (1999a) Disease outbreaks reported to the OIE in January–February 1999. *Bull. Off. Int. Epizoot.* **111**, 18.

Anon (1999b) Disease outbreaks reported to the OIE in July–August 1999. *Bull. Off. Int. Epizoot.* **111**, 379.

Anon (1999c) Disease outbreaks reported to the OIE in September–October 1999. *Bull. Off. Int. Epizoot.* **111**, 449.
Anon (1999d) Disease outbreaks reported to the OIE in November–December 1999. *Bull. Off. Int. Epizoot.* **111**, 543.
Anon (1999e) Bluetongue in Bulgaria: additional information. *Off. Int. Epizoot. Dis. Info.* **12**, 122.
Anon (2000a) Disease outbreaks reported to the OIE in January–February 2000. *Bull. Off. Int. Epizoot.* **112**, 16.
Anon (2000b) Disease outbreaks reported to the OIE in July–August 2000. *Bull. Off. Int. Epizoot.* **112**, 449.
Anon (2000c) Disease outbreaks reported to the OIE in September–October 2000. *Bull. Off. Int. Epizoot.* **112**, 552.
Anon (2000d) Disease outbreaks reported to the OIE in November–December 2000. *Bull. Off. Int. Epizoot.* **112**, 687–8.
Anon (2000e) Bluetongue in Algeria. *EMPRES Transboundary Anim. Dis. Bull.* **14**, 20–1.
Anon (2000f) Bluetongue in Greece: follow-up report. *Off. Int. Epizoot. Dis. Info.* **13**, 9–13.
Anon (2000g) Bluetongue in Tunisia: additional information. *Off. Int. Epizoot. Dis. Info.* **13**, 21.
Anon (2000h) Bluetongue in Greece: typing of virus strains isolated in 1998–1999. *Off. Int. Epizoot. Dis. Info.* **13**, 33–4.
Anon (2000i) Bluetongue in Tunisia: follow-up report no. 2. *Off. Int. Epizoot. Dis. Info.* **13**, 176.
Anon (2000j) Bluetongue in France: in the island of Corsica. *Off. Int. Epizoot. Dis. Info.* **13**, 195–7.
Anon (2000k) Bluetongue in Italy: follow-up report no. 1. *Off. Int. Epizoot. Dis. Info.* **13**, 209–10.
Anon (2000l) Bluetongue in Spain: in the Balearic islands. *Off. Int. Epizoot. Dis. Info.* **13**, 224.
Anon (2001a) Disease outbreaks reported to the OIE in January–February 2001. *Bull. Off. Int. Epizoot.* **113**, 17–18.
Anon (2001b) Bluetongue in France: in the island of Corsica. *Off. Int. Epizoot. Dis. Info.* **14**, 178.
Anon (2001c) Bluetongue in Greece and Italy. *Off. Int. Epizoot. Dis. Info.* **14**, 215–18.
Anon (2001d) Bluetongue in Bulgaria: follow-up report no. 1. *Off. Int. Epizoot. Dis. Info.* **14**, 230–1.
Anon (2001e) Bluetongue in Kosovo (FRY), territory under united nations interim administration. *Off. Int. Epizoot. Dis. Info.* **14**, 242–3.
Anon (2001f) Bluetongue in Yugoslavia. *Off. Int. Epizoot. Dis. Info.* **14**, 280–1.
Anon (2001g) Bluetongue in Greece: follow-up report no. 2. *Off. Int. Epizoot. Dis. Info.* **14**, 262–5.
Anon (2001h) Bluetongue in the former Yugoslav republic of Macedonia. *Off. Int. Epizoot. Dis. Info.* **14**, 234.
Anon (2001i) Bluetongue in Croatia: suspected outbreak. *Off. Int. Epizoot. Dis. Info.* **14**, 291–2.
Anon (2001j) Bluetongue in the former Yugoslav republic of Macedonia: follow-up report no. 1. *Off. Int. Epizoot. Dis. Info.* **14**, 265–8.

Anon (2001k) Bluetongue in Yugoslavia. *Off. Int. Epizoot. Dis. Info.* **14**, 252.
Anon (2001l) Bluetongue in Yugoslavia (additional information). *Off. Int. Epizoot. Dis. Info.* **14**, 280.
Anon (2001m) Bluetongue in Croatia: suspected outbreak. *Off. Int. Epizoot. Dis. Info.* **14**, 291.
Anon (2002) Bluetongue in Bosnia and Herzegovina. *Off. Int. Epizoot. Dis. Info.* **15**, 171.
Anon (2003a) Bluetongue in Israel. *Off. Int. Epizoot. Dis. Info.* **16**, 247.
Anon (2003b) Bluetongue in Israel (additional information). *Off. Int. Epizoot. Dis. Info.* **16**, 258.
Anon (2004a) Bluetongue in Morocco. *Off. Int. Epizoot. Dis. Info.* **17**, 273.
Anon (2004b) Bluetongue in Morocco (follow up report no. 1.). *Off. Int. Epizoot. Dis. Info.* **17**, 297.
Anon (2004c) Bluetongue in Morocco (follow-up report no. 2). *Off. Int. Epizoot. Dis. Info.* **17**, 318.
Anon (2004d) Bluetongue in Morocco (follow-up report no. 3). *Off. Int. Epizoot. Dis. Info.* **17**, 329.
Anon (2004e) Bluetongue in Morocco (follow-up report no. 4). *Off. Int. Epizoot. Dis. Info.* **17**, 346.
Anon (2004f) Bluetongue in Morocco (follow-up report no. 5). *Off. Int. Epizoot. Dis. Info.* **17**, 368.
Anon (2004g) Bluetongue in Morocco (follow-up report no. 6). *Off. Int. Epizoot. Dis. Info.* **17**, 383.
Anon (2004h) Bluetongue in France in the island of Corsica. *Off. Int. Epizoot. Dis. Info.* **17**, 266.
Anon (2006a) Bluetongue in Algeria (immediate notification). *Off. Int. Epizoot. Dis. Info.* **19**, 30.
Anon (2006b) Bluetongue in Morocco (immediate notification). *Off. Int. Epizoot. Dis. Info.* **19**, 44.
Anon (2006c) Bluetongue in France (immediate notification). *Off. Int. Epizoot. Dis. Info.* **19**, 35.
Anon (2006d) Bluetongue in Israel (immediate notification). *Off. Int. Epizoot. Dis. Info.* **19**, 48.
Anon (2007a) 13/10/2007: Bluetongue, Denmark (immediate notification). *Off. Int. Epizoot. Dis. Info.* **20**, 42.
Anon (2007b) 29/11/2007: Bluetongue, Czech Republic (immediate notification). *Off. Int. Epizoot. Dis. Info.* **20**, 48.
Anon (2007c) Bluetongue arrived on August 4/5, says DEFRA. *Vet. Rec.* **161**, 602.
Anon (2007d) 27/07/2007: Bluetongue, Spain (immediate notification). *Off. Int. Epizoot. Dis. Info.* **20**, 30.
Anon (2007e) 18/09/2007: Bluetongue, Portugal (immediate notification). *Off. Int. Epizoot. Dis. Info.* **20**, 39.
Anon (2007f) 18/09/2007: Bluetongue, France (immediate notification). *Off. Int. Epizoot. Dis. Info.* **20**, 48.
Anon (2007g) *Chapter 2.2.13 Bluetongue. Terrestrial Animal Health Code*. World Organisation for Animal Health, pp. 131–6.
Anon (2008) 17/01/2008: Bluetongue, Spain, (immediate notification). *Off. Int. Epizoot. Dis. Info.* **21**, 3.
Baylis, M., Mellor, P.S., Wittmann, E.J. and Rogers, D.J. (2001) Prediction of areas around the Mediterranean at risk of bluetongue by modelling the distribution of its vector using satellite imaging. *Vet. Rec.* **149**, 639–43.

Boinas, F., Amador, R., Pereira da Fonseca, I., Nunes, T., Fevereiro, M. and Pinheiro, C. (2005) Perspectives of bluetongue control in Portugal. In: Proc. Third Vet. Sci. Cong. Santarém, 13–15 October 2005, Lisbon, Portugal: Sociedade Portuguesa de Ciências Veterinárias, p. 18.

Boorman, J., Mellor, P.S. and Scaramozzino, P. (1996) A new species of *Culicoides* (Diptera: Ceratopogonidae) from southern Italy. *Parassitologia* **38**, 501–3.

Braverman, Y., Baylis, M., Baylis, M., Tatem, A.J., Rogers, D.J., Mellor, P.S. and Purse, B.V. (2004) What factors determine when epidemics occur in the Mediterranean? Prediction of disease risk through time by climate-driven models of the temporal distribution of outbreaks in Israel. *Vet. Ital.* **40**, 235–42.

Breard, E., Hamblin, C., Hammami, S., Sailleau, C., Dauphin, G. and Zientara, S. (2004) The epidemiology and diagnosis of bluetongue with particular reference to Corsica. *Res. Vet. Sci.* **77**, 1–8.

Burgu, I., Urman, H.K., Akca, Y., Yonguc, A.D., Mellor, P.S. and Hamblin, C. (1992) Serological survey and vector surveillance for bluetongue in southern turkey. In: T.E. Walton and B.I. Osburn (eds), *Proc. Second Int. Symp. Bluetongue, African Horse Sickness & Related Orbiviruses*. Boca Raton, Fl: CRC Press, pp. 168–74.

Calistri, P., Giovannini, A., Conte, A., Nannini, D., Santucci, U., Patta, C., Rolesu, S. and Caporale, V. (2004) Bluetongue in Italy: part 1. *Vet. Ital.* **40**, 243–51.

Callot, J. and Kremer, M. (1969) Description d'un *Culicoides* nouveau: *C. jumineri* (Diptera, Ceratopogonidae) trouve en Tunisie. *Bull. Soc. Pathol. Exot.* **62**, 1112–118.

Callot, J., Kremer, M. and Juminer, B. (1964) Contribution a l'etude des *Culicoides* (Diptera, Ceratopogonidae) de Tunisie. *Arch. Inst. Pasteur Tunis* **41**, 357–64.

Campano Lopez, A. and Sanchez Botija, C. (1958) L'epizootie de fievre catarrhale ovine en Espagne (blue tongue). *Bull. Off. Int. Epizoot.* **50**, 65–93.

Campbell, J.A. and Pelham-Clinton, B.A. (1960) A taxonomic review of the British species of *Culicoides latreille* (Diptera: Ceratopogonidae). *Proc. R. Soc. Edinb, B* **67**, 181–302.

Caracappa, S., Torina, A., Guercio, A., Vitale, F., Calabro, A., Purpari, G., Ferrantelli, V., Vitale, M. and Mellor, P.S. (2003) Identification of a novel bluetongue virus vector species of *Culicoides* in Sicily. *Vet. Rec.* **153**, 71–4.

Chaker, E. *Memoire presente en vue de l'obtention du diplome d'etudes et de recherches en biologie humaine*. Universite Louis Pasteur de Strasbourg, p. 196.

Defra (2007). News release (Ref: 316/07, 22 September 2007): Bluetongue detected in Suffolk. http://www.defra.gov.uk/news/2007/070922a.htm.

De Liberato, C., Purse, B.V., Goffredo, M., Scholl, F. and Scaramozzino, P. (2003) Geographical and seasonal distribution of the bluetongue virus vector, *Culicoides imicola*, in central Italy. *Med. Vet. Entomol.* **17**, 388–94.

De Liberato, C., Scavia, G., Lorenzetti, R., Scaramozzino, P., Amaddeo, D., Cardeti, G., Scicluna, M., Ferrari, G. and Autorino, G.L. (2005) Identification of *Culicoides obsoletus* (Diptera: Ceratopogonidae) as a vector of bluetongue virus in central Italy. *Vet. Rec.* **156**, 301–304.

Di Ventura, M., Tittarelli, M., Semproni, G., Bonfini, B., Savini, G., Conte, A. and Lika, A. (2004) Serological surveillance of bluetongue virus in cattle, sheep and goats in Albania. *Vet. Ital.* **40**, 101–104.

Dilovski, M., Nedelchev, N. and Petkova, K. (1992) Studies on the species composition of *Culicoides* – potential vectors of the virus of the bluetongue in Bulgaria. *Vet. Sci.* **26**, 52–6.

Djuricic, B., Nedic, D., Lausevic, D. and Pavlovic, M. (2004) The epizootiological occurrence of bluetongue in the central Balkans. *Vet. Ital.* **40**, 105–107.

Dragonas, P.N. (1981) Evolution of bluetongue in Greece. *Off. Int. Monthly Epizoot. Circ.* **9**, 10–11.

EFSA (2007a) Bluetongue Serotype 8 Epidemic Bulletin 15. http://www.efsa.europa.eu/etc/medialib/efsa/in_focus/bluetongue.Par.0041.File.dat/bluetongue_update_1_February_2007.pdf.

EFSA (2007b) Report on Epidemiological analysis of the 2006 bluetongue virus serotype 8 epidemic in north-western Europe. http://www.efsa.europa.eu/EFSA/DocumentSet/Report_bluetongue_S8_en,0.pdf.

Fevereiro, M., Luis, T., Vaz, A., Ramos, F., Fagulha, M.T., Barros, S., Duarte, M.M. and Cruz, M.B. (2005) Bluetongue virus serotype 4 in Portugal In: *Proc. Third Vet. Sci. Cong. Santarém*, *13–15 October 2005*, Lisbon, Portugal: Sociedade Portuguesa de Ciências Veterinárias, p. 107.

Gerry, A.C. and Mullens, B.A. (2000) Seasonal abundance and survivorship of *Culicoides sonorensis* (Diptera: Ceratopogonidae) at a southern California dairy, with reference to potential bluetongue virus transmission and persistence. *J. Med. Entomol.* **37**, 675–88.

Gibbs, E.P.J., Lawman, M.J.P. and Herniman, K.A.J. (1979) Preliminary observations on transplacental infection of bluetongue virus in sheep – a possible overwintering mechanism. *Res. Vet. Sci.* **27**, 118–20.

Gildernew, M. (2008) Ministerial Statement to Assembly: Update on bluetongue. Official Report, Northern Ireland Assembly, 19 February 2008.

Giovannini, A., Calistri, P., Conte, A., Savini, L., Nannini, D., Patta, C., Santucci, U. and Caporale, V. (2004a) Bluetongue surveillance in a newly infected area. *Vet. Ital.* **40**, 188–97.

Giovannini, A., Calistri, P., Nannini, C., Paladini, C., Santucci, U., Patta, C. and Caporale, V. (2004b) Bluetongue in Italy. *Vet. Ital.* **40**, 252–9.

Gloukova, V.M., Nedelchev, N.K., Rousev, I. and Tanchev, T. (1991) On the fauna of blood-sucking midges of the genus *Culicoides* (Diptera: Ceratopogonidae) in Bulgaria. *Vet. Sci.* **25**, 63–6.

Goffredo, M., Conte, A. and Meiswinkel, R. (2004) Distribution and abundance of *Culicoides imicola*, obsoletus complex and pulicaris complex in Italy. *Vet. Ital.* **40**, 270–4.

Goffredo, M., Satta, G., Torina, A., Federico, G., Scaramozzino, P., Cafiero, M.A., Lelli, R. and Meiswinkel, R. (2001) The 2000 bluetongue virus (BTV) outbreak in Italy: distribution and abundance of the principle vector *Culicoides imicola* Kieffer. In: *Proc. Tenth Int. Symp. Amer. Assoc. Vet. Lab. Diagnosticians (AAVLD),* Salsomaggiore, Parma, 4–7 July, Ames: AAVLD, pp. 308–9.

Gomez-Tejedor, C. (2004) Brief overview of the bluetongue situation in Mediterranean Europe, 1998–2004. *Vet. Ital.* **40**, 57–60.

Gomulski, L.M., Meiswinkel, R., Delecolle, J.-C., Goffredo, M. and Gasperi, G. (2005) Phylogenetic relationships of the subgenus avaritia fox, 1955 including *Culicoides obsoletus* (Diptera: Ceratopogonidae) in Italy based on internal transcribed spacer 2 ribosomal DNA sequences. *Syst. Entomol.* **30**, 619–31.

Gorman, B.M. (1990) The bluetongue viruses. *Curr. Top. Microbiol. Immunol.* **162**, 1–19.

Gubbins, S., Carpenter, S., Baylis, M., Wood, J.L.N. and Mellor, P.S. (2008) Assessing the risk of bluetongue to UK livestock: uncertainty and sensitivity analysis of a temperature-dependent model for the basic reproductive number. *J. R. Soc. Interface* **5**, 363–71.

Hammami, S. (2004) North Africa: a regional overview of bluetongue virus, vectors, surveillance and unique features. *Vet. Ital.* **40**, 43–6.

Hassan, A. (1992) Status of bluetongue in the Middle East and Africa. In: T.E. Walton and B.I. Osburn (eds), *Bluetongue, African Horse Sickness and Related Orbiviruses. 2nd Int. Symp,* Paris: CRC Press: pp. 38–41.

Hayashi, K., Suzuki, H. and Asahina, S. (1979) Notes on the transoceanic insects-captured on East China sea in 1976, 1977, and 1978. (In Japanese with English summary). *Trop. Med.* **21**, 1–10.

Herniman, K.A.J., Boorman, J.P.T. and Taylor, W.P. (1983) Bluetongue virus in a Nigerian dairy cattle herd. 1. Serological studies and correlation of virus activity to vector population. *J. Hyg. Camb.* **90**, 177–93.

Herniman, K.A.J., Gumm, I.D., Owen, L., Taylor, W.P. and Sellers, R.F. (1980) Distribution of bluetongue virus and antibodies in some countries of the eastern hemisphere. *Bull. Off. Int. Epizoot.* **92**, 581–6.

Hoogendam, K. (2007) *International Study on the Economic Consequences of Outbreaks of Bluetongue Serotype 8 in North-Western Europe*. Leeuwarden: Van Hall Institute.

Howell, P.G. (1963) *Bluetongue*. *In*: *Emerging Diseases of Animals*. Rome: FAO, pp. 111–53.

International Society for Infectious Diseases (2006a) Bluetongue, ovine – Netherlands: confirmed. ProMED-mail 2006; 18 August: 20060818.2311. http://www.promedmail.org.

International Society for Infectious Diseases (2006b) Bluetongue – Netherlands, Belgium, Germany (06): BTV-8. ProMED-mail 2006; 28 August: 20060828.2448. http://www.promedmail.org.

International Society for Infectious Diseases (2006c). Bluetongue – Europe (21): Bulgaria, BTV-8 suspected. ProMED-mail 2006; 24 November: 20061124.3347. http://www.promedmail.org.

International Society for Infectious Diseases (2007). Bluetongue – Europe (14): BTV-8, Germany (Nordrhein-Westfalen), confirmed. ProMED-mail 2007; 13 June: 20070613.1928), http://www.promedmail.org.

Jennings, D.M. and Mellor, P.S. (1988) The vector potential of British *Culicoides* species for bluetongue virus. *Vet. Microbiol.* **17**, 1–10.

Jones, R.H. and Foster, N.M. (1971) Transovarial transmission of bluetongue virus unlikely for *Culicoides variipennis. Mosq. News* **31**, 434–7.

Kirkland, P.D. and Hawkes, R.A. (2004) A comparison of laboratory and 'wild' strains of bluetongue virus – is there any difference and does it matter? *Vet. Ital.* **40**, 448–55.

Knowles, N.J.D. and Davies, P.R. (2000) Origin of recent outbreaks of foot-and-mouth disease in North Africa, the Middle East and Europe. Report of the Session of the Research Group of the Standing Technical Committee of the European Commission for the Control of Foot-and-Mouth Disease, 3 edn., Borovets, Bulgaria. FAO Rome, pp. 39-45.

Kremer, M., Leberre, G. and Beaucournu-Saguez, F. (1971) Notes sur le *Culicoides* (Diptera, Ceratopogonidae) de Corse. Description de *C. corsicus* n.sp. *Ann. Parasitol. Hum. Comp.* **46**, 635–60.

Labrovic, A., Poljak, Z., Separovic, S., Jukic, B., Lukman, D., Listes, E. and Bosnic, S. (2004) Spatial distribution of bluetongue in cattle in southern Croatia in the last quarter of 2002. *Vet. Ital.* **40**, 217–20.

Listes, E., Bosnic, S., Benic, M., Lojkic, M., Cac, Z., Cvetnic, Z., Madic, J., Separovic, S., Labrovic, A., Savini, G. and Goffredo, M. (2004) Serological evidence of bluetongue and a preliminary entomological study in southern Croatia. *Vet. Ital.* **40**, 221–5.

Lysyk, T.J. (2007) Seasonal abundance, parity, and survival of adult *Culicoides sonorensis* (Diptera: Ceratopogonidae) in southern Alberta, Canada. *J. Med. Entomol.* **44**, 959–69.

Maan, S., Samuel, A.R., Maan, N.S., Attoui, H., Rao, S. and Mertens, P.P.C. (2004) Molecular epidemiology of bluetongue viruses from disease outbreaks in the Mediterranean Basin. *Vet. Ital.* **40**, 489–96.

Manso-Ribeiro, J., Rosa-Azevedo, J.A., Noronha, F.M.O., Braco-Forte-Junior, M.C., Grave-Pereira, C. and Vasco-Fernandes, M. (1957) Fievre catarrhale du mouton (Bluetongue). *Bull. Off. Int. Epizoot.* **48**, 350–367.

Manso-Ribeiro, J. and Noronha, F.M.O. (1958) Fievre catarrhale du mouton au Portugal (blue tongue). *Bull. Off. Int. Epizoot.* **50**, 46–64.

McLachlan, N.J., Barratt-Boyes, S.M. and Brewer, A.W. (1992) Bluetongue virus infection of cattle. In: T.E. Walton and B.I. Osburn, (eds.), *Bluetongue, African horse sickness and related Orbiviruses,* CRC Press, Boca Raton, pp. 725–736.

Meiswinkel, R., Gomulski, L.M., Delecolle, J.-C., Goffredo, M. and Gasperi, G. (2004) The taxonomy of *Culicoides* vector complexes – unfinished business. *Vet. Ital.* **40**, 151–9.

Meiswinkel, R., van Rijn, P., Leijs, P. and Goffredo, M. (2007) Potential new *Culicoides* vector of bluetongue virus in northern Europe. *Vet. Rec.* **161**, 564–5.

Mellor, P.S. (2000) Replication of arboviruses in insect vectors. *J. Comp. Pathol.* **123**, 231–47.

Mellor, P.S. (2004) Infection of the vectors and bluetongue epidemiology in Europe. *Vet Ital.* **40**, 167–74.

Mellor, P.S. and Jennings, D.M. (1988) The vector potential of British Culicoides species for bluetongue virus. In: P. Roy and B. Osburn (eds.), *Proceedings of a BTV Workshop, Double Stranded RNA Virus Symposium,* Oxford, 9-13th September 1986, pp. 12–21.

Mellor, P.S. and Boorman, J. (1995) The transmission and geographical spread of African horse sickness and bluetongue viruses. *Ann. Trop. Med. Parasitol.* **89**, 1–15.

Mellor, P.S., Boorman, J. and Baylis, M. (2000) *Culicoides* biting midges: their role as arbovirus vectors. *Ann. Rev. Entomol.* **45**, 307–40.

Mellor, P.S., Jennings, M. and Boorman, J.P.T. (1984) *Culicoides* from Greece in relation to the spread of bluetongue virus. *Rev. Elev. Med. Vet. Pays. Trop.* **37**, 286–9.

Mellor, P.S. and Pitzolis, G. (1979) The transmission and geographical spread of African horse sickness and bluetongue viruses. *Ann. Trop. Med. Parasitol.* **89**, 1–15.

Mellor, P.S. and Wittmann, E.J. (2002) Bluetongue virus in the Mediterranean Basin 1998–2001. *Vet. J.* **164**, 20–37.

Melville, L.F. and Gard, G.P. (1992) Investigations of the natural infection with Orbiviruses on reproduction in cattle. In: T.E. Walton and B.I. Osburn, (eds.), *Bluetongue, African horse sickness and related Orbiviruses,* CRC Press, Boca Raton, pp. 744–750.

Melville, L.F., Hunt, N.T., Davis, S.S. and Weir, R.P. (2004) Bluetongue virus does not persist in naturally infected cattle. *Vet. Ital.* **40**, 502–7.

Miranda, M.A., Borras, D., Rincon, C. and Alemany, A. (2003) Presence in the Balearic islands (Spain) of the midges *Culicoides imicola* and *Culicoides obsoletus* group. *Med. Vet. Entomol.* **17**, 52–4.

Monteys, V.S., Aranda, C., Escosa, R., Pages, N. and Ventura, D. (2004) Results of current surveillance of likely bluetongue virus vectors of the genus *Culicoides* in Catalonia, Spain. *Vet. Ital.* **40**, 130–2.

Monteys, V.S.I. and Saiz-Ardanaz, M. (2003) *Culicoides* midges in Catalonia (Spain), with special reference to likely bluetongue virus vectors. *Med. Vet. Entomol.* **17**, 288–93.

Monteys, V.S.I., Ventura, D., Pages, N., Aranda, C. and Escosa, R. (2005) Expansion of *Culicoides imicola*, the main bluetongue virus vector in Europe, into Catalonia, Spain. *Vet. Rec.* **156**, 415–17.

Murray, M.D. and Nix, H.A. (1987) Southern limits of distribution and abundance of the biting-midge *Culicoides brevitarsis* Kieffer (Diptera, Ceratopogonidae) in south-eastern Australia – an application of the growest model. *Aust. J. Zool.* **35**, 575–85.

NASA and Earthsat (2004) World Imagery Base Maps. http://www.geographynetwork.com.

Nevill, E.M. (1971) Cattle and *Culicoides* biting midges as possible overwintering hosts of bluetongue virus. *Onderstepoort J. Vet. Res.* **38**, 65–72.

Nunamaker, R.A., Sieburth, P.J., Dean, V.C., Wigington, J.G., Nunamaker, C.E. and Mecham, J.O. (1990). Absence of transovarial transmission of bluetongue virus in *Culicoides variipennis*: immunogold labelling of bluetongue virus antigen in developing oocytes from *Culicoides variipennis* (Coquillett). *Comp. Biochem. Physiol. A* **96**, 19–31.

Osmani, A., Murati, B., Kabashi, Q., Goga, I., Berisha, B., Wilsmore, A.J. and Hamblin, C. (2006) Evidence for the presence of bluetongue virus in Kosovo between 2001 and 2004. *Vet. Rec.* **158**, 393–6.

Patta, C., Giovannini, A., Rolesu, S., Nannini, D., Davini, G., Calistri, P., Santucci, U. and Caporale, V. (2004) Bluetongue vaccination in Europe: the Italian experience. *Vet. Ital.* **40**, 601–610.

Placidi, L. (1957) La bluetongue au Maroc. *Bull. Acad. Vét. Fr.* **30**, 79–84.

Purse, B.V., Mellor, P.S., Rogers, D.J., Samuel, A.R., Mertens, P.P.C. and Baylis, M. (2005) Climate change and the recent emergence of bluetongue in Europe. *Nature Rev. Microbiol.* **3**, 171–81.

Purse, B.V., Nedelchev, N., Georgiev, G., Veleva, E., Boorman, J., Veronesi, E., Carpenter, S., Baylis, M. and Mellor, P.S. (2006) Spatial and temporal distribution of bluetongue and its *Culicoides* vectors in Bulgaria. *Med. Vet. Entomol.* **20**, 335–44.

Purse, B.V., Tatem, A.J., Caracappa, S., Rogers, D.J., Mellor, P.S., Baylis, M. and Torina, A. (2004) Modelling the distributions of *Culicoides* bluetongue virus vectors in Sicily in relation to satellite-derived climate variables. *Med. Vet. Entomol.* **18**, 90–101.

Savini, G., Goffredo, M., Monaco, F., Di Gennaro, A., Cafiero, M.A., Baldi, L., De Santis, P., Meiswinkel, R. and Caporale, V. (2005) Bluetongue virus isolations from midges belonging to the *Obsoletus* complex(*Culicoides*, Diptera: Ceratopogonidae) in Italy. *Vet. Rec.* **157**, 133–9.

Savini, G., Goffredo, M., Monaco, F., Di Gennaro, A., de Santis, P., Meiswinkel, R. and Caporale, V. (2004) The isolation of bluetongue virus from field populations of the obsoletus complex in central Italy. *Vet. Ital.* **40**, 286–91.

Savini, G., Goffredo, M., Monaco, F., de Santis, P. and Meiswinkel, R. (2003) Transmission of bluetongue virus in Italy. *Vet. Rec.* **152**, 119.

Scaramozzino, P., Boorman, J.P.T., Vitale, F., Semproni, G. and Mellor, P.S. (1996) Entomological survey on Ceratopogonidae in Central-Southern Italy. *Parassitologia* **38**, 1–2.

Sellers, R.F. (1975) Bluetongue in Cyprus. *Aust. Vet. J.* **51**, 198–203.

Sellers, R.F., Gibbs, E.P., Herniman, K.A., Pedgley, D.E. and Tucker, M.R. (1979b) Possible origin of the bluetongue epidemic in Cyprus, August 1977. *J. Hyg. (Lond.)* **83**, 547–55.

Sellers, R.F. and Maarouf, A.R. (1991) Possible introduction of epizootic haemorrhagic-disease of deer virus (serotype-2) and bluetongue virus (serotype-11) into British-Columbia in 1987 and 1988 by infected *Culicoides* carried on the wind. *Can. J. Vet. Res.* **55**, 367–720.

Sellers, R.F. and Mellor, P.S. (1993) Temperature and the persistence of viruses in *Culicoides* during adverse conditions. *Off. Int. Epizoot. Rev. Sci. Tech.* **12**, 733–55.

Sellers, R.F., Pedgley, D.E. and Tucker, M.R. (1979a) Possible windborne spread of bluetongue to Portugal, June–July 1956. *J. Hyg. (Lond.)* **81**, 189–96.

Shimshony, A. (1987) Bluetongue activity in Israel, 1950–1985: the disease, virus prevalence, control methods In: W.P. Taylor (ed.), Bluetongue in the Mediterranean region. Proc. Meeting in the Community Programme for Coordination of Agricultural Research. Istituto Zooprofilattico Sperimentale dell' Abruzzo e del Molise, 3–4 October 1985, Teramo, Italy: Commission of the European Communities, Brussels, Luxembourg, pp. 1–22.

Shimshony, A. (2004) Bluetongue in Israel - a brief historical overview. *Vet. Ital.* **40**, 116–118.

Singer, R.S., MacLachlan, N.J. and Carpenter, T.E. (2001) Maximal predicted duration of viraemia in bluetongue virus-infected cattle. *J. Vet. Diagn. Invest.* **13**, 43–9.

Takamatsu, H.-H., Mellor, P.S., Mertens, P.P.C., Kirkham, P.A., Burroughs, J.N. and Parkhouse, R.M.E. (2003) A possible overwintering mechanism for bluetongue virus in the absence of the insect vector. *J. Gen. Virol.* **84**, 227–35.

Taylor, W.P. (1987) Bluetongue in Syria and Jordan. *Proc. Meeting in the Community Programme for Coordination of Agricultural Research. Istituto Zooprofilattico Sperimentale Dell' Abruzzo E Del Molise, 3–4 October 1985,* 39–47, ed.

Taylor, W.P. and Mellor, P.S. (1994a) Bluetongue virus distribution in Turkey 1978–1981. *Epidemiol. Infect.* **112**, 623–33.

Taylor, W.P. and Mellor, P.S. (1994b) The distribution of Akabane virus in the Middle East. *Epidemiol. Infect.* **113**, 175–85.

Taylor, W.P., Sellers, R.F., Gumm, I.D., Herniman, K.A.J. and Owen, L. (1985) Bluetongue epidemiology in the Middle East In: T.L. Barber and M.M. Jochim (eds), *Bluetongue and Related Orbiviruses*. New York: Alan R. Liss Inc, pp. 527–30.

Torina, A., Caracappa, S., Mellor, P.S., Baylis, M. and Purse, B.V. (2004) Spatial distribution of bluetongue virus and its *Culicoides* vectors in Sicily. *Med. Vet. Entomol.* **18**, 81–9.

Urman, H.K., Mille, U.È., Mert, N., Berkin, S.Ë., Kahramen, M.M., Yue, H. and Avvurran, H. (1980) Tuerkiye'de buzagilarda konjenital epizootik arthrogryposis ve hydranencephalie olaylari. Ankara universitesi. *Veteriner Fakgltesi Dergisi* **26**, 287–95.

Vassalos, M. (1980) Cas de fièvre catarrhale du mouton dans l'ile de Lesbos (Grèce). *Bull. Off. Int. Epizoot.* **92**, 547–55.

White, D.M. and Mecham, J.O. (2004) Lack of detectable bluetongue virus in skin of seropositive cattle: implications for vertebrate overwintering of bluetongue virus. *Vet. Ital.* **40**, 513–19.

White, D.M., Blair, C.D. and Beaty, B.J. (2006) Molecular epidemiology of bluetongue virus in northern Colorado. *Virus Res.* **118**, 39–45.

Wilson, A., Carpenter, S., Gloster, J. and Mellor, P.S. (2007) Re-emergence of BTV-8 in northern Europe in 2007. *Vet. Rec.* **161**, 487–9.

Wilson, A. and Mellor, P.S. (2008) Bluetongue in Europe: vectors, epidemiology and climate change. *Parasitol. Res.*, in press.

World Organisation for Animal Health (2006) Bluetongue detected for first time in Northern Europe. http://www.oie.int/eng/press/en_060823.htm.

Yonguc, A.D., Taylor, W.P., Csontos, L. and Worrall, E. (1982) Bluetongue in western Turkey. *Vet. Rec.* **111**, 144–6.