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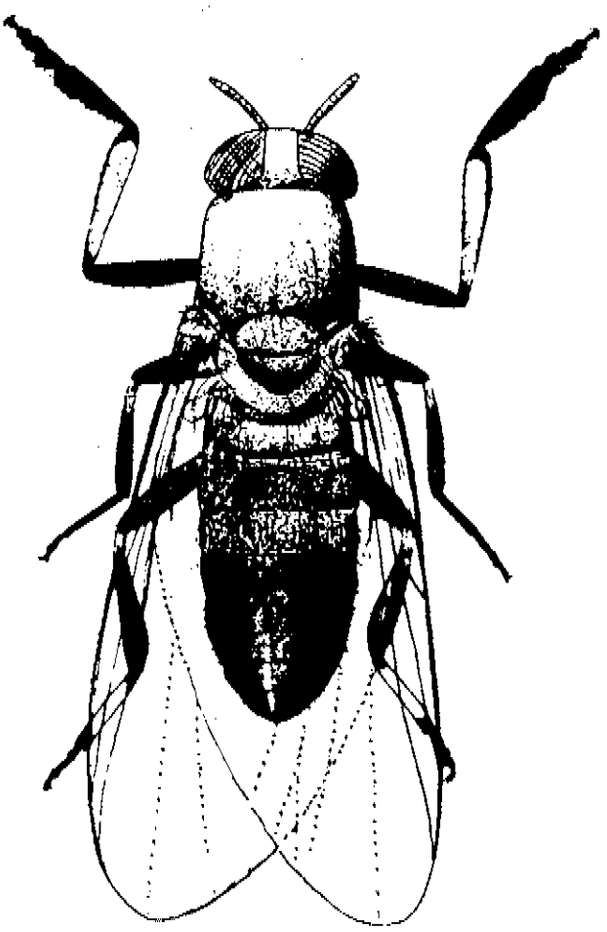
SIMULIUM

Training and Information Guide



WORLD HEALTH ORGANIZATION

Division of Control of Tropical Diseases

1991

FOREWORD

Since 1970 the former Division of Vector Biology and Control, now incorporated into the Division of Control of Tropical Diseases, of the World Health Organization, has prepared, with collaborators outside the Organization, a number of papers on vector biology and control. The Technical Report Series, No. 561: "Ecology and Control of Vectors in Public Health", WHO, 1975, recommended that such documents - general reviews of the ecology and control of individual vector groups - be continued and reviewed from time to time to provide workers with current, practical information on this particular subject.

With the greater demand for this material for use as training and information guides by different categories of personnel, particularly in the developing countries, it was decided to develop separate series of these documents: an advanced series for Masters of Science students in medical entomology and professional staff and a middle-level series for less specialized workers in the community.

The advanced series cover the relevant subjects in more detail and at a higher technical level, than the intermediate series. It is believed that this type of information will assist vector control specialists in acquiring the knowledge required for their daily work.

In order to improve the value and usefulness of this guide, evaluation forms are attached and users are requested to send the completed forms to the Training Unit, Division of Control of Tropical Diseases Division, World Health Organization, Geneva, so that their comments may be taken into consideration when the guide is revised.

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WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTE

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ORIGINAL: ENGLISH

SIMULIUM - VECTORS OF ONCHOCERCIASIS ADVANCED LEVEL TRAINING AND INFORMATION GUIDE

CORRIGENDUM

The following errors need to be corrected as indicated:

- page 28: Figure 4.3, caption, third line, "(Sanehun)" should be replaced by "(Senehun)".

- page 58: Figure 7.2, caption should be replaced by "A sample form for recording the results of Simulium catches and dissections at one site for one day. Data are recorded in numbered boxes or columns to facilitate transfer to a computer data base if required. All necessary information about each fly occupies half a line and the totals required to calculate monthly transmission criteria appear at the bottom left."

- page 64: Figure 8.1, end of caption, "Suzaki" should be replaced by "Suzuki".

- page 82: last formula, section X.7, "1 x 0.05 x 10 x 6) - 25 = 0.12 litres" should be replaced by
(1 x 0.05 x 10 x 6) ÷ 25 = 0.12 litres."

- page 88: Figure 10.2, end of caption, "Kurtakl" should be replaced by "Kurtak".

- page 93: TAXONOMY, "Classification of simuliid into species and genera by entomological names." should be replaced by "The science of classification as applied to living organisms."

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SIMULIUM - VECTORS OF ONCHOCERCIASIS

by

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ACKNOWLEDGEMENTS

We would like to thank our colleagues who contributed suggestions and text to this work. In particular, Dr J. F. Walsh, for an unpublished draft text on Vector Control and Insecticides; Dr D. Baldry, Dr R. Le Berre, Dr A. B. Knudsen and Mr J. D. Marr, for the section on Recent Developments and Conclusions in OCP from the period 1975-1991; Dr A. J. Shelley, for a draft key to the Simulium of Ecuador; Dr T. Suzuki, for permission to reproduce Fig. 8.1; Dr J. N. Raybould, for meticulous comments on the manuscript; and lastly, Mr J. Hayworth and Mrs N. Valabrègue, for their editorial work.

* * * *

C O N T E N T S

I. INTRODUCTION

This Training Manual is concerned with a single genus of vectors and one specific disease, onchocerciasis. The Simulium vectors have a very specialized biology, which is intimately associated with their efficiency as transmitters of onchocerciasis, and is exploited by man in controlling them.

Over the last 15 years, knowledge of the Simulium species which are the vectors of onchocerciasis has increased enormously, largely due to the impetus provided by the WHO Onchocerciasis Control Programme, which is the largest single on-going human disease vector control operation in the world. Originally, it was thought that about eight distinct species were involved in onchocerciasis transmission worldwide. It is now clear that most of these 'species' are a complexes of species, the members of which differ in their habits and ability to support the parasite. There is also evidence that there may be several geographical strains of the parasite, Onchocerca volvulus, and that these also interact differently with the various species within each vector complex.

In order to understand this complexity, the life cycle of the parasite, the disease, and the vector-parasite relationships have been covered in some detail. There is also a detailed section on morphology and taxonomy, because it is essential to understand the variations that may be found, and what techniques may be used in identification, even if some of the techniques are beyond the scope of this manual.

There is another unusual aspect to onchocerciasis in that, although it is theoretically possible for a person to get the disease from a single infected bite, as with malaria or trypanosomiasis, it usually takes many infected bites before sufficient worms become established for the symptoms of the disease to appear. Yet more bites again are required before the infection becomes heavy enough for blindness to set in. Once infected however, the adult female worms may continue to produce the microfilariae* that cause the damage for another 8 to 15 years. Thus there is a situation where a comparatively long period of contact with the vector is required for the initial infection, and an even longer period for the infection to die out naturally since the disease in itself is not fatal. During this time the infected persons are fully infective to any vectors that may bite them. It is this extended time scale that presents the greatest problems in planning and executing onchocerciasis control, whether by attacking the vector or by chemotherapy.

II. ONCHOCERCIASIS*

Onchocerciasis, or "river blindness", is a major endemic, parasitic disease caused by the filarial parasite Onchocerca volvulus (Leuckart, 1892), which in addition to causing human suffering is an obstacle to socioeconomic development. It is found in the Americas, in the south-western part of the

Arabian peninsula and in East, Central and West Africa. It is estimated that altogether about 18 million people are infected by onchocerciasis, and about 86 million live in endemic areas, but statistics are often unreliable and some foci have probably not yet been discovered. It therefore seems likely that the true figures will be higher.

II.1 Distribution of onchocerciasis

Latin America (Fig 2.1.A). The foci are small or medium-sized and generally well defined. In Mexico there is one focus in Oaxaca State and two in Chiapas State in a medium altitude area of coffee plantations. Seven of the 22 administrative districts of Guatemala contain endemic foci. Colombia has a small focus in the river Micay basin near the Pacific coast, not far from a small focus in Ecuador. In Venezuela two foci at medium altitude have been identified in the coastal region. There is another focus in the far south of the country around the sources of the Orinoco, which extends into Brazil and affects the Yanomami Indians of the Parima highlands.

Arabian peninsula (Fig. 2.1.B). Onchocerciasis is endemic in the Taiz region of southern Yemen, but this focus may well be larger and extend into Saudi Arabia.

Africa (Fig. 2.1.B). The endemic area lies betwen 15°N and 14°S. North of the equator there is a series of generally large and often contiguous foci from eastern Senegal to Ethiopia. South of the equator the foci appear to be smaller, separate and scattered, but there is still little information on many of them and they may be more extensive than indicated.

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* Terms marked with an asterisk are defined in Section XI - Glossary of terms.

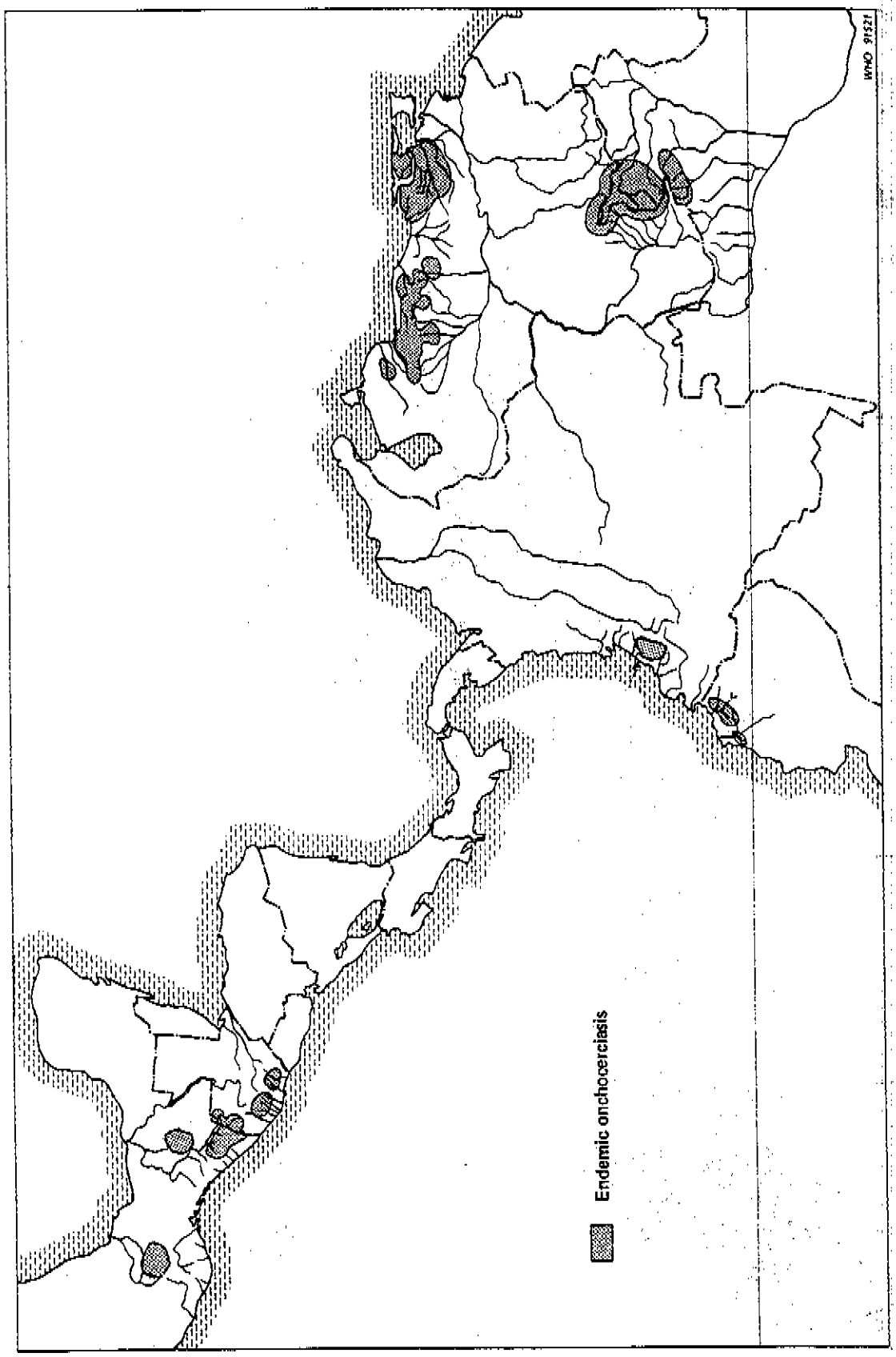

Figure 2.1A Geographical distribution of onchocerciasis in Latin America

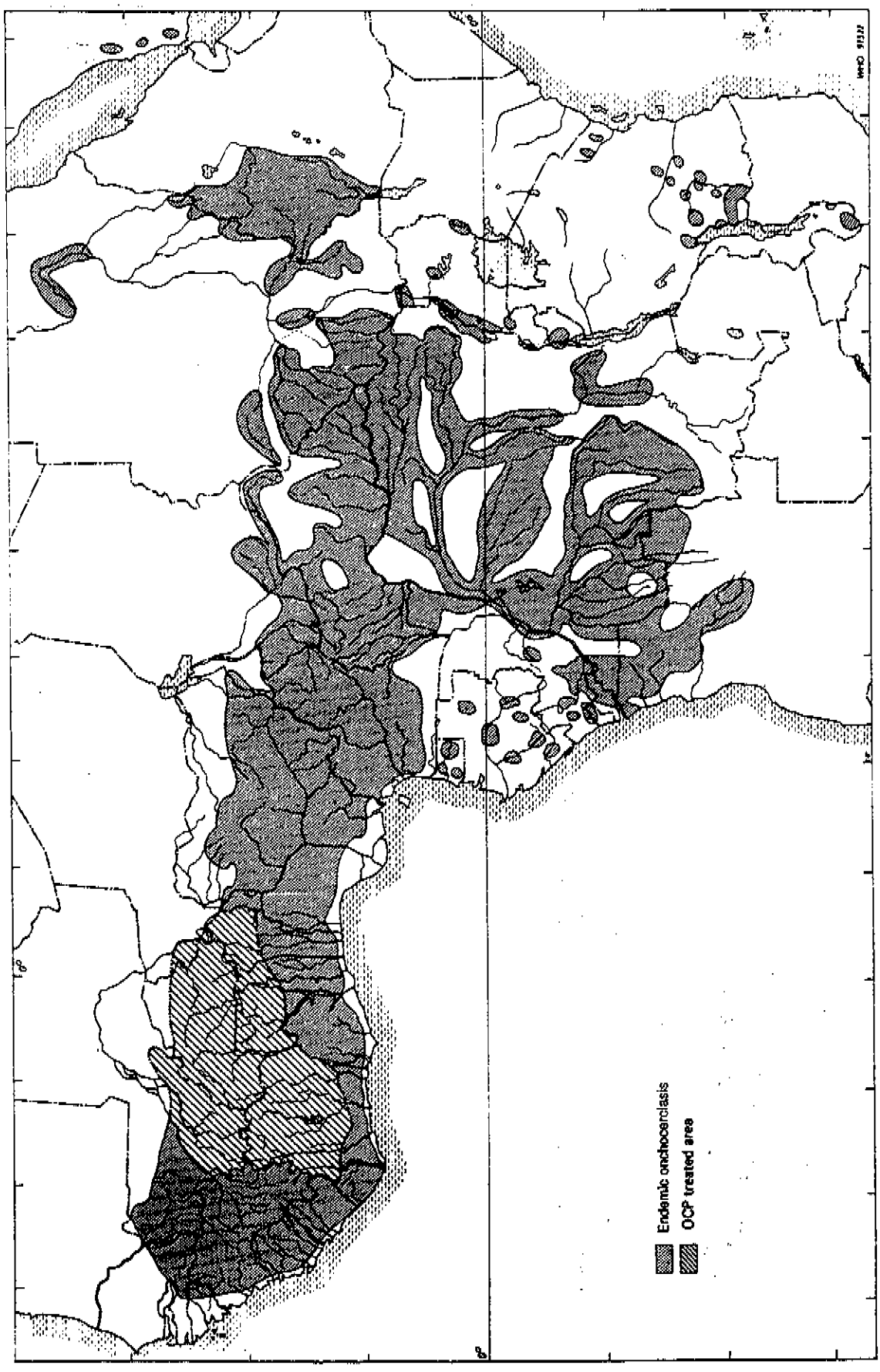

Figure 2.1B Geographical distribution of onchocerciasis in Africa and the Arabian Peninsula

II.2 Socioeconomic impact

Onchocerciasis is rife only in rural areas and up to about 20 km each side of the rivers or streams where the vector flies are found. The disease is most serious in small remote and isolated communities where it helps to destroy the already precarious balance of their subsistence economy by affecting the sight and reducing the efficiency of the most productive members of the community. This deterioration in the living conditions of the communities adjacent to rivers is one of the factors responsible for the desertion of the riverside lands by the villagers, who retreat to the areas between rivers. In the savanna it is not uncommon for valleys to be uninhabited for several kilometres either side of the major watercourses. Onchocerciasis is not always the initial cause of valley depopulation, as other diseases, such as trypanosomiasis, and historical factors, play a role. However, it represents a major obstacle to settlement and agricultural development of the area.

In the present economic and demographic context in Africa, it would obviously be important to reclaim this readily irrigable land. However, in the absence of blackfly control any move towards resettlement, whether spontaneous or organized, would entail considerable health risks for the resettled populations and result in ultimate failure. Onchocerciasis control is therefore essential both for bringing a very serious health situation to an end and for guaranteeing the success of reclaiming deserted valleys by making them healthy to live in.

In the Americas, because the sufferers from onchocerciasis are relatively few, the overall socioeconomic impact is less. Nevertheless, it is an important problem in the coffee-growing areas of Mexico and Guatemala. There are fears that these infected areas are expanding, while the opening of the forested areas of the Amazon basin, and coastal forests of Ecuador, Colombia and Mexico may cause the disease to spread to regions where suitable vectors exist, but the disease so far has not been introduced.

II.3 The disease

Onchocerciasis is caused by the filarial worm *Onchocerca volvulus* which inhabits the subcutaneous and deeper tissues of the human host. The worm is viviparous* and for the greater part of its life (around 12 years), emits embryos or microfilariae into the dermal and other tissues, where they provoke itching and skin lesions; these microfilariae may invade the eye, causing severe eye disorders culminating in blindness. It is the microfilariae which are the pathogenic stage of the parasite.

When some of these microfilariae are ingested by flies of the genus *Simulium* they develop into infective larvae, which find their way to the mouthparts of the insect, and may be inoculated into man during a subsequent bite. This passage through the vector is an essential stage in the spread of onchocerciasis.

The distribution of the disease is therefore dependant on the presence of suitable vectors which have an aquatic stage requiring flowing water; thus the disease is usually associated with watercourses, hence its name "river blindness".

II.4 Symptoms and therapy

Visible nodules surrounding one or more adult worms are located in human beings mainly around the hips, rib-cage and knees but also quite often on the head, particularly in the American form of the disease. They are generally small, 1-2 cm in diameter, but may sometimes exceed 5 cm, and when palpated they roll beneath the finger within the dermis. Other worms may also be found deep in the tissues where they cannot be palpated.

The clinical manifestations of onchocerciasis are primarily due to the microfilariae. The frequency and severity of the symptoms are closely correlated with the number of microfilariae, which depends on the number of adult filariae. This, in turn, is governed by the number of infective larvae received by the subject, that is the number of bites received from infected blackflies (as Simulium are popularly called) in relation to the length of time spent in an endemic area. Clinical onchocerciasis manifests itself only after an accumulation of infections over several years.

The most common skin symptom is a rash accompanied by violent and persistent itching which often disturbs sleep. The skin of the parts of the body where microfilariae are abundant, especially the legs, becomes atrophied* and in some places depigmented. Eye lesions are the most serious consequences of onchocerciasis. They result from the invasion of different parts of the eye by the microfilariae.

Since onchocerciasis is an accumulative disease the ocular complications appear only after a number of years, the higher the rate of transmission, the earlier they occur. In certain hyperendemic* areas blindness appears between the ages of 30 and 39 years but may occur earlier in some subjects.

While the discovery of subcutaneous nodules indicates the presence of the disease, it is not in itself sufficient evidence for a diagnosis. The diagnosis is based primarily on the presence of microfilariae in standardized skin snips taken from the iliac crest, calf, shoulder or outer canthus of the eye.

The means of treatment are at present inadequate. Two compounds have proved active to date but have severe limiations. These are diethylcarbamazine, (DEC)* which kills the microfilariae with no effect on the adult filariae, and suramin, which kills the adults worms. However, neither of these two drugs is appropriate for large-scale curative or preventative use since they have very serious side-effects, and may even lead to death in the case of suramin. More recently, ivermectin* has become available as a benign alternative to DEC and since it has few side-effects could become widely accepted. It is an excellent microfilariacide, but it does not kill adult worms (see Section X).

II.5 The parasite cycle

The filaria Onchocerca volvulus has no common animal reservoir (it has been found in wild gorillas). It develops only in man, although it has been transmitted experimentally to the chimpanzee. The parasite has up to now been regarded as a single species, but it is posible that work now in progress may confirm the existence of several forms with different epidemiological effects among the organisms at present covered by the name O. volvulus.

The filaria passes through several stages during a development cycle which takes place partly in man and partly in the blackfly (Fig 2.2).

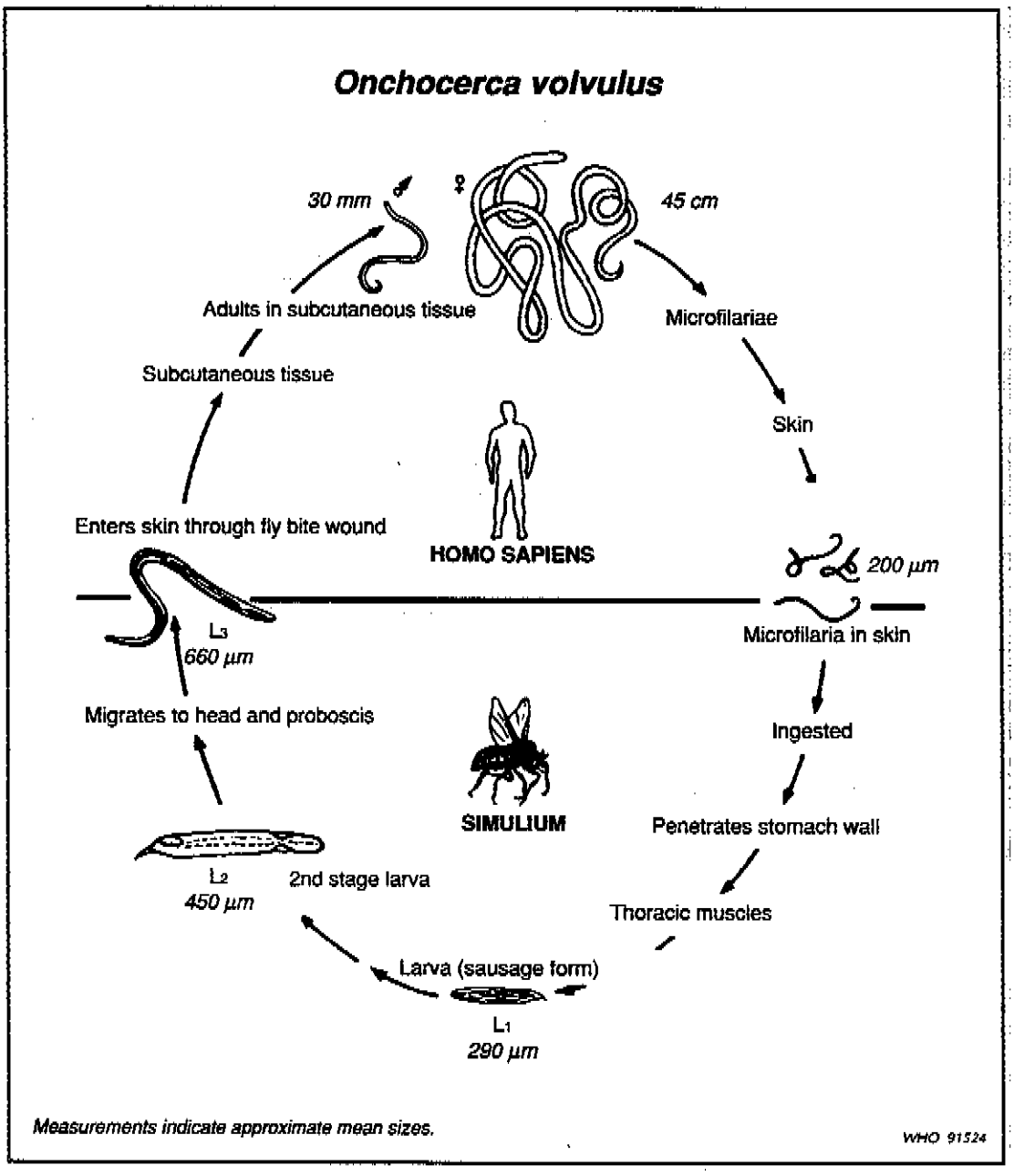

Figure 2.2 Life cycle of Onchocerca volvulus

When a blackfly infected with O. volvulus bites man to take a blood meal, it deposits the infective parasite larvae (known as L_3) in the wound. These larvae penetrate the superficial layers of the skin but it is not yet known precisely how they move and develop within the subcutaneous layer. Between one and three years after infection, onchocercal nodules appear within the skin and subcutaneous tissues. These are cysts consisting of a fibrous capsule, developed by the human body, which contain a variable number of worms of both sexes. When they become mature, adult male worms are 2-5 cm in length and 0.02 mm in diameter, while the females are 50-70 cm in length and 0.04-0.06 mm in diameter. The fertilized female gives birth to live microfilariae, measuring on average 330 microns. She emits 500 000 to 1 million of these each year throughout her sexually active life, which lasts from 8 to 12 years. In a severely infected individual there may be 50 to 200 million microfilariae distributed throughout the dermis*, the eyes and elsewhere in the body and they are sometimes excreted in the urine. The microfilariae can live for 1.5 to 3 years.

Only the female Simulium bites man, or animals, as it needs blood for the maturation of each batch of eggs laid. When the female bites a highly infected individual she may ingest as many as several hundred microfilariae present in the dermis. Once the blood arrives in her stomach most of the microfilariae are trapped by the peritrophic membrane*, are digested and disappear, but a few may pass through the intestinal wall and reach the abdominal cavity (Fig. 2.3). They then migrate to the thoracic muscles where they develop. After two morphologically different intermediate stages (L_1 and L_2), the microfilariae become infective larvae (L_3) measuring 600-680 microns. These find their way to the mouthparts, and may thus be transmitted to man during a subsequent blood meal (Fig. 2.3). The number of infective larvae in one blackfly is generally less than ten and in most cases from one to three. The maturation cycle of the larvae in the blackfly takes about seven days at 27-30°C, but can last up to 10 or 12 days at lower temperatures. Development stops when night temperatures are sustained at less than 16°C.

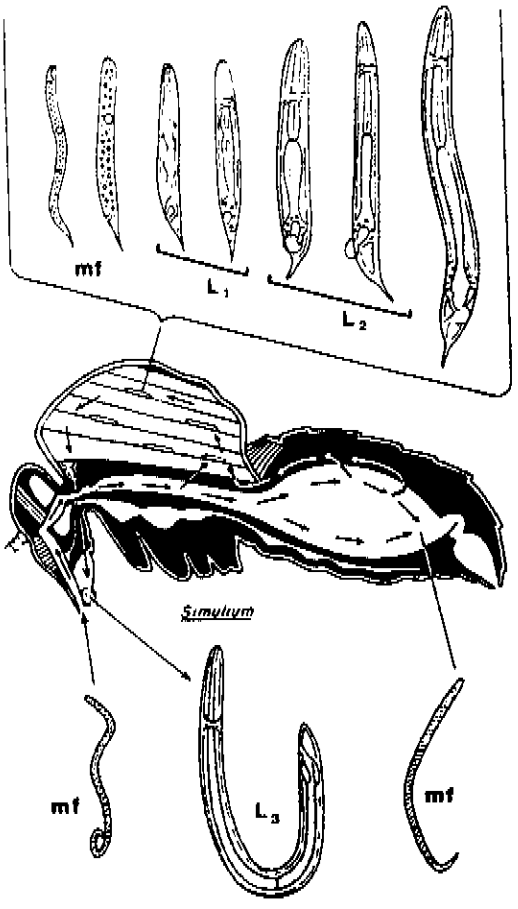

Figure 2.3 The development of Onchocerca volvulus in the vector. Arrows indicate the migration route of the larvae.

(After Ramírez-Pérez (1977), courtesy of the Pan American Health Organization)

III. VECTOR LIFE-CYCLE AND MORPHOLOGY

The interhuman transmission of Onchocerca volvulus requires only a single vector in which the whole metamorphosis* of the parasite from microfilaria to third-stage infective larva occurs. There are several species of vector, but they are all small insects belonging to the Simuliidae, a family of Diptera (see classification given in No. VII of this series - "The Housefly" - WHO/VBC/86.937 p.5) in which the adult females largely feed on blood from warm-blooded vertebrates. This bloodsucking habit is the means by which the microfilariae of O. volvulus, found in the skin and not actually carried in the blood stream, obtain entry to their vectors.

Members of the family Simuliidae are popularly called blackflies, particularly in North America, but as this name is often given to certain unrelated agricultural insect pests, the informal name simuliids or the generic name Simulium will generally be used in this guide.

The juvenile stages in the simuliid life-history are aquatic, and running water is essential for them. Nevertheless, the Simuliidae have an almost worldwide distribution and the family is found not only in flowing streams and rivers but in minor water sources in deserts, high polar latitudes, coral islands. About 1520 species of simuliids are currently recognised, but only about 20 of these are involved as vectors of human onchocerciasis.

The family Simuliidae is very homogeneous*, with great structural uniformity amongst both adult flies and juvenile stages, which means that groups of species may be characterized by relatively small morphological differences. So while some authorities may place the vectors in more than one genus, in this guide all vector species are considered to belong to a single genus Simulium sensu lato (s.l.).

III.1 The simuliid life-cycle

The life-cycle in all simuliids passes through the egg-larva-pupa-adult (imago) sequence of metamorphic stages. Figure 3.1 shows the cycle for the Simulium damnosum vector complex. All stages except the adult are aquatic, and eggs are laid into running water. To allow for growth the larval stage sheds its skin (cuticle), including the hardened head-capsule, several times. Between each such moult the larva is in a particular instar of its development.

Larval instars. Simuliidae are almost unique amongst Diptera for the high and variable number of larval instars, ranging from 6-9 in different species and sometimes varying within the same species. Determining the number of instars is difficult except when each is clearly marked by a distinct size-range. Seven larval instars occur in Simulium damnosum s.l., but reliable data for other vector simuliids do not exist.

Pharate* stages. The basic outline of development is complicated by pharate (hidden) stages in which the fly has physiologically reached the pupal stage (pharate pupa), but for a time keeps the larval morphology, or has moulted to the adult fly (pharate adult) within the pupal cuticle, but remains for a time imprisoned under water in the pupal morphology. The pharate pupa (or so-called 'pre-pupa'), because of its larval shape, is usually regarded as the fully grown or 'mature' larva; it looks exactly like the larva and

continues to feed as such. At this stage the simuliid has the gills of the future morphological pupa very much darkened and coiled up on each side of the thorax within the moulting fluid between the old larval cuticle and the concealed inner pupal cuticle (see stage VII, Fig. 3.1). It is usually called the 'gill-spot larva' and this is a convenient term for use in vector studies even if not strictly correct. After a short pupal period, the adult fly emerges under water, floats to the surface, and takes flight to mate, feed and continue the life-cycle. Pupal life is always short, ranging from 2-17 (usually 3-6) days. Durations of egg and larval stages vary greatly between species living under different environmental conditions; in the warm tropics the egg stage may last one or two days and the larval stage about one to two weeks.

The reported development times in various vectors are as follows. Simulium damnosum complex (West Africa): egg 1-3 days, larva 4-18 (usually 7-12) days, pupa 2-5 days. S. neavei group (East Africa): egg unknown in nature, larva between 26 and 72 days, pupa 8-10 days. S. ochraceum complex (Guatemala): egg 3-10 days, larva 7-15 days, pupa 4-6 days. S. metallicum complex (Guatemala): egg 3-20 days, larva 6-20 days, pupa 4-10 days. In the S. damnosum complex differences in development times of the four life stages appear to be related to species identity and to temperature. They often complete the life-cycle from egg to adult fly in about two weeks, and under some circumstances in as little as one week.

There are usually many generations per year which overlap and cannot be readily distinguished except in special circumstances (e.g. the first two or three generations of S. damnosum s. l. seasonally re-establishing themselves when the rivers flow again in the wet season following a drought period).

III.2 Recognition of the genus Simulium s.l. and the external anatomy

Nearly all simuliids that suck the blood of man belong to the genus Simulium s.l. and this is the only genus with man-biting members that is present in the endemic human onchocerciasis areas. Therefore a blood sucking fly caught on man in an onchocerciasis area that conforms to the following short description belongs to Simulium s.l.

The body shape is shown in Figs.3.1 and 3.2. The antennae are short and approximately cigar-shaped, with 11 segments and without whorls of long hair (cf. mosquitos and ceratopogonids). The mouthparts form a short, inconspicuous, downwardly-directed proboscis (cf. long fine forwardly-directed proboscis of mosquitos and tsetse flies). The wings are broad, colourless and without scales, with the wing shape and venation as in Fig. 3.2. The thorax on each side in front of the wing base has a characteristic large membranous* area (pleural membrane, Fig 3.2). The legs are rather short and stout without scales; the hind leg has inner apical* projection (calcipala) on the basitarsus* and a groove across the upper surface of the second tarsal segment (pedsiculcus).

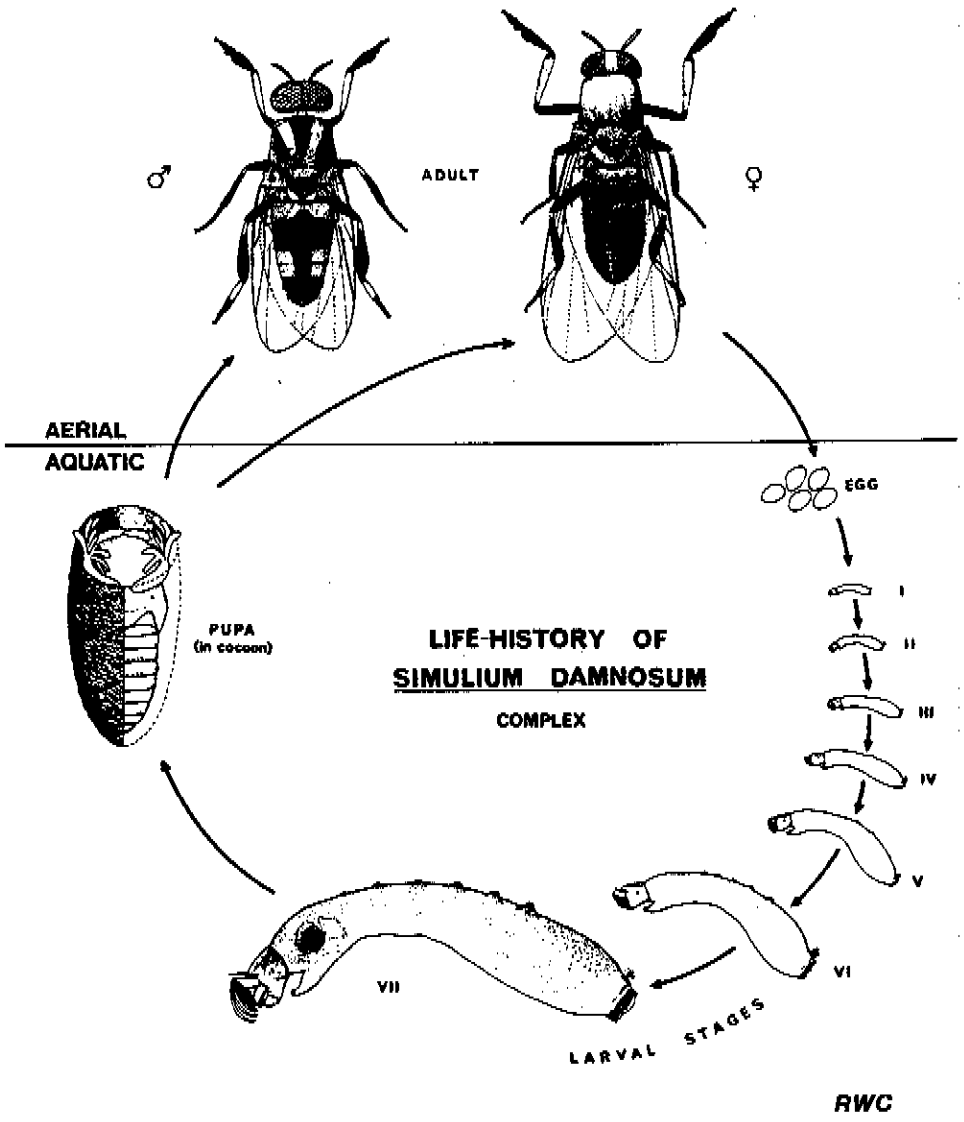

Figure 3.1 Stages in the life-history of S. damnosum
(Original R. W. Crosskey, with acknowledgements to the Wellcome Museum)

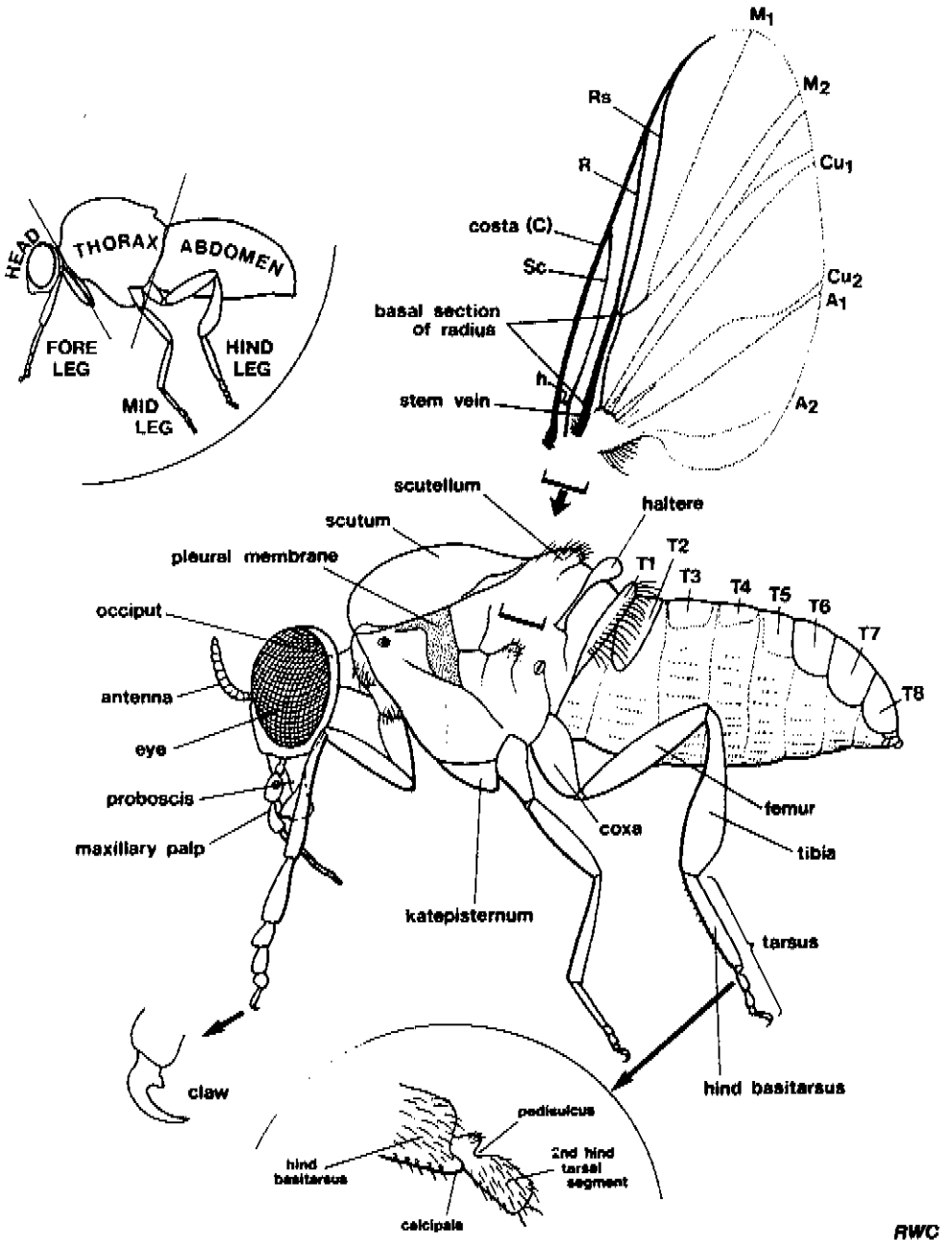

Figure 3.2 External morphology of the female Simulium
T1-T8 are the tergites of each abdomenal segment.
Lettered signs on the wing refer to the standard notation of the veins.
The tuft on the stem vein is on the dorsal surface.
(Original by R. W. Crosskey)

Egg. The shape is eccentric-ovoid (Fig.3.3.G), with a smooth eggshell, without morphological features at normal magnifications. It is white when fresh, darkening to brown with advancing embryogenesis*. The length is about 0.1-0.4 mm.

Larva. The larva is easily recognized by its characteristic shape (Fig. 3.3.A & B). The body is elongate, slightly or strongly swollen posteriorly and with two attachment organs, an anteroventral* proleg crowned with hooks on the thorax and a posterior circlet formed by a crown of hooks around the end of the body. The body cuticle is either bare or with small hairs or scales. The dorsal* outline of the body is usually smooth, with occasionally paired dorsal protuberances ('tubercles') present on the first five or six abdominal segments (e.g. in S. damnosum complex). The head is formed by a strong head-capsule, with a pair of very large cephalic fans which form hemispherical baskets when open. The upper surface of the head has a pair of slender three-segmented antennae. On each side of the head there is a pair of small black pigmented marks ('eye-spots'). Older larvae and pharate pupae ('gill-spot' larvae) show histoblasts* of developing legs and wings on each side of the thorax, and darkening histoblasts of the pupal gill (the 'gill-spot'). The length of the gill-spot larva varies from 4.5-11.0 mm according to species.

Pupa. The pupa is recognized by its characteristic body shape, the possession of a pair of gills anterodorsally* on the thorax, and the enclosure of the body within a cocoon* (Fig. 3.3 C to F). Gills occur in many very varied forms. The abdomen has nine segments and bears an arrangement of hooks for locking the pupa into the cocoon.

Adult fly. The eyes of the female are separated by a frons* above the antennae (head dichoptic*, Fig. 3.1 and 3.4); the eyes of the male meet above the antennae and frons are absent (head holoptic*; very rarely the male head is dichoptic), the male eyes have upper facets* enlarged, but the lower facets are small like those of the female. The antennae of both sexes are short and stout, with 11 almost equal segments. The proboscis is directed downwards, and is short, with anterior an apically-toothed labrum, large labella and paired mandibles and maxillae. The thorax has a large membraneous area on each side (pleural membrane) below the scutum and before the wing base. The wings are broadest near the base and have very weak posterior veins with a very uniform venation (Fig. 3.2). The legs have paired apical claws; the claws of the female with or without a single tooth (if a tooth is present, it is on the claws of all legs); the hind leg has calcipala and pedisulcus (Fig. 3.2). Males of some species have very prominent silver markings on the scutum which in S. damnosum has been used to separate geographical races of some cytospecies*. The front five tarsal segments may be enlarged or swollen in some vector species, e.g. damnosum and guianense. The abdomen is short, with nine distinct segments, sides and venter* almost all membranous the first tergite* forms a prominent basal scale bearing a hair fringe; the other tergites are small except those of segments 6-8 (Fig. 3.2). Males have genitalia in the form of a compact hypopygium.

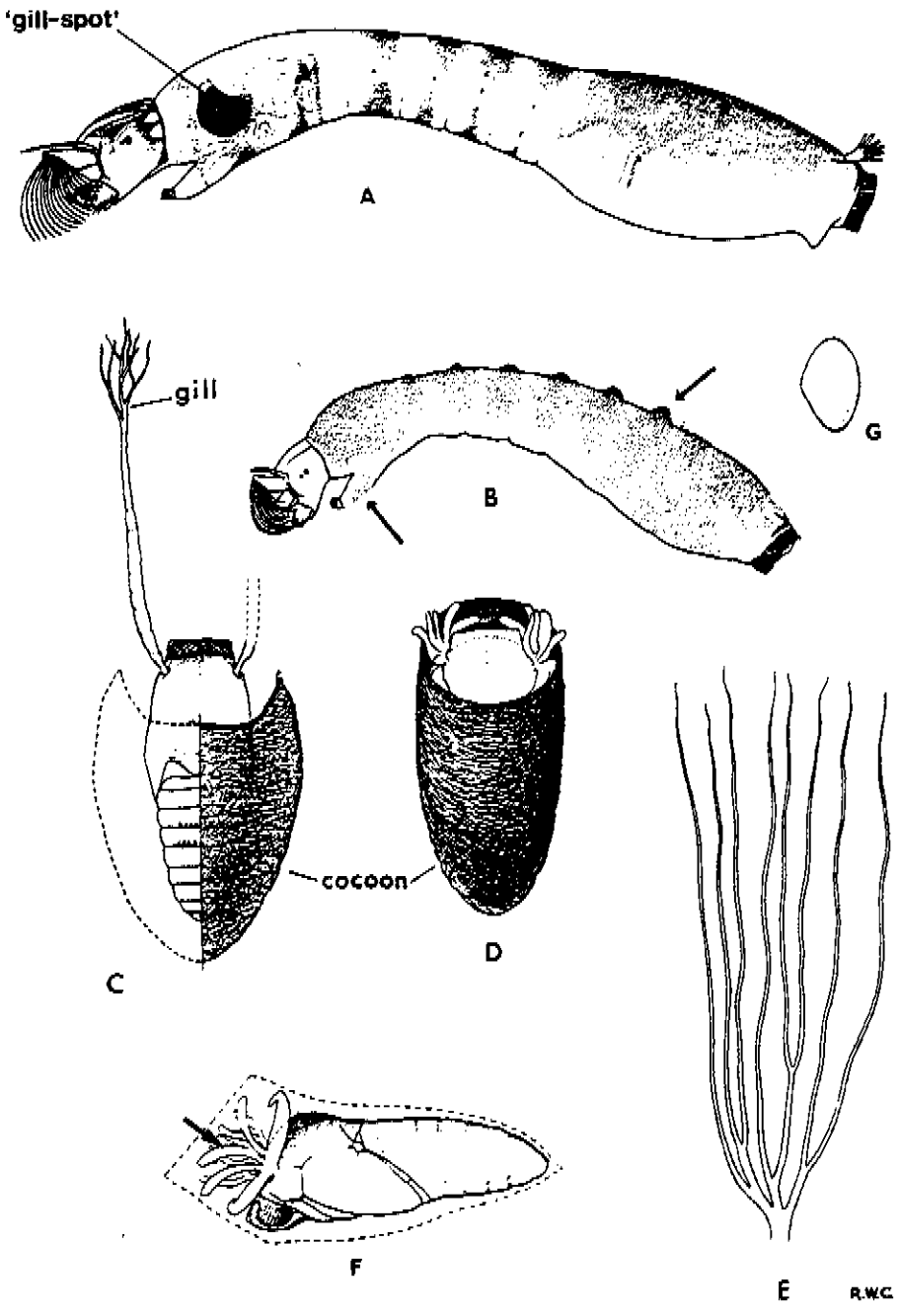

Figure 3.3 The aquatic stages of Simuliidae.

A. Seventh stage larva showing well-developed "gill spot".
B. Larva of S. damnosum s.l. showing well-developed tubercles and covering of short spines, particularly on the proleg.
C. Cut-away of pupa in its cocoon.
D. & F. Pupa and cocoon of S. damnosum s.l.
E. Pupal gill of S. neavei group.
G. Typical egg, showing eccentric oval shape.

(Figs. A-E & G reproduced with permission of the Trustees of the British Museum (Natural History); Fig. F original by R. W. Crosskey)

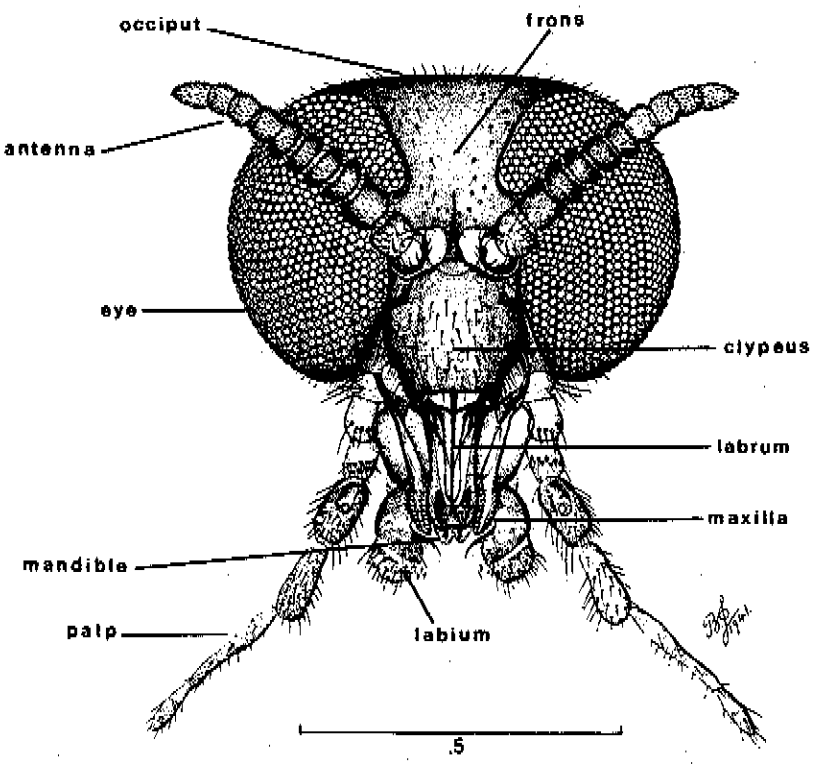

Figure 3.4 Anterior view of the head of a typical Simulium female (After Jobling (1987). Reproduced with permission of the Wellcome Trust.)

III.3 Internal anatomy of adult female fly and larva

Features of the internal anatomy of adult female vector flies, and to a lesser extent of larvae, that are commonly examined in the course of vector studies are briefly described below. The structures concerned are shown semi-diagrammatically in Figures 3.5 and 3.6. A meticulous fully- illustrated study of the anatomy of the adult female head and mouthparts has been produced by Wenk (1962). Ramírez-Pérez (1977) has provided a comprehensive illustrated review of anatomy applied to the vector species S. metallicum.

Adult female fly (Fig. 3.5)

Alimentary system. This consists essentially of a straight tube from the food channel in the head running to the anus, differentiated into the following sections:- (i) cibarium, a strengthened tube orientated vertically in the head, its floor occasionally provided with teeth (cibarial armature) at the dorsal end where it angles to the pharynx; (ii) pharynx, a short strengthened tube running backwards from the cibarial junction, always without an armature; (iii) oesophagus, a short tube in the neck and anterior thorax; (iv) mid-gut, a long tube divided into a tubular forward part in the thorax and an expanding rear part (stomach) in the mid-abdomen; (v) hind-gut, a short tube divided into a narrow anterior colon and an expanded rectum opening to the terminal anus. A crop duct leads from the floor of the posterior oesophagus to a large diverticulum*, the crop, lying anteroventrally in the abdomen (thin-walled and capable of great expansion). The junction of the oesophagus and the mid-gut is marked by an invagination*, the oesophageal valve. Four long narrow doubled-back excretory Malphigian tubules enter the gut in pairs immediately behind the junction of the stomach and colon.

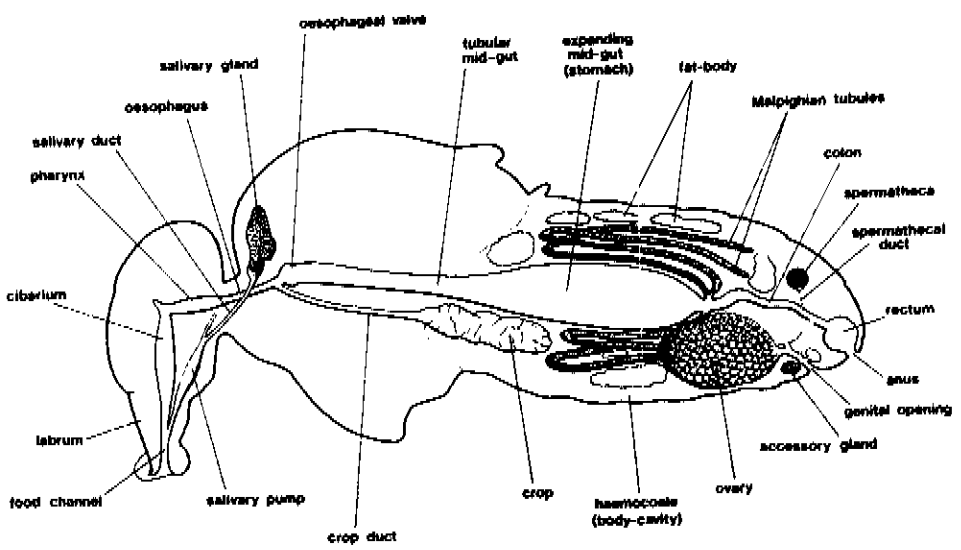

Figure 3.5 Internal anatomy of a female Simulium
(Original R. W. Crosskey)

Salivary system. This comprises paired U-shaped salivary glands lying at the front of the thorax with a duct from each gland passing through the neck, the salivary ducts uniting beneath the oesophagus as a salivary pump which opens by a duct into the hypopharynx.

Reproductive system. This comprises (i) large paired ovaries containing very many ovarioles* lying posteriorly in the abdomen when undeveloped but occupying most of the greatly expanded abdomen when the fly is gravid*; (ii) a short membranous oviduct from each ovary uniting into a short vagina leading to the genital opening; (iii) paired accessory glands opening by very short ducts into the genital chamber; and (iv) one almost spherical or oval spermatheca opening to the genital chamber by a long narrow spermathecal duct. The wall of the vagina is supported by the basal stem of a strengthened Y-shaped rod (genital fork) that is conspicuous in slide preparations of terminalia*.

Body-cavity. The abdominal organs lie in a blood-filled cavity (haemocoele) which in the young fly contains much loose white tissue, the fat body, around and between the viscera*.

Larva (Fig. 3.6.)

Alimentary system. The mouthparts form a funnel shaped space leading food to the mouth and short cibarium and then to the parts of the gut, namely: (i) the pharynx and slightly widened oesophagus that are only weakly distinguished from one another, the latter passing from the head into the thoracic region and ending at an oesophagal valve at the junction with the (ii) mid-gut, a very large straight tube extending from the thoracic region towards the end of the abdomen; and (iii) the hind-gut, beginning in the swollen posterior part of the abdomen and divided into a looped colon and a short tubular rectum which opens at the anus situated on the dorsal body surface. Four sac-like diverticula (gastric caeca) open from the anterior mid-gut just behind the oesophageal valve, and (as in the adult fly) two pairs of Malphigian tubules open into the anterior colon. An eversible* trilobed* rectal organ, in which the three lobes are often subdivided into many finger-like secondary lobules, arises from the floor of the rectum; in preserved larvae it is often visible emerging from the anus.

Silk-producing system. This consists of a pair of long tubular silk glands that lie doubled back in the lateroventral* regions of the thorax and abdomen. The ends of the glands are tapering* and filamentous*. Ducts from the glands meet in the head to form a common silk duct which opens on the hypopharynx. Enlarged cells of the silk gland walls with prominent nuclei contain polytene* chromosomes* which are the chromosomes studied in routine cytotaxonomy* of the Simulium damnosum vector complex and other simuliids. The glands containing these chromosomes should properly be known as silk glands, but are often mistakenly called salivary glands. The glands in the larvae have the sole function of silk production.

Gonads. The rudimentary gonads* (male testis subglobular*, female ovary sausage-like) lie in the abdomen of the well developed 'gill-spot' larvae. They enable larvae to be sexed for cytogenetic studies.

Fat body. Much of the larval abdomen is filled with loose fat body tissue (which carries over through the pupal stage to the young adult fly).

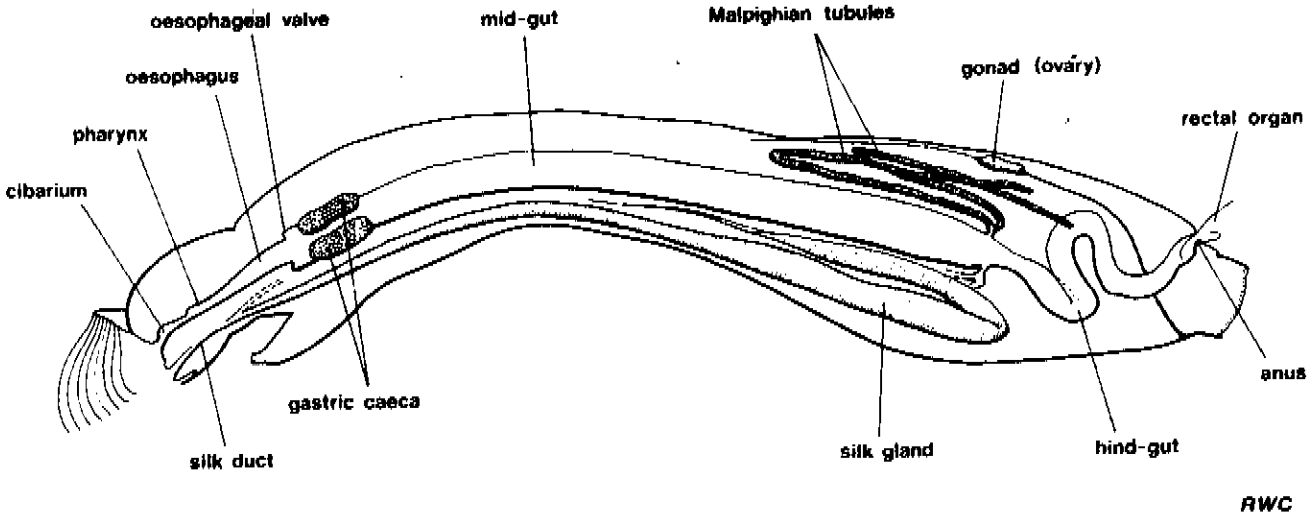

Figure 3.6 Internal anatomy of a Simulium larva
(Original R. W. Crosskey)

IV. VECTOR BIOLOGY

IV.1 Aquatic stages

(a) Egg biology

The simuliid egg must be laid in water and no vector species is known that has a truly drought-resistant egg. Most simuliids, including S. damnosum s. l., affix egg masses to firm substrates, and a falling water level which exposes the eggs to air-drying is an important factor in mortality.

The hatching of eggs is often not synchronous* from the same oviposition, particularly when communal egg-laying activity by many flies has resulted in superimposed layers of eggs. Eggs in the uppermost stratum may hatch normally, but embryonation may be delayed in the lower layers or remain uncompleted. To hatch, the larva ruptures the eggshell with a blunt prominence ('egg-burster') on top of the head, a feature by which a very small larva is easily recognized as being in the first instar.

(b) The larval habitat

The places where larvae occur are known as 'breeding sites' (in French 'gites'). Breeding sites are always in running water, and range from the largest rivers (such as the Nile and Zaire, e.g. S. damnosum s.l.); but a few species such as S. ochraceum s.l. may occur in small seepage channels on hillsides.

Habitats include special kinds of niches such as cascades, lake outlets and springs. In such special habitats one species often occurs alone, but in generalized river or stream habitats several species may live together. Simuliid juvenile populations can be very high, and the Simuliidae form a major component of running-water faunae in most parts of the world.

Vector species have decided breeding-site preferences, and the kind of aquatic habitat occupied by the larvae of a particular vector is of the utmost importance in relation to its insecticidal control. Breeding sites of the S. damnosum complex in West Africa exist mainly in broken water of rapids in larger rivers (over 10 m wide) and there is little or no breeding in minor side-streams. Thus prospection of 'white-water' in rapids enables potential breeding sites to be readily discovered. By the strongest possible contrast, S. ochraceum in Guatemala and Mexico breeds in multitudes of tiny streams and even in infant trickles that run across the forest floor without defined stream-beds, and is therefore an 'area' breeder not susceptible to aerial prospection of restricted breeding sites. Other vectors breed in intermediate conditions between these extremes, members of the S. neavei group mainly in heavily shaded and turbid* medium-sized streams, and S. metallicum s.l. in small to large streams (i.e. in larger and better defined watercourses than those occupied by S. ochraceum). In Brazil the vectors breed in large turbid rivers of the peri-Amazonian rain forest. With S. damnosum s.l. an unfortunate effect of the habitat preference is that it can be accidentally simulated by man-made objects such as causeways for dry season river-crossings and, by dam spillways.

Larvae of vectors show wide tolerances to some of the hydrochemical and physical characteristics of their preferred habitats. S. damnosum s.l. has been reported from current velocities between 0.4-2.4 metres per second and water temperatures of 17°C to 33°C. Within the S. damnosum complex, however, the water temperature, current speed, turbidity, pH and conductivity factors appear to play some part in restricting the geographical or seasonal distribution of the larval habitats of different cytospecies. Vector species and other simuliids are sensitive to chemical pollutants in the larval habitats, and absence of larvae from apparently suitable watercourses can be a useful pollution indicator.

Almost any fixed submerged surface may be used for larval attachment and typically include water plants, trailing roots and branches, fallen leaves, stones, inclined rock surfaces and lips of waterfalls (Fig. 4.1.A). Clean surfaces are usually preferred, and larvae will readily attach to artificial substrates such as plastic ribbons used for experimental purposes. Larvae tend to occupy substrates near the surface of the water, but in S. damnosum s.l., which typically occurs on superficial trailing vegetation in disturbed waters of rapids, use of artificial substrates has shown that larvae may occur to a depth of at least three metres. Larvae may be found in enormous aggregations on the preferred substrate, particularly at lake outfalls or dam spillways and on cascade substrates covered by thin water films.

Each larva secures itself in the current by latching the hooklets of the abdominal posterior circlet to a pad of silk spun from the silk glands and adhered to the substrate. The body points in the direction of the current with the head downstream. The larva can move from one secured position to another either by looping like a caterpillar or drifting on a long anchored silk thread fixed to the original substrate, which acts as a safety line until the larva can secure a hold on a new substrate further downstream. The S. neavei vector group, and other phoretic* simuliids differ from normal simuliids in that the larvae attach to movable substrates provided by the bodies of their arthropod carriers.

(c) Larval feeding and diet

Nearly all simuliid larvae, including those of all vectors, have cephalic fans (Fig. 3.3A) with which they strain suspended matter from the water. The head-downstream orientation ensures that the current loads the fans with suspended matter, and they thus rely on the water flow to bring them their food. Larvae may also graze on the surface, and such behaviour has been reported in the S. neavei vector group and in temperate simuliid species. The filtering mechanism of the cephalic fans, and other aspects of larval feeding are of utmost importance in relation to the formation and application of anti-Simulium larvicides for use in vector control. The subject is reviewed by Colbo & Wotton (1981).

(d) Phoretic* associations with other arthropods

The only vectors involved in phoretic relationships are members of the S. neavei group in which the larvae and pupae live on river crabs of the genus Potamonautes. Several species of crab can act as carriers for the larvae and

pupae. Eggs are not laid on the crabs and first instar larvae have not been found on them. Several parts of the crab are favoured as the larval or pupal substrate, including the limb-joints, eye stalks and sides of the carapace* (Fig. 4.1.C). Larvae of the S. neavei group require to survive a range of circumstances not met by other tropical African simuliids, because the crab carriers may lie for long periods in still water or in the damp substratum beneath dried-out streams, and the crabs sometimes travel overland between streams at night. Such crab behaviour poses special problems for vector control (Raybould and Mhiddin, 1978) as well as for simuliid survival. The unusually prolonged larval life in S. neavei group could be because crabs may remain semi-dormant for a long time in concealed places in still water when conditions are adverse. The duration of larval life has been reported as 30-41 days for S. neavei in Uganda, and 28-72 days for S. woodi/S. nyasalandicum at Amani in the United Republic of Tanzania.

(e) Pupation, the pupa and adult emergence

When ready for change to the morphological pupa, the gill-spot larva (i.e. pharate pupa) spins the cocoon with silk exuded by the silk glands, taking about 40-70 min to complete its construction. The transition from larva to pupa usually takes about 45 min. Cocoons are usually built with the open end downstream or angled across the current so that the gills of the pupa escape the full force of the water.

Pupae are usually found in the same sites as larvae, but in some species the gill-spot larva seeks more protected sites (for example the lower edges instead of the upper surfaces of stones) on which to pupate. Pupae lie motionless in their fixed cocoons, and darken progressively until the time for adult fly emergence (eclosion). At this time, air appears within the pupal skin that envelops the pharate adult and the pressure of this forces a longitudinal split along the pupal thoracic skin. Thus freed, the adult floats immediately to the water surface and breaks free to become the aerial fly. Empty cocoons left by emerged flies do not survive long but can be useful evidence for the occurrence of a particular simuliid species in a stream.

Light and perhaps temperature appear to influence the time of day when emergence occurs. Daytime emergence is usual and in S. damnosum s.l. emergence activity shows an early morning peak and sometimes a lesser one in the evening. Male and female flies usually emerge at about the same time, but instances of male emergence largely preceding female emergence have been recorded.

IV.2 Adult flies

(a) Swarming and mating

Mating occurs soon after emergence from the pupa at or near breeding sites and many simuliids form male mating swarms that orientate to a 'marker' object such as a tree, house-corner, vehicle or animal's head. The swarms are seldom dense and in vectors are very rarely seen. Data on swarming vector species exist only for S. damnosum s.l, which are reported to occur near trees at 2-4 metres above ground in West Africa, and beneath trees at less than one metre above the ground in northern Sudan.

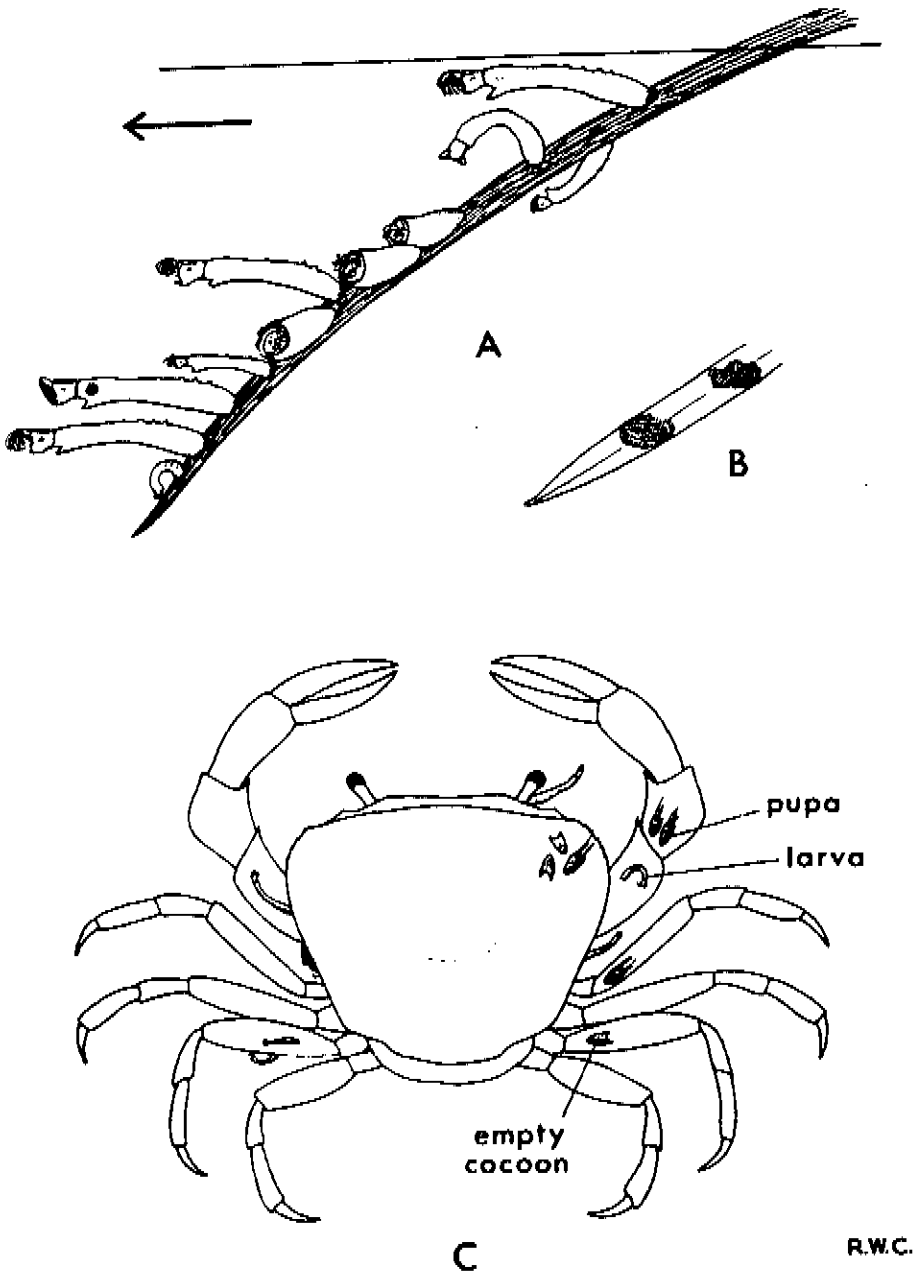

Figure 4.1 Attachment sites of eggs and larvae of Simulium

A. Larvae and pupae of S. damnosum on a grass leaf, showing typical orientation to the water current as indicated.
B. Egg masses of Simulium spp.
C. Larvae, pupae and empty cocoons of S. neavei s.l. attached to a crab at typical attachment sites.

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Male flies secrete a capsule (spermatophore*) around the sperms, which are thereby transferred as a single package to the female genital chamber during copulation* to be stored as free sperms in the female's spermatheca. It is believed that simuliid females mate only once. Spermatophores are sometimes seen attached to wild-caught females, providing evidence that the flies are young and nulliparous*.

(b) Host selection and feeding site preferences

Both sexes feed on plant juices, but females also suck the blood of warm-blooded vertebrate animals. Many bloodsucking species have a strong or exclusive preference for feeding either on mammals or birds, but some feed readily on both host groups. A wide range of hosts is attacked. Mammals include man, monkeys, domestic animals (particularly cattle and horses), and many small mammals such as rabbits; birds include domestic poultry, wild ducks, hawks, hornbills and many small birds.

No simuliid feeds exclusively on man. Although vector species must be anthropophilic* to be vectors at all, each is to a greater or lesser extent also zoophilic*. Zoophily is an important aspect of vector behaviour, firstly because bloodmeals taken from animals lower the likelihood of infection of the vector with O. volvulus (which occurs only in man), and secondly because filariae of animal origin can occur in anthropophilic vector flies and can be confused with O. volvulus.

A few vectors are strongly anthropotrophic*. The most definite trophic response to man amongst vectors is shown by S. ochraceum s.l.in the Meso-American* onchocerciasis zones, even so, 15 alternative hosts have been recorded, including all common domestic animals (Dalmat, 1955). Similar long lists of hosts exist for S. metallicum s.l. and S. callidum, the secondary vectors in Guatemala. These species are anthropophilic but not anthropotrophic, though S. metallicum s.l.in particular might seem so because of the enormous numbers in which it sometimes bites man; in fact both these vectors show an outstanding preference for feeding on horses and other equines rather than man.

Simulium damnosum s.l. host selection varies in different parts of its range. The complex is extensively anthropophilic in West and equatorial Africa and the Nile valley but is mainly zoophilic in East Africa and entirely so in southern Africa (Fig. 4.2). Anthropophily and zoophily, particularly in eastern Africa, are closely linked to cytospecies. Most of the geographically restricted cytospecies of eastern Africa are zoophilic. In West Africa S. damnosum s.l. is anthropophilic virtually throughout its range, and very nearly anthropotrophic in many areas, but zoophilic populations occur where human settlement is lacking. In settled areas the complex also feeds on animals, several hosts having been recorded that include bovines, dogs and domestic and wild birds. Precipitin testing* in this and other vectors is potentially useful for identifying animal hosts, but is constrained by difficulty in finding bloodfed flies when they are not actually feeding.

The complex factors involved in host location and selection of feeding site on the host have been reviewed by Wenk (1981). The only vector as yet critically studied is S. damnosum s.l. In forested areas of the Cameroon they are attracted to man primarily by scent and exhaled breath, whereas flies in

savanna areas probably locate a human host visually. Body odour is apparently the main attractant in the forest. Sweat from the lower body is the most positively attractive human secretion, probably accounting for the well established fact that S. damnosum s.l., irrespective of the cytospecies, shows a very strong preference for feeding on legs below the knee. Preference for feeding on the lower parts of the human body is shown equally by most other vectors. In all these vectors some 70-95% of bites are on the legs below knee level. A reverse preference is shown by S. ochraceum, the primary vector in Mexico and Guatemala, in which about 70% of bites are above waist level and mainly on the torso*, and by S. oyapockense s.l.in Brazilian foci where only about a tenth of bites are below the knee.

(c) Blood engorgement

Simuliids are described as 'pool-feeders'. The biting mouthparts produce a skin laceration from which seeping blood is sucked. The simuliid proboscis is short but it penetrates deeply, to about 0.4mm depth in S. damnosum s.l. Blood, with any contained microfilariae from the skin wound, is imbibed by muscular pumping of the cibarium and pharynx and passes rapidly from the food-channel in the proboscis to the mid-gut where its increasing volume greatly dilates the stomach and visibly expands the abdomen. If a cibarial armature is present the blood flows over it as it enters the pharynx. Anticoagulant* saliva from the salivary glands is discharged into the feeding pool from the hypopharynx during bloodsucking and infective O. volvulus larvae apparently escape from the fly's head into the skin wound at the same time.

On the favoured host, bloodsucking is very determined and the fly is not easily disturbed once feeding has started. The time required for full engorgement, depending on adequate blood flowing into the feeding wound, varies from 0.5-20 min, but engorgement time in vectors is typically about 3-6 min. S. damnosum s.l. takes longer to engorge when feeding on a subject with onchocercal skin thickening than when feeding on a normal skin. Most simuliids appear to take a little more than their own body weight in blood at a full feed. In savanna S. damnosum s.l. the bloodmeal volume has been recorded as averaging 1.02 mm^3.

Shortly after engorgement the stomach epithelium begins to secrete a hardened lining, the peritrophic membrane. A strong initial membrane may be formed in 30 min to 3 h, but successive layers of material may continue to be laid down over 24 h during the early stages of bloodmeal digestion. In S. damnosum s.l. the bloodmeal is fully digested in 72 h, but disintegration of the membrane begins after about 48 h.

(d) Ovarian development and gonotrophic cycles

Female simuliids normally produce several egg-batches a few days apart. All vector species require a blood meal to trigger the maturation of the ovaries and in these there is gonotrophic concordance*. Changes in the ovaries associated with the gonotrophic cycle* and other physiological changes such as loss of body fat and increasing transparency of the Malphigian tubules, allow female flies to be age-graded, that is differentiated into those that have not laid an egg batch (nulliparous flies or nullipars) and those that have (parous* flies or pars). In parous flies the number of separate egg batches that the fly might have laid cannot be reliably determined. Probably very few pars complete more than four or five gonotrophic cycles because each cycle takes a few days and flies seldom live for more than a very few weeks.

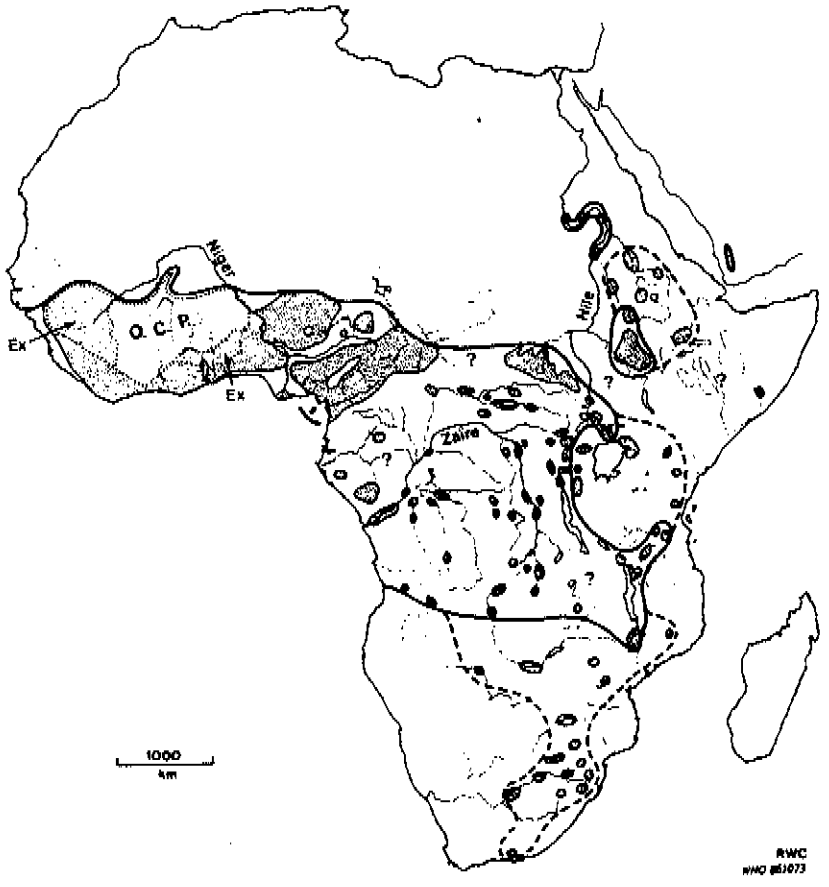

Figure 4.2 Approximate known distribution of Simulium damnosum s.l. (shaded areas)

(Solid line indicates the areas in which members of the complex are anthropophilic and can be expected to act as onchocerciasis vectors; broken lines indicate additional range over which the complex is zoophilic.

The length of the gonotrophic cycle in vectors is less than that of O. volvulus development within the fly from microfilaria to infective larva in the head (which is usually 6-8 days). Hence a fly is most unlikely to become infective within the time-span of one gonotrophic cycle, and one found to be infective (harbouring infective (L_3) O. volvulus larvae in its head) has almost certainly undergone two or more cycles.

Ovarian development usually takes from 2-5 days in vectors, and 3-6 days is the usual time for a complete gonotrophic (= inter-ovipositional) cycle - the extra day being the approximate period between an oviposition and the next bloodmeal. In S. damnosum s.l. the inter-bloodmeal period is 3-5 days.

The length of the gonotrophic cycle in S. ochraceum s.l. is usually stated as being 2-4 days, but one report gives a minimum of five days. An unusually prolonged gonotrophic cycle can occur if there is a substantial delay between the time when a fly becomes gravid and when the eggs are laid. In the laboratory, oocytes of S. woodi have been observed to mature by 60 h post-bloodmeal, but eggs were not laid until 4-6 days after feeding.

Five stages in the development of the ovaries have been defined in S. damnosum s.l., and in S. ochraceum s.l. The ovarioles at different physiological stages in ovarian development are figured by Lewis (1958, 1960b) for S. damnosum s.l. and by Lewis (1960a) for S. woodi (as neavei). The ovaries of parous flies have follicular relics in the ovarioles which confirm their parity and differentiate them from nulliparous flies. The works cited should be consulted for further details.

(e) Nulliparity and parity

Studies on the S. damnosum complex in Cameroon have shown that in the savanna parous flies tend to stay near to, or to move along, the rivers, whereas in forest regions the proportions of parous flies are highest away from the rivers. As only parous flies can be infective this has clear implications that man-fly contact at the riverside is potentially most dangerous for transmission in the savanna. Savanna and forest in West Africa are typically occupied by different cytospecies of S. damnosum s.l., and these findings are presumably cytospecies-related. They do not imply that parous flies in the savanna do not travel far from breeding sites, and in the Onchocerciasis Control Programme experience shows that the opposite is the case: over 90% of long-range migrant flies of savanna cytospecies are parous (Garms et al. 1979).

(f) Biting activity and biting rates

Simuliids bite by daylight and in the open. Where and when man biting occurs or is at its most intense varies greatly with geographical site, daily weather conditions, seasonal climatic changes and species identity. Biting activity is most easily considered as geographical, diurnal and seasonal.

Geographical. This is primarily related to vector breeding sites, at or near which biting frequently takes place. River banks and crossings are major points of man-fly contact. All vectors disperse from their breeding places, and biting activity often occurs several kilometres from the source; long-range migrant cytospecies of the S. damnosum complex may bite up to several hundred kilometres from their emergence sites. Vectors in forested areas are usually less mobile and bite mainly at riversides, edges of villages or clearings, or along tracks. In West African savannas S. damnosum s.l. often concentrates its

biting at sites in open or semi-open bush far from rivers (its biting behaviour is not so strongly associated with river banks as is often supposed). Simulium neavei on Mt. Elgon in Uganda bites in forest at higher altitudes than those at which early stages breed in more open areas in association with crabs. Amazonian vector species bite mainly near river banks and not in deep rain forest (but S. oyapockense has been observed biting in open high ground 3-4 km from the river (personal observation; Davies, J.B.). Outdoor biting is usual in vectors, but S. ochraceum s.l.shows a stronger tendency to bite in the shade of verandas, and occasionally indoor rooms, than other species.

Diurnal. Diurnal* biting begins at daybreak and continues until twilight, in tropical latitudes for about 12 h. Nocturnal* biting is virtually non-existent. A midday lull often occurs that gives rise to a bimodal* biting activity pattern that may be skewed towards either predominantly early morning or evening biting. In forested areas with relatively constant diurnal conditions of temperature, humidity and shade, biting usually continues steadily through the day (Fig. 4.3), but it may nearly cease from about noon to late afternoon in savanna or open grassland areas with little shade, high midday temperature and low midday humidity. Biting is inhibited by strong winds and heavy rain, but will continue in light rain and tends to be provoked by barometric changes associated with oncoming thunderstorms. A normally unimodal pattern of biting, if interrupted by heavy rain showers, can be transformed into an upsurge in biting activity when the rain has passed.

Seasonal. Annual climatic changes, especially well marked wet and dry seasons, have associated biting activity patterns. In West African savannas with severe drought seasons the biting of S. damnosum s.l. almost completely ceases for several months, resuming again with the coming of the rains and re-establishment of extensive breeding sites in the rivers; biting is heavily concentrated on the wet season. In forested pre-coastal West Africa, where dry and wet season are less dramatically different, biting activity is spread more evenly through the year (Fig. 4.3). Similarly in the Brazilian rainforest region, where breeding is perennially sustained in major rivers and annual conditions are relatively uniform, biting activity continues thoroughout the year. Biting activity in most tropical areas tends to be maximal when rainfall is heaviest, but that of S. ochraceum s.l. shortly after the main rainfall period in Mexico and Guatemala is most pronounced in the early dry season when multitudes of infant streamlets are running over the ground to provide breeding sites. In S. damnosum s.l. biting activity in mid-wet season can be temporarily depressed if excessive flooding destroys many breeding sites.

Biting rates. The numbers of flies caught in a certain time period whilst settling to bite are routinely used for assessing vector 'densities', usually as flies/manhour (FMH) or flies/man-day (see Section VII). Density figures vary enormously within and between vector species because they reflect many interrelated variables that influence biting activity. In S. damnosum s.l. peak densities in West African savanna areas usually range from 30-60 FMH, but densities up to 200 FMH or more frequently occur in forest areas. New World vectors bite in conspicuously higher average densities than African vectors. For example, approximately 100-1000 FMH for S. ochraceum in Guatemala, and 300-400 FMH for S. oyapockense s.l. in Brazil. Such figures are a striking contrast to the yearly average of 3.2 FMH and the maximum of 9.4 FMH that have been recorded for S. woodi in the Amani focus of the United Republic of Tanzania.

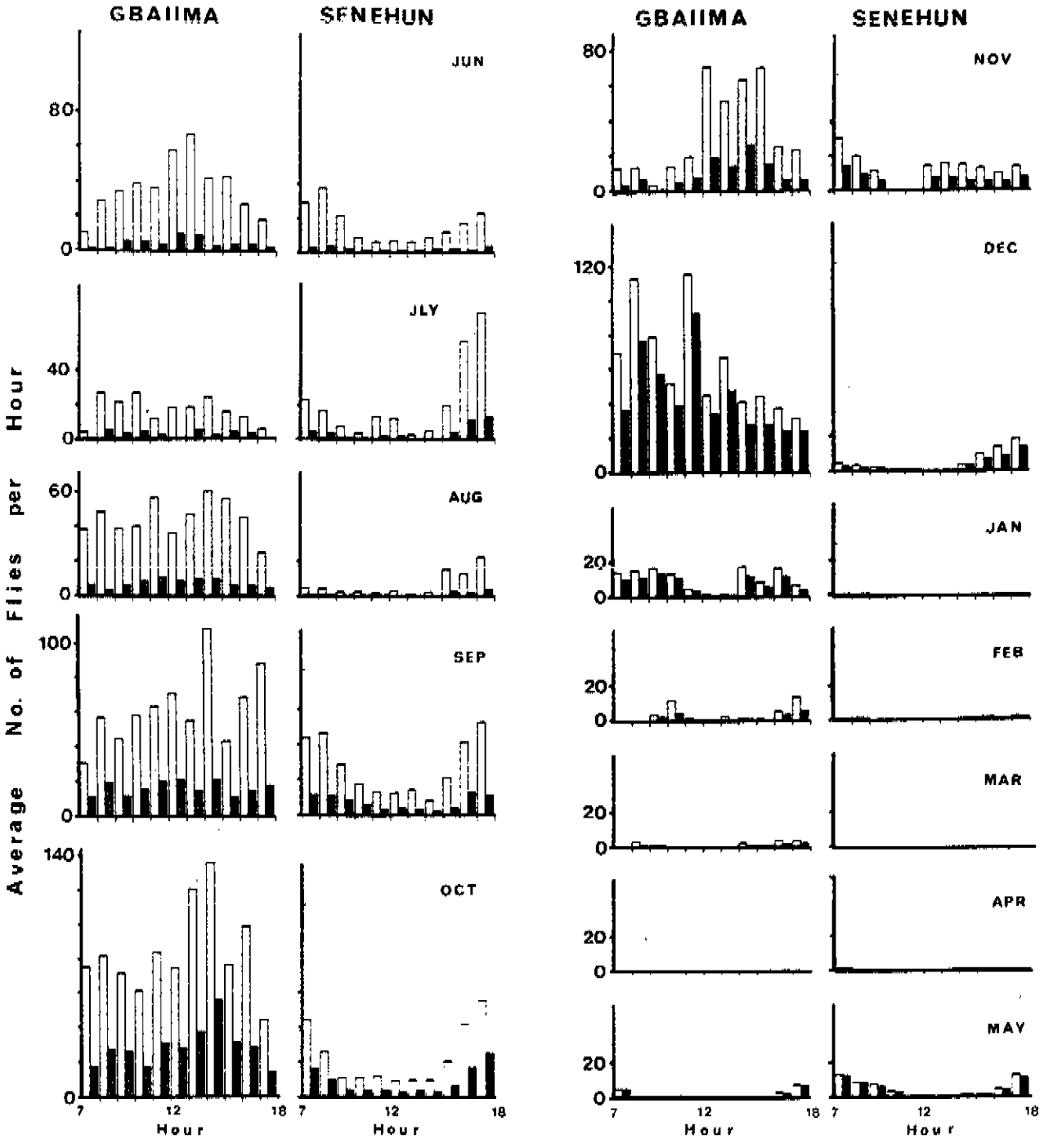

Figure 4.3 Seasonal biting activity in a forest area of Sierra Leone

Numbers of all S. damnosum s.l. (open columns) with numbers of parous flies (black column) caught biting each hour at a semi-shaded riverside site (Gbalima) compared with an open rice field site (Sanehun), each month of the year. Seasonal and diurnal differences in biting activity and age structure (dry season, October to May) are demonstrated.

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V. VECTOR TAXONOMY AND IDENTIFICATION

Onchocerciasis vector species seldom occur in isolation from other simuliids, and most onchocerciasis zones have a simuliid fauna consisting of many species of which the majority is completely harmless to man. There is thus a need for accurate differentiation of vector species (as larvae, pupae and adult flies) from innocuous species.

The problems of simuliid vector identification and approaches to their solution have been discussed by Townson and Meredith (1979). Problems differ between vectors. The African Simulium damnosum complex can be readily recognized by 'at sight' characters in larval, pupal and adult stages, but reliable identification of individual species within the complex remains difficult. In Guatemala, on the other hand, adult females of the three vectors, S. ochraceum, S. metallicum and S. callidum, can be immediately distinguished by obvious colour differences, whereas their larvae and pupae are much less obviously separable.

V.1 Species complexes

Species complexes are groups of species in which members can be reliably distinguished only by non-morphological means. The chief means of analysing species complexes and recognising their non-morphologically definable constituent members (sibling* species) is by cytotaxonomic study of giant (polytene) chromosomes found in the nuclei of simuliid larval silk gland cells. The chromosomes provide micromorphological criteria for diagnosing and discriminating the species (which, since the primary characters are cytological, are conveniently called cytospecies). Cytotaxonomic analysis of complexes is dependent on the larval life stage, and in the case of the S. damnosum vector complex is now being supplemented by isoenzyme studies of the adult flies in an attempt to discover a directly reliable means of identifying wild-caught female vector flies (Meredith & Townson, 1981).

Non-morphological studies of vectors other than S. damnosum s.l. have been few, and identification of all of them remains completely dependent upon conventional morphological characters. Some, however, belong to morphologically close-knit species-groups of vectors and non-vectors. In this category are S. neavei and S. woodi belonging to the Simulium neavei group in East Africa and S. metallicum s.l. and the very recently described species S. horacioi (formerly misidentified as metallicum) in Guatemala.

V.2 Published keys and descriptions

Modern comprehensive monographs by which the simuliid fauna in onchocerciasis areas can be identified are lacking, and field workers unless concerned with an already well-known vector should take the precaution of having specimen identifications verified or made by specialists. The following texts, though outdated by subsequent description of more species, can with caution be used as starting points for identification of Simulium s.l. species in onchocerciasis areas: for tropical Africa, Freeman and de Meillon (1953, pupae and adults), Crosskey (1960, larvae); for West Africa, Philippon and Séchan (1978); for Meso-America, Dalmat (1955, Guatemala) with coloured plates of vectors, Onishi et al. (1977, Guatemala, larvae and pupae), Vargas and Díaz Nájera (1957, Mexico); for South America, Ramírez-Pérez and colleagues (1982, Venezuela) and Shelley and collaborators (1988, Ecuador, with coloured

illustrations). Pictorial keys to the adults, larvae and pupae of Guatemalan species are given in Yamagata and others (1983, in English and Spanish), and for Venezuela by Ramírez-Pérez et al. (1985); and the S. amazonicum group by Shelley and colleagues (1982).

V.3 Lists of vector species

Simulium damnosum complex

A list of the species and species names that are included in the S. damnosum s.l. species complex are given in Table 5.1. This formidable list is included because these names appear in the literature and include accepted Latin-named species (e.g. S. damnosum sensu stricto) as well as 'common' named species of doubtful status even though they may be undoubted vectors (e.g. "Beffa"). The table clearly shows how 'common' named species eventually become formalized with Latin names once their status is confirmed by cytotaxonomic studies (e.g. S. yahense was formerly known as "Yah").

From the practical viewpoint they form three categories, according to the status of their classification and are so listed in order to help clarify the confusion of names in the literature:

(i) Formally Latin-named member taxa (sibling species or "cytospecies") based on chromosomal characters, but to some extent either morphologically or enzymatically* identifiable.

(ii) Formally Latin-named member taxa based on morphological characters as yet unsupported by chromosomal or enzymatic characters.

(iii) Vernacularly named member taxa based on chromosomal characters, poorly or not studied morphologically, and unknown enzymatically.

Category (1) includes the geographically widespread sibling species that have been much investigated in West Africa notably S. sirbanum, S. damnosum s.s., S. sanctipauli, S. soubrense, S. yahense, and S. squamosum. The first two of these six species are termed "savanna" species, and the other four "forest" species. S. sirbanum is the dominant, if not the only species in the northernmost areas of the Sudan savanna. S. damnosum s.s., although essentially a savanna-dwelling species, adapts itself to more wooded biotopes* and may form small permanent populations even in dense forest. The S. sanctipauli subcomplex, including S. sanctipauli, S. soubrense, S. soubrense "B", and the "Beffa", "Djodji" and "Konkouré" forms, is under revision; criteria and status need to be reassessed. The species of this subcomplex vary morphologically, and in their host preferences, vectorial roles and adaptation to the different bioclimatic zones. They are mainly forest species inhabiting large rivers, but in the rainy season some of them may extend their areas of distribution into the Guinea savanna (10°-11°N.) and swollen smaller rivers. S. yahense is associated with the rain-forest and generally breeds in small watercourses. S. squamosum, which is probably also a subcomplex, has a focal distribution, with a preference for small or medium sized rivers in hilly and mountainous areas in West Africa. However, in the east of Nigeria and west of Liberia it can also occur in large rivers.

Table 5.1 Synopsis of the S. damnosum complex: list of taxa names and their geographical distribution[A]

Taxon*	Distribution
	Category 1
S. damnosum s.s. syn. S. cingulatum syn. "Nile"	Senegal to Central African Republic (mainly savanna), Uganda and Sudan (Nile Basin) (vector)
S. dieguerense syn. "Dieguera"	Mali (savanna) (? vector)
S. kilibanum syn. "Nyamagasani" syn. "Kiliba"	Burundi, Malawi, United Republic of Tanzania, Uganda, E. Zaire (vector some areas)
S. mengense	N. and S. Cameroon, N.W. Central African Republic, United Republic of Tanzania (? vector)
S. rasyani	Yemen (vector)
S. sanctipauli[S] syn. "Bandama"	Sierra Leone/Guinea to S.W. Nigeria (forest) (vector)
S. sirbanum syn. S. sudanense syn. "Sirba"	Senegambia to N.W. Central African Republic (savanna), Sudan (vector)
S. soubrense[S] syn. "Soubre"	Sierra Leone/Guinea to Nigeria (forest/ forest-savanna mosaic) (vector)
S. soubrense "B"[S]	Sierra Leone (vector)
S. squamosum syn. "Bille"	Sierra leone to Central African Republic/W. Zaire (forest-savanna mosaic) (vector)
S. yahense syn. "Yah"	Sierra Leone/Guinea to Togo (forest) (vector)
	Category 2
S. buisseti	W. Zaire (probably non-vector)
S. juxtadamnosum syn. "Bubumu" syn. "Lutumgulu"	E. Zaire (probably non-vector)

.../Cont'd

Table 5.1, Continued

Taxon*	Distribution
"Kapere"	E. Zaire (forest) (? vector)
S. latipollex	South Africa (non-vector)
S. luadiense	S.W. Zaire (? vector)
S. maertensi	W. Zaire (? vector)
S. microlepidum	W. Zaire (? vector)
S. nganganum	S.W. Zaire (? vector)
S. repertum	W. Zaire (? vector)
S. wambanum	S.W. Zaire (? vector)
	Category 3
"Beffa"	Benin, Liberia, Nigeria, Togo (vector)
"Bole" (unpublished)	N.W. Central African Republic (? vector)
"Djodji"[S]	Togo/Ghana border (vector)
"Hammerkopi"	United Republic of Tanzania (Ruaha) (probably non-vector)
"Jimma"	S.W. Ethiopia (? vector)
"Jovi"	United Republic of Tanzania (probably non-vector)
"Kagera"	United Republic of Tanzania/Uganda (probably non-vector)
"Kaku"	Uganda (Kigezi) (probably non-vector)
"Ketaketa"	United Republic of Tanzania (Ruaha) (? vector in Kilosa)
"Kibwezi"	Kenya, United Republic of Tanzania (plain below E. Usambara) (probably local vector)
"Kipengere"	United Republic of Tanzania (Ruaha) (probably non-vector)
"Kisiwani"	Ethiopia, Kenya, United Republic of Tanzania (non-vector)
"Konkoure"	Guinea (vector)
"Kulfo"	Ethiopia (non-vector)
"Mutonga"	Kenya, (Mt. Kenya area) (non-vector)

.../Cont'd

Table 5.1, Continued

Taxon*	Distribution
"Ngaraba" (unpublished)	S. Central African Republic (? vector)
"Nkusi"	W. Kenya, United Republic of Tanzania, Uganda (? vector in United Republic of Tanzania, non-vector in Kenya)
"Sanje"	Malawi, United Republic of Tanzania (inc. E.Usambara) (? vector)
"Sebwe"	United Republic of Tanzania, Uganda (? vector)
"Turiani"	United Republic of Tanzania (prob. non-vector)

A Formally Latin-named and vernacularly named taxa are distinguished. Category indicates the nature of the taxonomic criteria (see text).
(? vector) indicates that the degree of anthropophily and involvement in transmission is uncertain.
(non-vector) indicates occurrence in onchocerciasis-free areas.
(probably non-vector) indicates that the taxon is largely or completely zoophilic.

S Member of the S. sanctipauli subcomplex, which is under revision.

X Also known morphologically, but status uncertain.

Category (2) includes morphospecies of uncertain validity from the Zaire basin.

Category (3) includes taxa from eastern Africa with restricted distributions (most of them are probably valid sibling species). A few taxa fall outside these main categories, e.g., the West African "Beffa" form, which has been chromosomally and morphologically characterized but remains unnamed because its taxonomic status is uncertain.

The total known distribution for S. damnosum s.l. is shown in Fig 5.1, with an indication of its anthropophilic range. Distribution maps of the common West African cytospecies can be found in W.H.O. (1987) and Crosskey, (1987); while East Africa is covered by Raybould and White, (1979).

Simulium neavei group

The S. neavei group includes all simuliids in which the larvae and pupae live in a phoretic association on river crabs. The group contains eight described species and some forms of uncertain taxonomic status. Three species are considered to be vectors. The structural uniformity of members of the S. neavei group and the lack of cytological or enzymatic studies makes the identification of potential vector species especially difficult. The larval cuticular microsculpture can be used to differentiate taxonomically most members of the group, but is difficult to use and the help of a specialist may be required.

The S. neavei group occurs widely but sporadically in Africa from Ethiopia southwards to Malawi and westwards through the Zaire river basin to Cameroon and Liberia (Fig 5.2). No vector species extends into West Africa. In many foci S. neavei s.l. and S. damnosum s.l. occur together and act in conjunction as vectors. This happens in the foci of eastern Zaire, the central highlands of the United Republic of Tanzania, southwestern Ethiopia, and two or three areas in Uganda. In such foci the S. neavei group species tend to be of greater relative importance at the high altitudes within the transmission area. A few foci are maintained entirely by S. neavei group vectors, notably the Mount Elgon and Bugoma foci of Uganda, where S. neavei s.s. is the only vector, and the Usambara (Amani) focus in the United Republic of Tanzania, where S. woodi is responsible for transmission. The situation is presented in detail by Raybould and White (1979). Man-biting S. neavei s.l. have been collected in Zambia where the presence of endemic onchocerciasis is suspected but as yet unproven.

American vectors

The species which are considered to be vectors in the Americas are given in Table 5.2, and are covered in detail in Section VI.

Table 5.2 Summary of the vectors of onchocerciasis in the Americas

Focus	Vector[a]	*Simulium* species
Guatemala, Mexico	1	*S. ochraceum*
	2	*S. metallicum*
	2	*S. horacioi*
	2	*S. callidum*
Colombia	1	*S. exiguum* s.l.
Ecuador	1	*S. quadrivittatum*
Venezuela		
Caripe & Altamira	1	*S. metallicum* s.l.
	?	*S. exiguum* s.l.
Brazil/Venezuela		
highland areas	1	*S. guianense (pintoi)*[b]
	?	*S. limbatum (incrustatum, yarzabali)*
lowland areas	1	*S. oyapockense* s.l. *(amazonicum, cuasisanguineum, minisculum, sanguineum)*
	?	*S. exiguum* s.l.

[a] 1 = primary vector,
2 = secondary vector,
? = suspected vector.

[b] included in parentheses are other names given to the species.

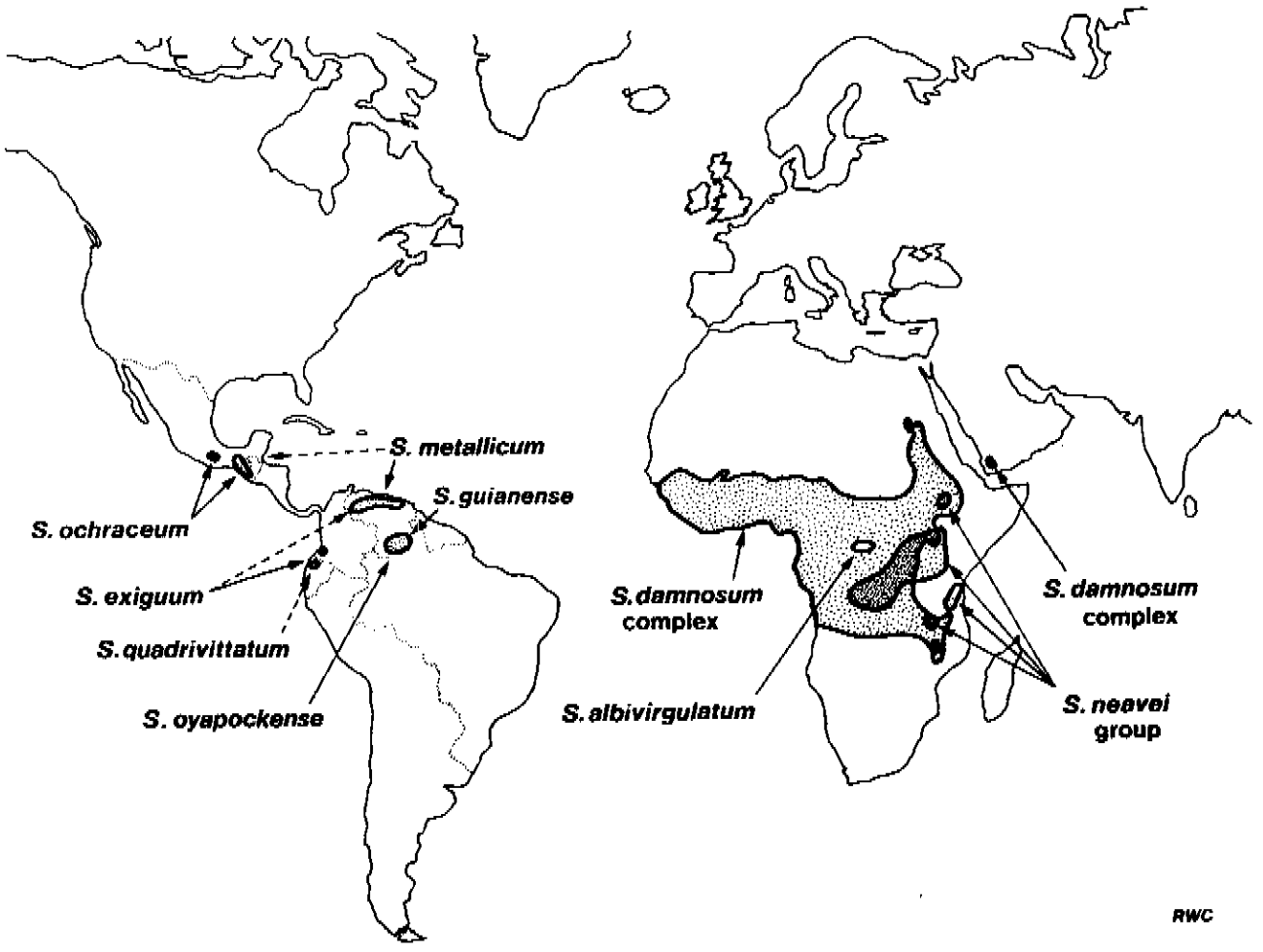

Figure 5.1 World distribution of the primary (solid arrows) and secondary (broken arrows) vectors of onchocerciasis.

(Original, R. W. Crosskey.)

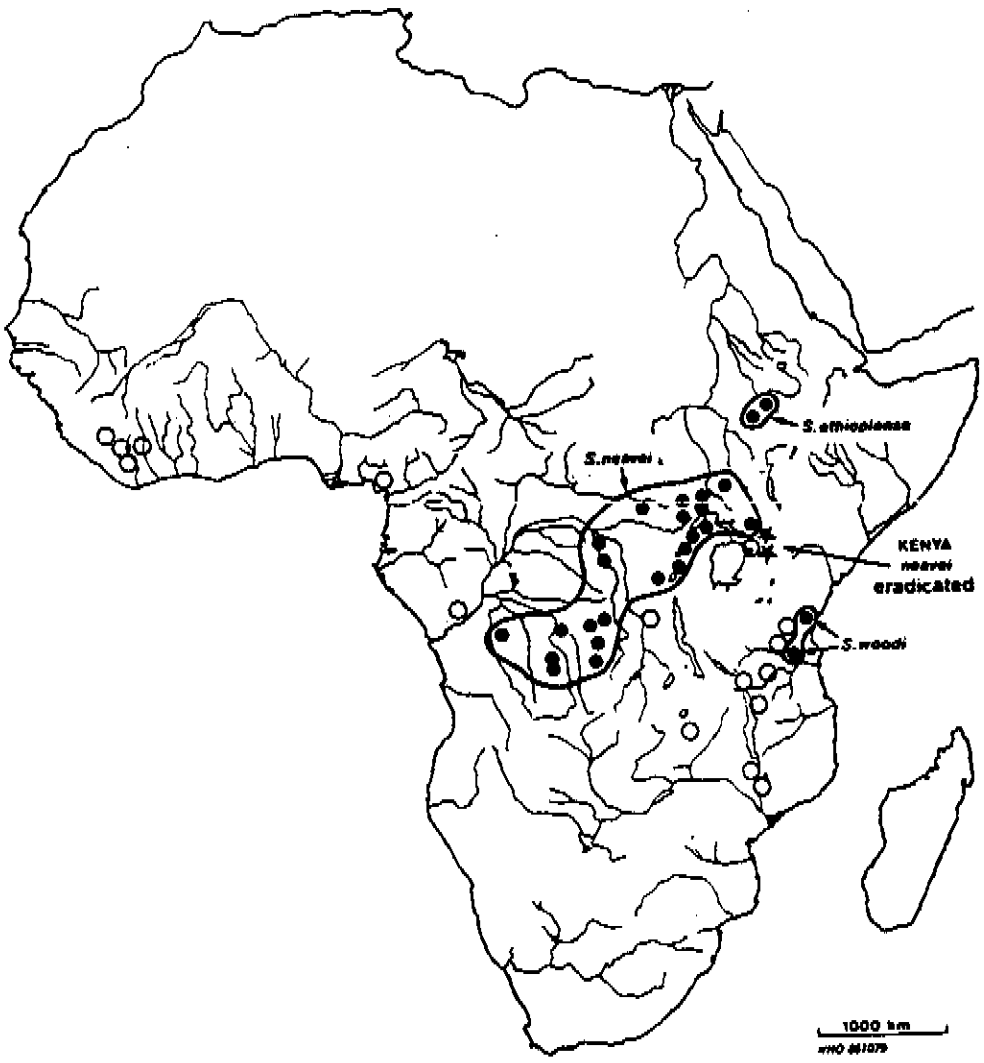

Figure 5.2 Geographical distribution of the Simulium neavei group

Solid lines enclose the known localities with vector species. Open circles outside these lines indicate localities with non-vector species.

(Original, R. W. Crosskey.)

V.4 Field keys to the common man-biting female Simulium species in onchocerciasis areas

The Keys refer to the common man-biting Simuliidae in each area but other species may bite rarely or in low numbers. The Keys have been made as simple as possible, so they are not as precise as the definitive keys published in the literature quoted above. Any specimens that do not key out easily should be checked by other keys or referred to a specialist. Wherever possible, characters have been used that can been seen through a hand-lens or dissecting microscope.

Keys A and B are based on those by Crosskey (1973); Key C is modified from Shelley et al. (1989), and Key D is original. Species names in brackets refer to synonyms or to closely related species that are morphologically inseparable at this level. Measurements refer to the total length of head, thorax and abdomen, unless otherwise stated.

A. Tropical Africa and Yemen

Note: The geographical areas cited are those in which man-biting is known, not the full range of the species distribution. Only S. damnosum s.l.and S. neavei s.l. commonly bite man. All other species bite man occasionally, or very locally, or at very low frequency.

1. Fore tarsus broad and flattened, with a conspicuous dorsal hair crest. Abdomen with silver or silver-yellow hairs arranged in flat clumps on the sides, and with the hind part shining black and bare (except for some minute erect dark hairs). Legs black with a characteristic wide and veryconspicuous creamy-white band on hind basitarsus. Medium-sized species,
wing-length 2.0-2.4 mm. (Commonest man-biting species, West to East Africa, Zaire Basin, Ethiopia, Sudan, Yemen)

damnosum s.l.

- Fore tarsus not conspicuously dilated and without obvious hair-crest. Abdomen with rather evenly distributed hair on whole surface, which is not aggregated into definite separated clumps at the sides. Legs usually either uniformly black or extensively pale. Size varied. ...2

2. Large black species with silvery-yellow to golden or partly black hair on thorax and abdomen. Scutum without trace of pattern. Wing length 2.6-3.6 mm. ...3

- Very small greyish species (blackish in wellmanni) with silvery hair on thorax and abdomen. Scutum with three fine longitudinal lines, best seen as fly is turned. Wing length 1.3-2.3 mm. ...6

3. Abdomen with evenly distributed silvery-yellow to golden hairing, sometimes coppery near the middle. Base of abdomen not strikingly contrasting with remainder. Ground colour of basal part of hind basitarsi paler than remainder of hind legs (inconspicuously so in neavei). (Eastern Zaire and East Africa). ...4

- Base of abdomen thickly covered with silvery-yellow to golden hairs and strikingly contrasting in colour with remainder of abdomen, which appears partly or entirely black with black hairs. Hind basitarsi entirely black like the rest of the legs. (Cameroon). ...5

4. Abdominal hairs uniformly silvery-yellow to golden. Paler part of hind basitarsus inconspicuous, not forming a definite pale band. (Zaire, Uganda, formerly Kenya)

neavei

- Abdominal hairs not all one colour, distinctly coppery or bronze coloured on middle part, which contrasts in colour with silvery-yellow hair on other segments. Hind basitarsus with definite pale yellow or reddish-yellow pale band in background colour of basal half or two-thirds. (Tanzania, Malawi)

woodi

5. Very black species with hair of frons, clypeus, prescutellar area of scutum, and scutellum black or bronze-black. Abdomen almost entirely black-haired, except for the contrasting yellow-haired basal two segments. (Forested parts of Cameroon)

dukei

- Not such conspicuously black species, hair of frons, clypeus, all of scutum and much of scutellum silvery-yellow to pale golden. Abdomen with conspicuous yellow hair on side of segments 3-6 (middle part), in addition to thick yellow hairs of basal two segments, and bronze-black to black haired only on terminal segments and mid-dorsally on segments 3-6. (Mainly savanna parts of Cameroon)

ovazzae

6. Claws with large basal tooth[1]. ...7

- Claws simple, without basal tooth. ...8

7. Wing with basal section of radius haired[1]. Pleural membrane haired. (Mainly northern savanna areas of West Africa)

adersi

- Wing with basal section of radius bare. Pleural membrane bare. (Mainly Sudan, also Nigeria)

griseicolle

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[1]High magnification is required to see these features adequately and slide preparations are useful (Fig. 3.2).

8. Legs and antennae entirely black. (Angola)

wellmanni

- Legs extensively pale or with at least a pale hind basitarsal band; antennae with first two segments reddish-yellow to dark reddish and conspicuously paler than remainder of antennae. ...9

9. Legs mainly pale reddish-yellow, only darkened brown to blackish-brown on tarsi and apices of tibiae, sometimes faintly on mid femora. (Mainly Nigeria)

bovis

- Legs dark brown or blackish-brown, only yellowish on basal two-thirds of hind basitarsi and sometimes paler brownish on bases of tibiae. (Zaire and Zambia)

albivirgulatum

B. Mexico and Guatemala

1. Scutum yellow or orange. Basal section of radius haired. ...2

- Scutum brownish-black or black in ground colour (narrowly orange-yellow on sides in veracruzanum). Basal section of radius bare. ...3

2. Scutum with a pattern of two curving silvery-grey pollinose* longitudinal bands running the length of the scutum. Legs mainly yellow, only brown on parts of tarsi and apically on hind femora and tibia. Abdomen yellow only on first two segments

callidum

- Scutum without a pattern of pollinose stripes (only two small silver spots on anterior half). Legs entirely dark brown. Abdomen yellow on basal half (first four tergites) and black on remainder, the colour contrast very conspicuous

ochraceum s.l.

3. Ground colour of scutum all dark, usually with pattern also. Abdomen entirely black. Scutellum brown or black. ...4

- Ground colour of scutum mainly black but orange-yellow on lateral margins and adjacent to humeri*; median black area divided into three by a pair of broad and boldly marked longitudinal stripes of silvery-grey pollinosity. Abdomen with two segments yellow. Scutellum yellow

veracruzanum

4. Hair of scutum all or mostly black. Scutellum without any pale hair. Legs dark brown or black except for conspicuous creamy-white band on each hind basitarsus. ...5

- Hair of scutum entirely silvery-yellow to golden. Legs mainly pale yellow, only parts of tarsi and hind legs noticeably dark brown. Scutellum with conspicuous long soft recumbent yellow or golden hair on each side.

...6

5. Scutum with pollinosity forming a pattern of three narrow stripes (outer pair convergent anteriorly) which appear black and darker than surrounding areas in some lights but reverse to appear silver-grey against a dark background as fly is turned into different lights. Hair of scutum all black, very short. Medium sized species, wing-length about 2.4 mm

metallicum s.l.
(horacioi)

- Scutum with pattern formed of two brilliant nearly parallel silver pollinose stripes, their appearance more or less fixed. Scutal hair mainly black, but posterior angles of scutum and the prescutellar area with conspicuous soft yellow recumbent hair. Small species, wing-length about 2 mm

quadrivittatum

6. Scutum without a pattern but bearing many discrete clumps of silver or greenish-yellow hairs. Hind basitarsus entirely yellow

exiguum s.l.
(gonzalezi)[2]

- Scutum with very bold pattern, two broad parallel silver-grey pollinose stripes which merge posteriorly with each other and with similar grey pollinose side-margins leaving a median and two sublateral broad black vittae. Hair of scutum evenly distributed. Hind basitarsus white on about basal two-thirds and black-brown on apical third

haematopotum

C. Ecuador and Colombia

1. Scutum orange with white pollinose pattern dorsally. ...2

- Scutum black with or without a silver pattern. ...3

2. Scutum with a pair of submedian greyish white bands running from anterior border for four fifths of the scutal length. Postnotum black with silver pollinosity. Abdomen with dorsal chequerboard pattern of prominent black markings on greyish background

escomeli

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[2]Mainly zoophilic.

- Scutum with a pair of submedian white pollinose comma-shaped bands beginning at posterior border of humeri and running half length of scutum. Postnotum orange. Abdomen dark brown with anterior segments orange (mainly zoophilic) bipunctatum

3. Scutum velvet black with a pattern of silver pollinose bands formed by a pair of submedian longitudinal stripes reaching silver hind margin together with a pair of sublteral bands. Abdomen black dorsally with transverse silver bands on anterior five tergites quadrivittatum.

- Scutum shiny without silver pattern, but with scattered clumps of silver or yellowish white hairs. Abdomen black without transverse silver bands. Legs mainly dark exiguum (gonzalezi)[3]

D. Venezuela and Brazil

All species in this key have a general black, grey or brown colouration.

1. Scutum with a prominent pattern of silver stripes, commas or triangles on a black velvet background. ...2

- Scutum without this pattern but possibly with faeint dark stripes where the pollinosity is absent. ...3

2. Very small, 1-2mm. Scutum velvet black with a broad U-shaped band of silver pollenosity running round the lateral and posterior margins. Centre of scutum either with two comma shaped silver marks touching the anterior margin or two parallel bands touching both anterior and posterior margins, (appearance depending on direction of light)

oyapockense s.l.
(amazonicum)
(cuasisanguineum)
(minusculum)
(sanguineum)

- Medium size, 2-2.5mm. Scutum black velvet colour with scattered single silver or golden hairs and a narrow area of silver pollenosity running round the lateral and posterior margins. Two triangular shaped areas of

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[3]Mainly zoophilic.

silver pollenosity lie one on each side of the mid-line against the anterior margin (not extending beyond centre of the disc whatever the direction of the light)

limbatum
(incrustatum)
(yarzabali)

3 Scutum black with greenish-yellow or silver hairs arranged in discrete clumps of 3-5 hairs. Hind basitarsus entirely yellow. Very small 1-2mm exiguum s.l.

- Scutum without discrete clumps of 3-5 hairs. ...4

4 Scutum with a silvery pollinosity, giving a general grey aspect, with an underlying pattern formed of three narrow stripes which appear black and darker than the surround, or appear silver against a dark background (depending on the light). Fore tarsi not enlarged. Medium size, 2-2.5mm (N.Venezuela)

metallicum s.l.

5 Scutum with a faint pollinosity but without underlying pattern, with scattered single short silver or brassy hairs, particularly on the lateral margins. Tarsi of forelegs very broad about twice as wide as tarsi of other legs) black, with long black hairs on the anterior margin. Abdomen dark above but pale below. Antennae generally pale, darkening towards the tip. Large, 2.5-3mm

guianense
(pintoi)

V.5 Identification of larvae and pupae of vector species

The separation of larvae and pupae of vector species from the many non-vector species living in the same rivers throughout the world is too large a subject to be covered in this Guide. However, in any specific area the number of possible non-vector species living in the same rivers as the local vectors is unlikely to be more than about 20, so it is quite feasible for an expert to make simple keys that would be applicable to any particular area. Indeed, one of the first priorities for working in a new area would be a survey to determine all simuliid species present in that area.

In Africa, it is fortunate that both the larvae and pupae of the S. damnosum s.l. complex are unique in structure and are easily recognised. In areas where adults of the S. neavei group are to be found biting, any larvae or pupae found on river crabs may be assumed to be the same species as the man-biting adults, until proved otherwise. The pupal gill is shown in Fig. 3.3.F.

Simple descriptions and figures of West African species are given (in French) by Philippon and Sechan (1978), of Guatemalan species (in English and Spanish) by Yamagata and et al. (1983). Other descriptions and keys are to be found in the works cited in Section V.2.

Recognition of the larvae of the S. damnosum s.l. complex

Mature larvae are relatively large compared to other species, and black in colour due to a dense covering of short broad black spines mostly on the dorsal and lateral surfaces and to a lesser extent on the ventral surface (Fig. 3.3.B). On the posterior half they are also interspersed with a scattering of a few broader spines. All the spines give the larva an 'unshaven' appearance which is visible on all instars. The dorsal surface usually carries five or six pairs of short conical tubercles* (also visible on instars 2 or 3 onwards). On late instars in some localities these tubercles may be visible to the naked eye, but in others may be so small as to be visible as small bumps only under the dissecting microscope. The only other African species so thickly covered in spines is S. albivirgulatum (also a vector in Zaire) which differs from S. damnosum s.l. by having spines only on the dorsal surface and in lacking the additional broader spines. Other black-looking larvae lack either the spines or the tubercles.

Recognition of the pupae of the S. damnosum complex

The pupa is shown in Fig. 3.3.D It is larger than most (but not all) non-vector species occupying the same habitat. The cocoon has a distinct collar which lifts the opening well above the substrate (Fig. 3.3.E). Within this opening can be seen the thorax of the pupa (Fig. 3.1). The 11 respiratory filaments are unusual in that they are short and banana-like and are scarcely long enough to extend beyond the edge of the opening of the cocoon (compared to the long thin filaments that may be as long as the whole pupa in many species). Three Y-shaped filaments usually lie close to the surface of the thorax. In fresh specimens these appear milky-white and as they are easily seen with a hand lens they provide a good character for field identification.

VI. VECTOR-PARASITE RELATIONSHIPS

VI.1 Primary and secondary vectors

The relative importance of a simuliid species as a natural vector varies from place to place, and the total geographical range of a vector species is often much more extensive than the range over which it is acting as a vector of O. volvulus. Even within this range the extent of involvement in transmission may vary from one site to another or at different seasons. Hence apparently the same species can be a major vector in one area but not in another (e.g. S. metallicum s.l. in Venezuela compared with Guatemala). It appears that in most if not all multi-vectorial situations, one primary vector species is the predominating transmission agent keeping the focus 'alive', or able to do so regardless of other vectors, whilst other secondary vectors contribute to the transmission of the disease, but are incapable of maintaining the focus on their own. These are indicated by broken arrows in Fig. 5.1, and detailed in Table 5.2.

VI.2 *Simulium damnosum* complex

(a) Vector-parasite relationships and transmission

All known West African species of the *S. damnosum* complex are able to transmit *O. volvulus* (however, no data are available for *S. dieguerense*.) Under experimental conditions, the most efficient vectors, both in terms of the proportion of flies that become infective and the parasite loads that develop in them, are, in order of importance, the *S. sanctipauli* subcomplex, *S. yahense*, *S. squamosum*, *S. damnosum* s.s. and *S. sirbanum*. However, under natural conditions there are great variations within and between species. These differences are determined by numerous factors, in particular longevity, host preferences, and relative abundance of hosts. Nevertheless all these species are capable of maintaining levels of transmission that are regarded as unacceptable at least in terms of savanna onchocerciasis. In ideal conditions, the highest loads of infective larvae for a given number of biting flies occur in forest populations of *S. yahense*, followed by members of the *S. sanctipauli* subcomplex at low latitudes, and to a far lesser extent by *S. squamosum*. *S. damnosum* s.s. and *S. sirbanum* have the lowest loads.

Although knowledge of the comparative vector potentials of the different members of the *S. sanctipauli* subcomplex is incomplete, and requires further study, it is clear that at the northern limits of their range in Burkina Faso, Côte d'Ivoire, Ghana and Mali, the ability of members of this subcomplex to act as vectors declines markedly.

The migratory populations of *S. damnosum* s.s. and *S. sirbanum* that seasonally reinvade the controlled savanna areas of the Onchocerciasis Control Programme are perfectly capable both of establishing themselves there and of transmitting the local parasite. However, there is evidence that some migrating populations are non-anthropophilic.

(b) Vectors and epidemiological characteristics

In West Africa it is customary to make an implicit association between the clinical and parasitological patterns of onchocerciasis and bioclimatic zones by referring to "savanna" and "forest" onchocerciasis. Foci of "savanna type" (with heavy microfilarial loads and associated high rates of serious ocular lesions and blindness due to onchocerciasis, and with lack of settlement in the main valleys) are only very exceptionally found south of latitude 8°N. The savanna area north of 8°N is the domain of *S. sirbanum* and *S. damnosum* s.s. and locally of small foci of *S. squamosum*. With the exception of a few unusual concentrations, especially in the rainy season, the *S. sanctipauli* subcomplex does not appear to play a significant role as a vector. South of 8°N the *S. sanctipauli* subcomplex, and *S. yahense* predominate as vectors (Fig. 6.1).

In West Africa and across the Sudan, no foci of "savanna type" onchocerciasis are known that are not associated with the presence of *S. sirbanum* and *S. damnosum* s.s. On the other hand, foci of "forest type" onchocerciasis are always associated with transmission by the *S. sanctipauli* subcomplex or *S. yahense* or both in West Africa, or with *S. squamosum* and *S. mengense* in Cameroon.

Nevertheless, where the forest merges gradually into the savanna (6-8°N), there is a wide zone in which foci of blinding hyperendemic onchocerciasis do occur, although the blindness rates are not usually as high as in the true savanna and the relationship between microfilarial loads and the frequency of serious ocular lesions is less clear. The "savanna dwelling" and "forest dwelling" vector species populations that coexist in this intermediate zone are each separately capable of accounting for the observed level of disease severity. Unfortunately there is, at present, no way of telling which of the putative forms or strains of O. volvulus the different vector species are transmitting in the wild in this zone.

In Togo, this intermediate zone extends to the southern limit of the distribution of the S. damnosum complex, and it reaches to the north of the 8°N on the Black Volta, in Côte d'Ivoire and Ghana and along the tributaries of the Upper Niger in Guinea. In these places, the S. sanctipauli subcomplex maintains high levels of transmission that must be regarded as unacceptable in terms of savanna onchocerciasis, irrespective of the annual transmission potentials of the "savanna-dwelling" species (S. damnosum s.s. and S. sirbanum) associated with it in the same areas.

(c) Onchocerca volvulus-Simulium complexes

There is much evidence concerning the heterogeneity of O. volvulus as a species. The existence of very high annual transmission potentials (see Section VII), sometimes up to 30 times greater than those in the worst savanna foci associated with typically forest forms of onchocerciasis, is well-known. It shows that the severity of the disease in relation to the eyes is not due to a greater intensity of transmission in savanna than in the forest.

In some species of the S. damnosum complex, poor experimental parasite yields have been found after ingesting microfilariae of O. volvulus strains whose geographical origin differs from that of the vector. This suggests that local parasite strains adapt genetically to the local vector species. Incompatibilities that were observed in Cameroon between vector-parasite pairs of the savanna and the forest have been confirmed in West Africa. Experimentally, the savanna dwelling vector species maintain low transmission of forest strains of O. volvulus, while S. yahense is a poor transmitter of savanna-dwelling strains of parasites. On the other hand, West African savanna O. volvulus seems to develop in species of the S. sanctipauli subcomplex as easily as the local forest strains.

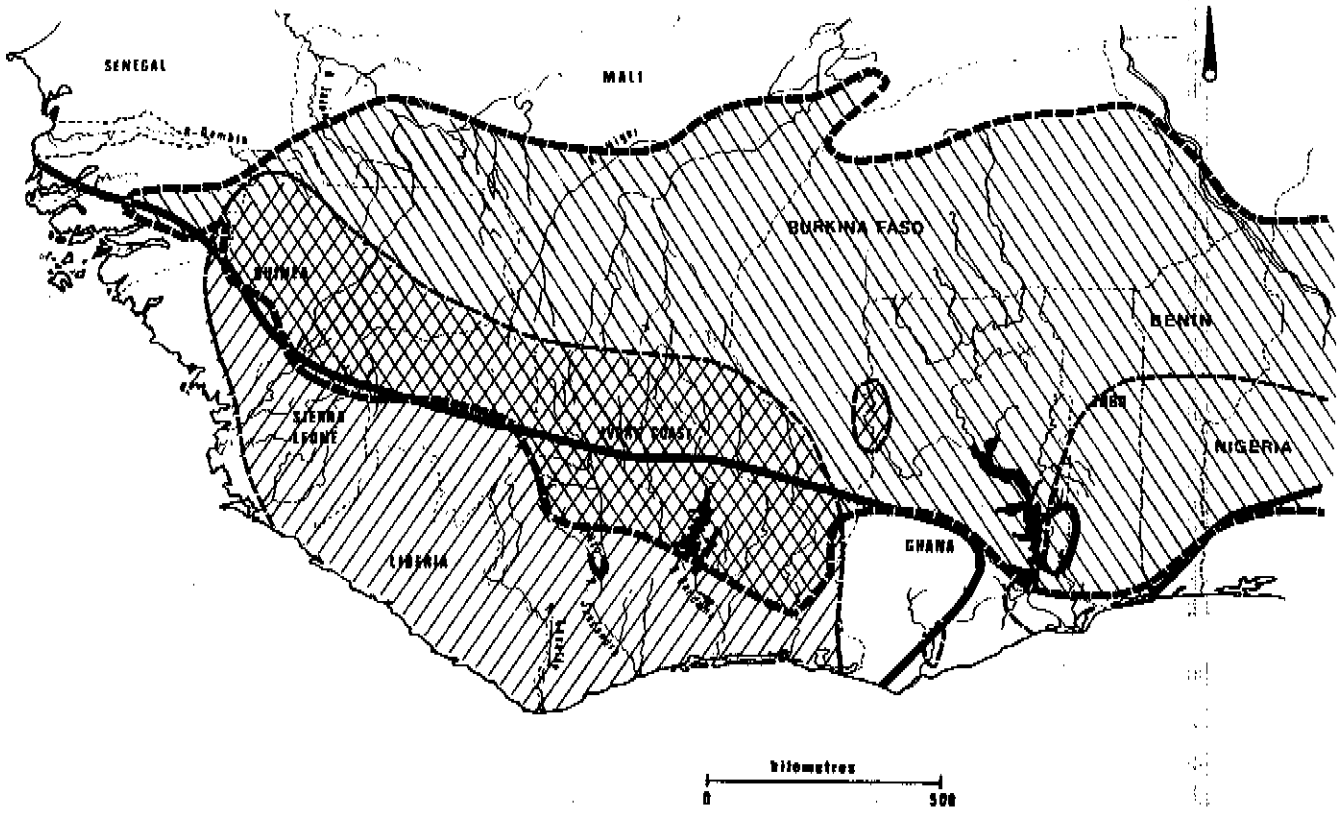


Figure 6.1 Outline map of West Africa comparing the overlapping distribution of the "forest" and "savanna" cytospecies of the S. damnosum complex

____ Northern limit of forest-savanna mosaic.

▪▪▪▪ and NW-SE shading. Combined distribution of the "savanna" species of S. damnosum and S. sirbanum.

---- and NE-SW shading. Combined distribution of the "forest" S. sanctipauli subcomplex species.

[After figures from Crosskey (1987).]

VI.3 Simulium neavei group

The three main vector species of S. neavei, S. ethiopiense and S. woodi breed in heavily shaded, small to medium-sized permanent streams passing though forest or woodland. They all depend heavily on the dense vegetational cover over the streams. Deforestation leads to the disappearance of these vectors or to a decrease in population density. The number of man-biting S. woodi in the Usambara Mountains in the United Republic of Tanzania has been falling for many years probably because deforestation has exposed forest streams and thus made them less suitable as breeding sites.

Although little is known about the dispersal of females of the S. neavei group the flight range is known to be much more limited than that of S. damnosum s.l. S. neavei bites mostly in the forest areas, while S. woodi prefers forest clearings. Little is known about the degree of zoophily of the S. neavei group of vector species. Man is the only known host of S. woodi, but infective filarial larvae other than O. volvulus have been found in S. neavei biting man in Uganda, suggesting that there are other animal hosts.

VI.4 Other vectors in Africa

The long-suspected vectorial role of S. albivirgulatum has recently been confirmed for the "Cuvette centrale" focus in Zaire. This species also occurs in Zambia.

VI.5 Guatemala and Mexico

(a) Simulium species and onchocerciasis

Of nearly 50 blackfly species known to occur in Guatemala, eight are anthropophilic in the endemic areas (S. ochraceum s.l., S. samboni (syn. colvini), S. horacioi, S. callidum, S. haematopotum, S. metallicum s.l., S. gonzalezi and S. veracruzanum). The principal vector of O. volvulus in Mexico is considered to be S. ochraceum s.l., because it is highly anthropophilic, reaches the highest biting densities in the endemic areas, bites the upper parts of the body where microfilariae are most numerous, and shows the best survival rate after infection with O. volvulus. Natural infections assumed to be due to O. volvulus were found in S. ochraceum s.l., while infective larvae probably of animal origin were only present in S. metallicum s.l. and S. callidum. Infection rates of S. ochraceum are highest in the dry season, when transmission is most intensive. The complex is also abundant in some areas where onchocerciasis does not occur; whether this can be explained by the existence of non-vector siblings of S. ochraceum, is being investigated. S. ochraceum was the main target of a pilot control scheme performed in Guatemala from 1979 to 1984.

(b) Bionomics and distribution of S. ochraceum s.l. in relation to control

Breeding sites of S. ochraceum s.l. are distributed in mountainous terrain at altitudes of 500-1500 m (Fig. 6.2.A). Immature stages are found in streams with a water discharge of less than 50 L/sec, but mainly in smaller streams with less than 10 L/sec. Larvae are sometimes even found in minute streams with a discharge of 0.1 L/sec. Maximum densities are recorded at water velocities of 50-60 cm/sec and a depth of less than 1 cm. The fine distribution of breeding has been studied in relation to geological features.

Biting of S. ochraceum occurs throughout the day with two peaks, the higher of the two in the morning and the lower in the afternoon. The gonotrophic cycle is 3-4 days at an altitude of 650 m, while O. volvulus completes its development to the infective stage within 8-9 days. At an elevation of 1480 m the gonotrophic cycle of S. ochraceum s.l.including the host-seeking and oviposition phases, was also 3-4 days. But, the development of O. volvulus requires about 11 days under these conditions. Thus at higher altitudes S. ochraceum s.l. normally has completed three gonotrophic cycles before reaching an epidemiologically dangerous age and, at the earliest, transmits the infection only when coming for its fourth bloodmeal.

VI.6 In South America

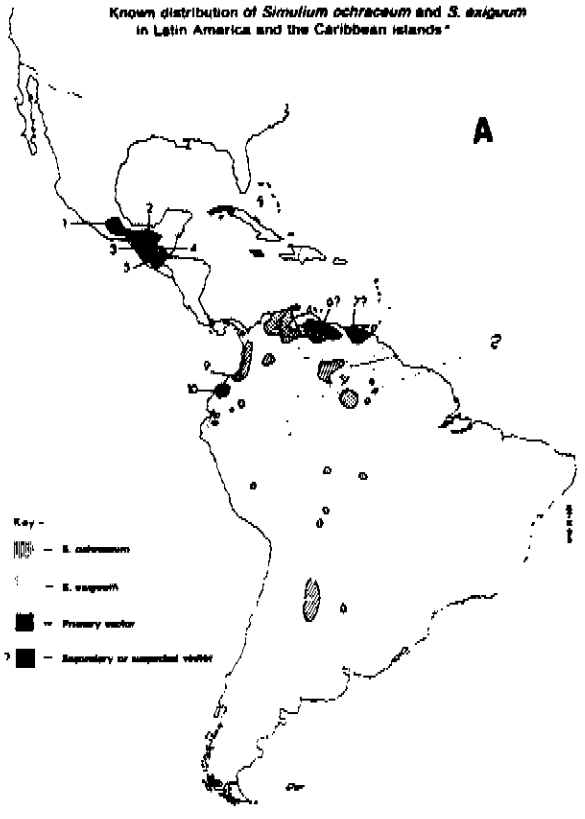
Simulium exiguum s.l. is widespread in South America, from Colombia and Venezuela in the north to Argentina and Bolivia in the south (Fig. 6.2.A), and occurs in all the foci of onchocerciasis. It is a species complex in Ecuador consisting of at least three cytotypes. It is the primary vector of onchocerciasis in the Santiago focus in Ecuador, the only vector in Colombia, and a presumed sporadic vector in the northern Venezuelan focus. In Ecuador, the high transmission potential is reflected in high natural infection rates and the rapid appearance of parasite transmission in new onchocerciasis foci resulting from the migration of infected Amerindians from the main focus of infection. S. exiguum s.l. may also act as a sporadic vector in lowland areas of the Brazil/Venezuela onchocerciasis focus.

S. exiguum s.l., like other Latin American vector species, ingests large numbers of microfilariae when feeding. This can cause the death of the fly, particularly in species that lack a cibarial armature (exiguum s.l., guianense, metallicum s.l.). S. exiguum s.l. bites man, but may also bite large domestic animals, and in some localities total zoophily occurs. Larvae and pupae of S. exiguum s.l. are typically found in the middle reaches of large rivers and their tributaries (wider than 5 m), attached to submerged vegetation in deeper parts of the rivers and in shallow water running over slate shingle beds.

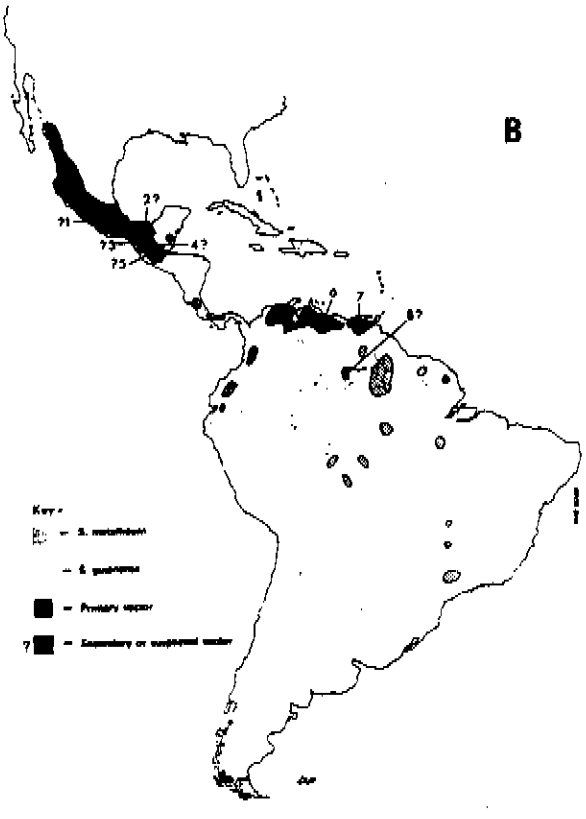
S. guianense has a wide distribution from southern Venezuela to southern Brazil and also occurs in the Guyanas (Fig. 6.2.B). It is a natural vector of O. volvulus in the Brazil/Venezuela onchocerciasis focus. Its predominance (with S. limbatum) in the mountainous (mainly hyperendemic) localities of the focus suggest that it is the primary vector. In the lowland localities because of its low anthropophily it is probably only a sporadic vector. Immature stages are typically found on emergent vegetation in large fast-flowing rivers particularly near waterfalls.

S. metallicum s.l. is commmon in Latin America, from the Andes as far south as Ecuador, to the northern coastal belt of Venezuela and some Caribbean islands (Fig. 6.2.B). Eleven cytospecies have been distinguished of which seven are currently accepted as sibling species. S. metallicum s.l. is the only proved vector of onchocerciasis in the northern Venezuelan foci of Altamira and Caripe. At low microfilarial intakes it is an efficient vector of O. volvulus, but its efficiency decreases when higher microfilarial intakes increase fly mortality. Larvae are found on submerged vegetation in small streams.

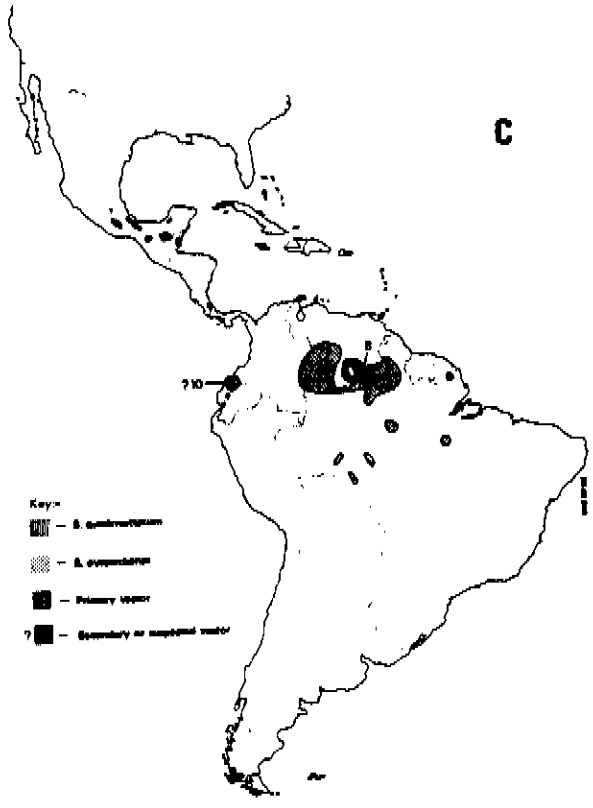
Known distribution of *Simulium ochraceum* and *S. exiguum* in Latin America and the Caribbean islands*



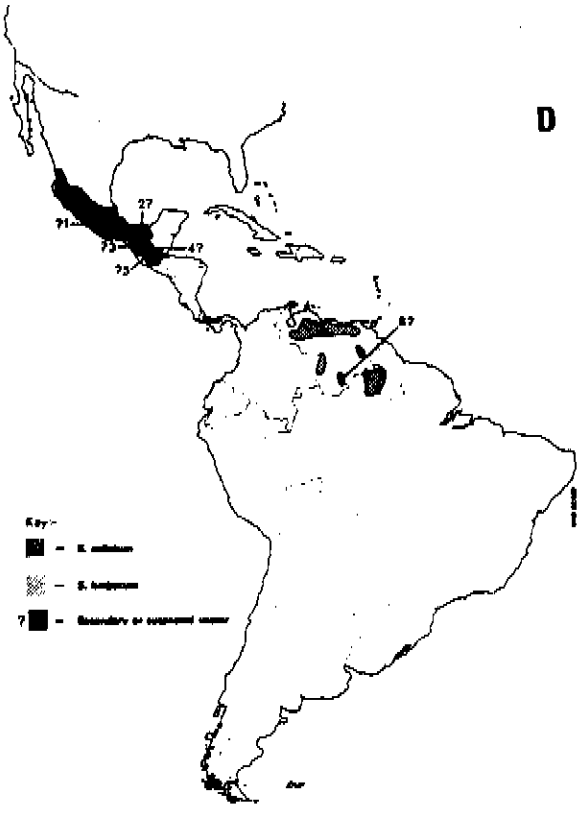
Known foci of *Simulium metallicum* and *S. guianense* in Latin America*



Known foci of *Simulium quadrivittatum* and *S. oyapockense* in Latin America and the Caribbean islands*



Known foci of *Simulium callidum* and *S. limbatum* in Latin America*



*Areas of proved or suspected transmission are shaded darker. Key to the onchocerciasis foci of Latin America: Mexico: Oaxaca (1); North Chiapas (2); South Chiapas (3), Guatemala: South Chiapas (3); Huehuetenango (4); Yepocapa (5); Venezuela: Altamira (6); Caripe (7). Amazonia (8); Brazil: Amazonia (8); Colombia: San Antonio (9); Ecuador: Esmeraldas (10).

S. oyapockense s.l. is one of the most common anthropophilic species of the lowland forests and savannas of the Amazon and Orinoco basins of Colombia, Brazil and Venezuela and is also present in the Guyanas (Fig. 6.2.C). Female S. roraimense are indistinguishable from S. oyapockense s.l. and, because of their sympatry in the Brazil/Venezuela onchocerciasis focus, references to S. oyapockense s.l. probably include S. roraimense as well. S. oyapockense s.l. is the only significant vector of onchocerciasis in the lowland forested areas of the Brazil/Venezuela focus. Biting occurs on all exposed parts of the body, with a slight preference being shown for areas above the waist.

S. quadrivittatum is a Central American species, and has only been recorded in South America from the coastal lowlands of Ecuador, west of the Andes (Fig. 6.2.C). It is a natural vector of O. volvulus in Ecuador where, because of its low biting rate, it plays a secondary role to S. exiguum s.l. at the end of the wet season. This species breeds in small shaded forest streams on submerged plants and debris.

Simulium limbatum is the only other species that is suspected of significantly contributing to transmission in the highland areas of the Brazil/Venezuela onchocerciasis focus. This species is commonly found throughout Venezuela, the Guyanas, and northern Brazil (Fig. 6.2.A). It is a suspected vector because of its high anthropophily in the focus.

VII. SURVEY TECHNIQUES

Surveys for Simulium provide base-line data on the geographical distribution and seasonal abundance of the vector species and are used to monitor the success of control measures. They are directed at both the adult and aquatic stages, firstly to determine when and where people may be at risk of contracting onchocerciasis, and secondly to determine when, where and how vector control may be attempted or how well it is succeeding. Surveys for aquatic stages also provide information on the other non-anthropophilic species in the rivers.

VII.1 Adult Simulium collections

(a) Methods

Because one objective is to assess the numbers of infective Onchocerca larvae being transmitted to man, and as no trap has yet been devised that is as efficient as human bait, there is still no alternative to human bait for estimating densities of biting adult Simulium and the numbers of infective larvae being transmitted to man.

S. damnosum are usually caught by one person seated on the ground or on a low stool with his legs bared below the knee. The flies coming to bite are caught alive before they have a chance to feed by the same person, who inverts a small vial over the resting fly. The disturbance caused by this act usually causes the fly to fly up inside the vial, which is then corked or plugged with cotton wool. Alternatively, a suction tube or "pooter"* may be used, provided it is remembered that when densities are high the pooter, being more efficient, will catch higher numbers than are caught using single vials. Also, flies crowded together in the same vial become over-active and do not survive as long as individually enclosed flies. Having many flies in the same vial makes counting more difficult.

In the Neotropics*, where the preferred biting sites of some vectors are on other parts of the body, the bait person usually sits with head, shoulders and legs bared while flies are caught by a colleague.

The choice of site must be determined by the habits of the vector species involved. Normally this is on the bank of a river, in the open and not densely shaded, and separated from crowded river crossings and washing places by at least 50 m. Cooking fires or smoking should not be permitted at the catching site.

The time and duration of the catch should be chosen to give as representative a sample of the fly population as possible. Because times of biting of parous and nulliparous flies may vary with season, temperature and sunshine, (Fig. 4.3) catches should ideally be made from dawn to dusk (or from 07h00 to 18h00 as in the Onchocerciasis Control Programme). Where biting is particularly intense, catches could be limited by catching for 15 to 20 min in each hour and the hourly total estimated proportionately. Because of the long catching day it is customary to employ two teams of collectors working alternate hours.

Flies are segregated and recorded at the catching site at the end of each hour, and the vials wrapped in damp cotton wool or cloth and conserved in an insulated container. Wet ice, if available, may be used for cooling, but the vials or the damp wrapping should not touch the ice. Times of heavy rain should also be noted. Other meteorological conditions such as temperature, humidity, cloud cover and wind speed may be recorded as required.

At the end of the day, all flies should be sent to a laboratory for dissection and recording. Occasionally it may be desirable to make dissections at the catching site, but care should be taken to ensure that the illumination provided for the dissecting microscope is adequate. Poor illumination can cause developing larvae of O. volvulus to be missed by the dissector. It may also lead to misidentification of morphologically similar species, and incorrect ageing of flies.

Traps to replace humans as bait have been extensively investigated, and have been reviewed by Service (1977, 1981, 1987). So far there have been no serious contenders. However, a useful trap for ovipositing female S. damnosum has been developed by Bellec (1976), and has proved able to catch flies (perhaps zoophilic) in areas of savanna West Africa where human bait caught nothing. A point to remember is that if fly catches are to be used to estimate transmission levels, then the use of human bait ensures that only anthropophilic flies are caught.

(b) Collection records

Every day that biting Simulium are caught (including days on which catches were attempted but no flies found), should be recorded separately. The data should include:

(i) date and number of hours worked;
(ii) name(s) of vector collectors;
(iii) name of the site (and code No. if extant);
(iv) number of flies caught each hour and their identity if more than one recognisable species is present; and
(v) observations on weather, especially rainfall.

It is useful to have a small card printed with a table including a line for each hour, which can be filled in by the vector collector as the day progresses.

(c) Dissections - live flies

As parous determinations can only be made on living flies, these should be dissected immediately on arrival at the laboratory. If this is not possible they should be held in a refrigerator at just above +4°C for not more than two days. If a refrigerator is not available they may be held in the insulated container overnight for dissection the next day. Flies should be kept at room temperatures for as short a period as possible because Onchocerca larvae will continue to develop to the infective stage, leading to inflated records of numbers of infective larvae.

Dissections should be made under a good quality binocular dissecting microscope at about 25-30x magnification and with a good constant light source. (A fibre-optic lamp with two lenses, allowing both incident and transmitted illumination without heat, is the best.)

Each fly is anaesthetized in its vial with chloroform vapour, or by drowning in saline and detergent, and quickly placed in a drop of normal saline on a slide. It should be checked for sex and species identification, and any cytospecific morphological characters, such as the colour of the hairs of the stem vein tuft (Secton X.10), noted. Using fine needles and forceps, the abdomen is slit down the ventral or lateral surface and the ovaries separated from the other viscera. The characteristics which are used to identify parous S. damnosum are given in Table 7.1. The presence of retained eggs is the most easily observed character and is a certain indicator of parity. To see the follicular relics it is necessary to stretch the ovary and disrupt the outer membrane so that the follicles separate. A fresh relic consists of a sac-like follicular epithelium which persists for a few hours after ovulation before shrinking to its normal tube-like shape (Lewis, 1960b, Fig. 3K, p.214). More commonly the relic consists only of granular matter lying just below the next developing follicle and is often very difficult to see (Fig.7.1).

When stretching the ovary, the experienced dissector will obtain a good indication of the age of the fly. Nulliparous ovaries are small, compact and clear, and, although elastic when slightly stretched, will tear when over-stretched. Parous ovaries never shrink to their original size after ovulation, and are therefore larger, flaccid, have lost their elasticity and may be slightly yellow, or opaque, or granular in appearance. Flies infected with Onchocerca larvae cannot be nulliparous, and those with mermithid* larvae are unlikely to be parous.

In practice, it will at times be necessary to form an opinion of the state of the fly using all the characters listed in Table 7.1, because no certain indicator is visible. All flies definitely classed as parous, as well as those of uncertain status, should be examined further for the presence of Onchocerca larvae. This is done by separating the head from the thorax, and teasing it out. Any third stage larvae will usually be readily visible, and will usually separate itself from the debris by slow movement. Sites in which they may remain hidden are in the mouthparts, palps and antennae.

Table 7.1

Characters used for age-grading S. damnosum s.l. females and probably also applicable to other species

Character	Nulliparous	Parous	Reliability
Follicular relicts	Never present	Present	Certain when present
Ovaries elasticity	Tight, elastic but disrupt if overstretched	Loose, easily stretched, do not regain original size	Good
appearance	Translucent	Granular, yellowish	Good
retained eggs	Never present	May be present	Certain when present
Spermatophore	May be found in 'early nullipars'	Never present	Certain when present
Fat body	Numerous large cells present	Reduced or absent	Unreliable unless abundant
Malpighian tubules	Dense, contents unbroken	Progressively clearing with age	Fair
Meconium*	May be found in 'early nullipars'	Never present	Certain when present
Mermis larvae	May be present	Very rare	95% certain if present

Expanded from Wenk (1981)

Once the head has been dissected, the thorax may be teased out separately. Care should be taken to split up the bundles of flight muscles until they are thin enough to be transparent, as 1st and 2nd stage larvae normally lie between and parallel to the muscle fibres and are not always sufficiently mobile to extricate themselves, unlike late 2nd and 3rd stage larvae. Also the blocks of muscle which adhere to the inside of the mesonotum* and to the bases of the legs must be broken up.

Apart from special studies, it will not normally be necessary to dissect more than 60 flies from each day's catch. Where the number of flies caught exceeds the number dissected, the dissection should be made on a proportion of each hour's catch. For days on which none, or only part, of the catch is dissected, records should cover the number of flies caught per hour, the total, and the number of hours worked for each day's catch. Where more than one vector species or species group is present, the data for each should be recorded separately. Representative samples of adults not used for dissection should be preserved dry on micro-pins and in 80% alcohol for later reference or dissection. As the state of knowledge of cytospecies advances it must be possible to refer back to early collections which can then be determined more accurately. Without reference collections of such 'voucher'* or reference specimens it would be impossible to refer back and identify pre-control populations, for example. (See Section X.9 for guidance on preserving reference specimens).

(d) Preserved flies

Flies that have been preserved in 70% or 80% ethanol can be dissected to determine whether they are carrying Onchocerca larvae by staining the entire fly before dissection using the haemalum staining technique (Section X.1).

(e) Dissection records

Good record keeping is of paramount importance. The larger the scheme, the more difficult it becomes to keep track of dissection and fly density data. A

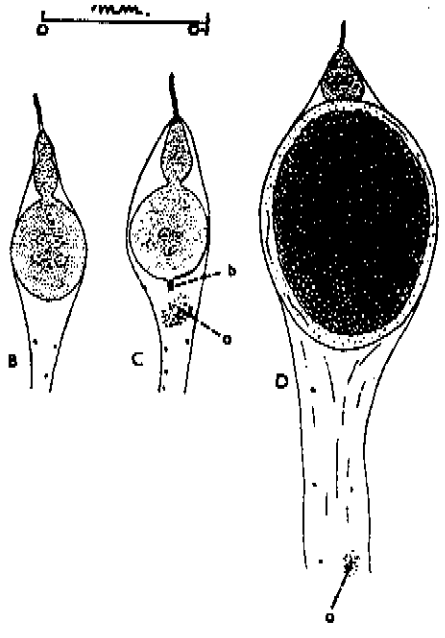


Figure 7.1

Ovarian follicles of S. damnosum s.l.
B - nulliparous ovariole;
C - parous ovariole;
D - parous ovariole at a late stage of development;
O - follicular relic;
b - funicle.

[From Lewis (1958); with permission of the Editors, Annals of Tropical Medicine and Parasitology.]

basic dissection record sheet is given in Fig. 7.2. This has been modified from the standard form in the Onchocerciasis Control Programme to make it usable for any species of Simulium. The most common mistakes stem from transcription errors, so the form is intended to be completed by the dissector, only essential data such as the totals being transcribed for accumulating monthly and annual data. All data are arranged so that the information on each dissected fly, and daily catches which are not dissected, can be recorded.

VII.2 Calculation of biting and transmission indices

The criteria of most value in planning and in assessing the progress of a control scheme are the Annual Biting Rate (ABR) and the Annual Transmission Potential (ATP). The basic criterion is the Monthly Biting Rate (MBR) which is the theoretical number of Simulium bites that would be received by a person who remained stationary at a catching site during almost all the hours of daylight for a complete month. The Annual Biting Rate is the sum of the twelve MBRs. The Monthly Transmission Potential (MTP) and the Annual Transmission Potential are the number of infective 3rd stage larvae indistinguishable from O. volvulus that would be received by the same person. Some workers have based their calculations of ATP on the total number of infective larvae in each fly, regardless of location. In the Onchocerciasis Control Programme, however, the calculation is based on the number of larvae found in the head capsule only, because with a large number of dissectors, working under differing conditions, the use of head larvae gives the most consistent results; also the number of larvae in the head approximates to the number of larvae voided in a bite. There is no reason, however, to prevent other workers from using all larvae (it has the advantage of providing slightly higher numbers which are statistically more acceptable when biting densities are low) provided that the basis of the calculation is made clear. Ideally both criteria should be calculated.

The formulae for ABRs and ATPs are given below and should be calculated separately for each catching site.

$$\text{MBR} = \frac{\text{No. of flies caught} \times \text{No. of days in month}}{\text{No. of catching days}}$$

MBRs may be averaged for groups of sites if required, but should not be added.

$$\text{MTP (H)} = \frac{\text{MBR} \times \text{no. of 3rd stage larvae in the head}}{\text{No. of flies dissected}}$$

$$\text{MTP (A)} = \frac{\text{MBR} \times \text{no. of all 3rd stage larvae}}{\text{No. of flies dissected}}$$

The above are the most commonly quoted criteria. It should be stressed that ABR and ATP are indices which are useful for comparative purposes only. They are not estimates.

Other criteria which may be of value from time to time are:-

The Monthly Infective Biting Rate (MIBR)

$$\text{MIBR (H)} = \frac{\text{No. of flies with 3rd stage larvae in head x days in month}}{\text{No. of catching days}}$$

for MIBR (A) use number of flies with third stage larvae anywhere, in the head, thorax or abdomen.

Since only parous flies can transmit onchocerciasis, the monthly parous biting rate (MPBR) is becoming more widely used, particularly close to large breeding sites, where a large proportion of the biting flies can be expected to be newly emerged nullipars, or in areas of reinvasion where the proportion of parous flies can be unusually high.

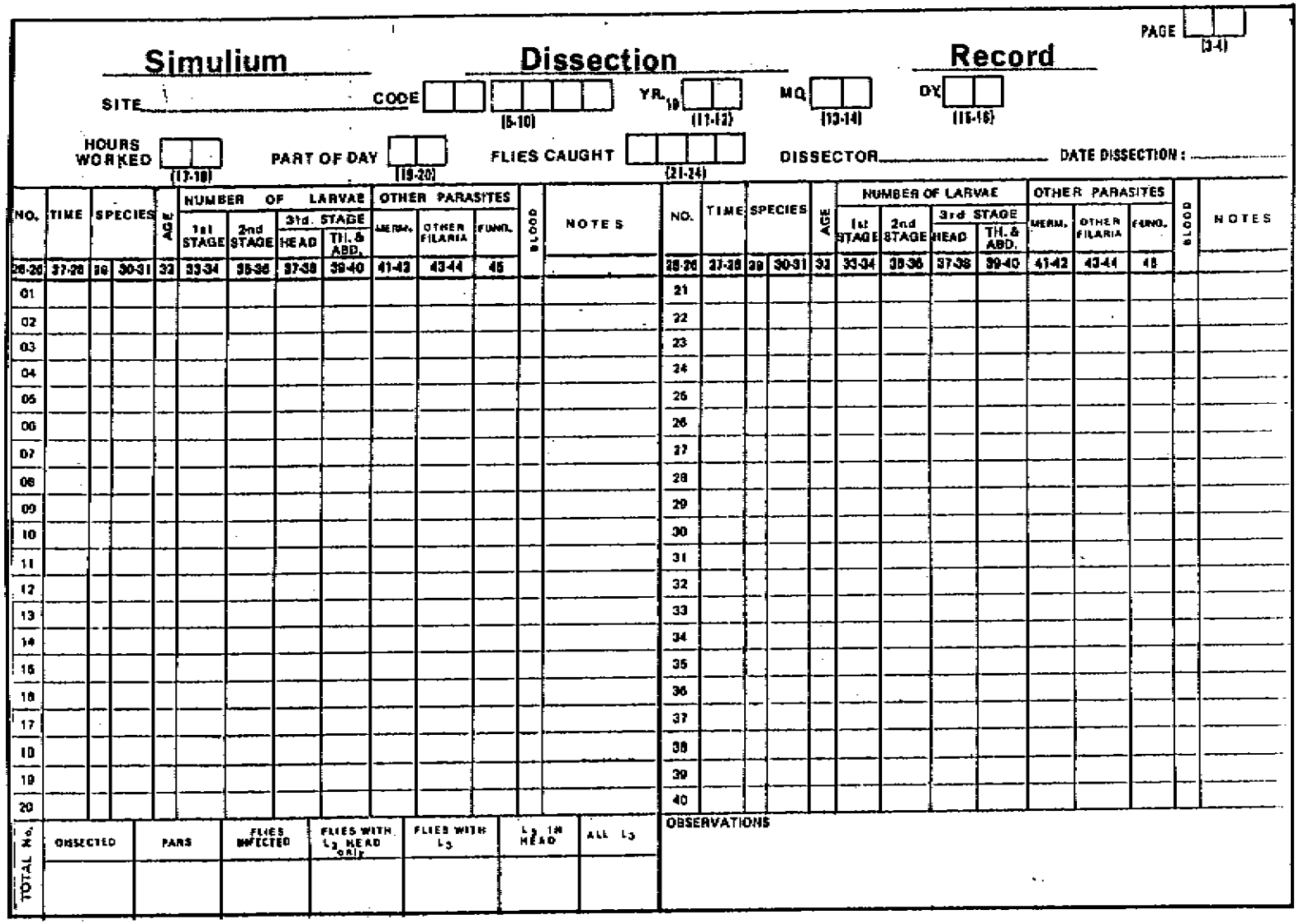
$$\text{MPBR} = \frac{\text{No. of parous flies x days in month}}{\text{No. of catching days}}$$

MIBR (H), MIBR (A) and MPBR can be summed to give the equivalent annual criteria.

Another useful ratio is the number of infective larvae per 1000 parous flies.

$$L_3/1000 \text{ pars} = \frac{\text{No. of } L_3 \text{ larvae recovered from the sample x 1000}}{\text{No. of parous flies in the sample}}$$

This gives an indication of the efficiency of the transmission under the conditions in which the sample of flies was collected and is not affected by the number of nullipars in the population.



Simulium Dissection Record

PAGE [ ][ ] (3-4)

SITE _____ CODE [ ][ ][ ][ ] (5-10) YR 19[ ][ ] (11-12) MO [ ][ ] (13-14) DY [ ][ ] (15-16)

HOURS WORKED [ ][ ] (17-18) PART OF DAY [ ][ ] (19-20) FLIES CAUGHT [ ][ ][ ][ ] (21-24) DISSECTOR _____ DATE DISSECTION : _____

NO.	TIME	SPECIES		AGE	NUMBER OF LARVAE				OTHER PARASITES			BLOOD	NOTES
					1st STAGE	2nd STAGE	3rd STAGE HEAD	3rd STAGE TH. & ABD.	MERM.	OTHER FILARIA	FUNG.		
25-26	27-28	29	30-31	32	33-34	35-36	37-38	39-40	41-42	43-44	45		
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NO.	TIME	SPECIES		AGE	NUMBER OF LARVAE				OTHER PARASITES			BLOOD	NOTES
					1st STAGE	2nd STAGE	3rd STAGE HEAD	3rd STAGE TH. & ABD.	MERM.	OTHER FILARIA	FUNG.		
25-26	27-28	29	30-31	32	33-34	35-36	37-38	39-40	41-42	43-44	45		
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35													
36													
37													
38													
39													
40													

OBSERVATIONS

TOTAL No.	DISSECTED	PARS	FLIES INFECTED	FLIES WITH L_3 HEAD only	FLIES WITH L_3	L_3 IN HEAD	ALL L_3

SEE CORRIGENDUM

Figure 7.2 A sample form for recording the results of Simulium catches and dissections at one site for one day. Data are recorded boxes or columns to facilitate transfer to a computer data base if required. All necessary information about each fly occupies hal the totals required to calculate monthly transmission criteria appear at the bottom left.

(Courtesy of the Director, Onchocerciasis Control Programme.)

VII.3 Surveys for aquatic stages

In the pre-control baseline data collection, situation surveys for aquatic stages (also known as larval surveys or prospections) are the only way to map the distribution of the breeding sites of the vector species. Because the distribution may change with the climate, particularly rainfall, it is usually necessary to carry out such surveys between two and four times in the year. In very large control schemes such as the Onchocerciasis Control Programme, surveys may be simplified by visiting the suspect sites by helicopter, but most projects will have to rely on ground transport by four-wheel drive vehicles and foot. It is most important that all breeding sites in the area are identified, as their location must be known before the control operation is planned.

The breeding sites of S. damnosum s.l. can usually be identified by the presence of "white water" in the rapids of larger rivers, but in the wet season breeding may extend into swollen smaller tributaries. In mountainous areas of East and Central Africa potential vector species of the complex may be found in fast flowing small streams only a metre or so in width which may be covered in vegetation, a situation that is similar to the sites of the vectors in Guatemala and Mexico. In forest areas, breeding sites, even whole rivers, may be obscured from the air by the forest canopy.

The favoured substrates for each species should be carefully examined. In all but the smallest streams this requires searchers to wade in the river or use boats to reach inaccessible rocks and islands. Simulium larvae or pupae should be removed from sticks, leaves, small stones or other substrates, and kept damp in a plastic bag which is labelled with the site, date and time. All free water in the bag should be drained off, and the bag and its contents kept cool until examination in the laboratory. If a laboratory is not within easy access, specimens may be picked off the substrate with fine forceps at the river bank and placed in 80% alcohol or Carnoy's fixative (Section X.3) for later examination. In many cases, some larvae and pupae can be immediately identified by an experienced operator, using a hand lens.

The results of all surveys must be recorded on a survey sheet. Wherever possible all the species found should be identified using published keys, and their abundance noted using a five-point scale of - to +++ corresponding to absent, scarce, few, common, heavy. Because of the frequent difficulty in finding the aquatic stages and their patchy distribution in the river, numerical counts are difficult to standardize and may be misleading. Negative sites must be recorded with equal diligence. The positions of identified breeding sites should be marked on maps, with a separate map for each season. In this way the distribution of the sites, and their seasonal variation can be easily seen. A technique for rearing adult flies from mature pupae can be found in Section X.2

In the case of the S. neavei group, it will be necessary to obtain information on the distribution and abundance of the species of crabs to which the larvae and pupae of this group are attached (see Section X.5).

VIII. CONTROL OF ONCHOCERCIASIS VECTORS

VIII.1 Considerations

The following facts must be considered before deciding how best to control onchocerciasis.

(i) The adult female parasite lives in the human host for 10 to 15 years;
(ii) The adult female O. volvulus is found deep in the tissues as well as in surface nodules;
(iii) Many infective bites are usually required to establish an infection;
(iv) There is at present no practical macrofilaricide*;
(v) The microfilaricides*, DEC and ivermectin do not kill the adult worms;
Killed microfilariae (mf) are gradually replaced by new mf. over 6-18 months;
DEC is contra-indicated in heavy infections;
Some treated people may still be able to infect flies;
(vi) The vector is found in easily identified breeding sites (in Africa at least);
(vii) The vector larvae are sedentary filter feeders and very susceptible to insecticides; and
(viii) There is no common animal reservoir.

As nodulectomy* and chemotherapy still only provide a partial solution, the most effective means of controlling the disease at present is by breaking the transmission cycle by vector control, combined with use of the microfilariacide, ivermectin.

VIII.2 Options for vector control

(a) Physical separation of man and fly

(i) Siting communities away from high vector densities is not much employed in Africa because one objective of onchocerciasis control is to encourage repopulation of deserted river valleys. However, siting a village a kilometre away from a badly infested river in the West African savanna could reduce the level of transmission in the community by about 90%, if alternative sources of water could be provided, although high risk occupations such as fishing and boating would not be affected.

(ii) Personal preventive measures. Simuliids cannot bite through most clothing (although they will penetrate thin cloth if it is tightly stretched against the skin). Where vectors bite low down, wearing trousers, socks and shoes will protect adequately. However, clothing is expensive in many areas and uncomfortable to work in in the humid tropics, so this measure is probably more applicable to visitors than residents.

(b) Environmental manipulation

(i) Vegetation removal. Felling the riverine forest was used in the small Riana focus of Kenya in 1950 to eradicate S. neavei by removing its preferred shady habitat. However, for environmental reasons, this method cannot be

recommended. Breeding of S. damnosum s.l. was considerably reduced in the southern Sudan by removal of weed and vegetation from a short length of river, but a similar attempt against S. ochraceum s.l. in Mexico was unsuccessful. Deforestation may encourage the spread of S. damnosum s.l. (and possibly S. ochraceum s.l.) by providing the open habitat that they prefer.

(ii) Dams and impoundments. Flooding river valleys and rapids by the construction of large hydroelectric dams can eliminate many kilometres of breeding. But conversely, breeding may be encouraged on spillways and enhanced downstream by stabilizing the river flow over most of the year. Insecticides can be efficiently introduced into the dam outflow, however, and on smaller dams downstream breeding could be controlled by shutting off the flow for one day each week. The construction of small earth dams for irrigation purposes in arid areas may serve to extend the range of S. damnosum s.l. by providing new breeding sites on and below the spillways (e.g. in northern Burkina Faso and parts of Mali).

(iii) Debris and obstructions. Broken and disused bridges and causeways can provide breeding places in otherwise unsuitable terrain by forming small rapids. If possible they should be repaired or removed.

(c) Biological control

(i) Parasites and predators. So far, no promising candidate parasites or predators have been found, partly due to our inability to maintain laboratory colonies on which they could be tested. The mermithid worm Isomermis lairdi can be found naturally in over 50% of some adult S. damnosum s.l. populations, and is lethal, but even so appears to have little regulatory effect.

(ii) Genetic control has not been attempted due to lack of laboratory colonies on which to experiment. The possibility of replacing efficient vector species with less efficient or non-vector species has been considered but no suitable candidates have been found.

(d) Chemical control

Population reduction by insecticides directed at the larval stage has been the only method attempted on any large scale so far.

(i) Larvicides are the chosen means of vector control in most of the 50 or more schemes that have been initiated since 1950. Details of techniques, and insecticides are given below.

(ii) Adulticides. The use of these is limited by the long flight range of some species and by lack of knowledge of adult behaviour, particularly resting sites. Aerial fogging with HCH* was used successfully on the River Zaire at Kinshasa for many years from 1948, and deltamethrin, applied aerially as an ultra-low-volume fog for tsetse control, reduced S. damnosum s.l. in an experimental trial in Burkina Faso. However, accidental killing of fish by minute quantities of insecticide falling on the water surface was unacceptably high.

(e) Integrated control

This has not yet been attempted as a primary technique in Simulium control, but the possibility of combining the selective use of insecticides with environmental management, chemotherapy and community cooperation is becoming increasingly attractive. It is probably the only approach to maintaining disease-free areas after the parasite has been eliminated by long-term vector control or mass chemotherapy.

VIII.3 Insecticides and formulations

The formulations of the insecticides that are used for large-scale campaigns have to satisfy a wide range of requirements. They must be highly effective against the vectors, but must be safe for the rest of the environment. Each formulation must be specially devised for blackfly control, the supply of insecticide must be guaranteed over a long period of time, and the cost kept as low as possible. Biodegradable constituents are required, but there must also be a maximum carry downstream from the point of application. In addition, since the vectors are under constant insecticide pressure in very extended control zones, alternative larvicides must be available, preferably belonging to different chemical classes, so that any resistance to one or more compounds can be dealt with promptly. Some measure of resistance is sure to occur sooner or later, and susceptibility levels to all likely insecticides must be determined before treatments begin, and whenever an increase in tolerance is suspected. The techniques are outlined in Section X.6.

(a) Insecticides currently in use

Temephos. After the first control campaigns that used organochlorines, in particular DDT, the organophosphorus compound temephos (ABATE®) became the preferred larvicide. A 20% emulsifiable concentrate of temephos has been specially developed for Simulium control and has been widely used in Africa, especially in the Onchocerciasis Control Programme (O.C.P.) area. Experience has shown that formulation plays an important part in the efficiency and selectivity of the active ingredient, so each formulation has to be carefully adapted to suit different ecological conditions. In West Africa, temephos is applied at a dosage of 0.05 mg/l (or 0.05 ppm) for 10 min in the wet season and at 0.1 mg/l in the dry season. During the wet season the carry of the formulation in large rivers is 20-40 km downstream from the application point. When the water-levels are low, the carry is reduced and the fewer breeding sites have to be treated individually. Temephos is considered to act largely as a stomach poison.

In Guatemala, slow-release, solid formulations and water dispersible powders of temephos have been used against S. ochraceum s.l. (Takaoka et al. 1981).

Chlorphoxim. Following the local development of resistance to temephos by some members of the S. damnosum complex in West Africa and Cameroon, a 20% emulsifiable concentrate of the organophosphorus compound chlorophoxim was chosen as an alternative. Although this formulation is less selective than temephos, it has proved to be operationally acceptable when used infrequently. It is suspected of acting more as a contact insecticide than a stomach poison.

Bacillus thuringiensis serotype H-14. This control agent, which is specific for Diptera, has very little impact on non-target fauna. It is in operational use by the O.C.P., although formulations are not yet ideal because

of the high dosages required. Nevertheless substantial improvements in formulation have already been achieved and more are in prospect. It is supplied as a peanut butter-like soft paste of bacterial residue which needs to be mixed with water before application. The larvae ingest these particles and the toxins destroy the stomach lining.

Permethrin and carbosulfan. Permethrin (pyrethroid) and carbosulfan (carbamate) are both in operational use by the O.C.P., although they are less selective as regards the non-target invertebrate fauna than the organophosphates discussed above.

Insect growth regulators. Another group of compounds that seem to be promising for the control of onchocerciasis vectors is the insect growth regulators, that inhibit the production of chitin. Small-scale field trials have been already carried out with such agents and some of them have been found to be effective against blackfly larvae.

(b) Mode of action

It is most probable that the lower carry of insecticide at slower water velocities is due to adsorption of the insecticide onto silt and other particles in the water and on the riverbanks and vegetation. This is demonstrated in Fig. 8.1, which shows the difference in recovery rates of temephos after contact with fine and coarse sands at two water velocities. Although initial loss was about equal for both sands, the coarser sands became more saturated and retained less insecticide as the volume of treated water increased, in contrast with fine sands which had a much greater capacity for adsorbing the insecticide. The degree of adsorption varies greatly between insecticides, and may explain the reduced selection of the pyrethroids and carbamates mentioned above.

VIII.4 Principles of larviciding

Larviciding relies on the river to carry the insecticide to the larvae. Thus when rivers are flowing well, a single application may kill all Simulium s.l. larvae over 20-40km as the section of treated water passes downstream. It is generally accepted that DDT and temephos act primarily as stomach poisons and it is probable that these insecticides become adsorbed onto particles in the water which are then filtered out by the larvae and eaten. In this way, although the concentration of insecticide may be very low, small doses are accumulated by the larva until a lethal level is reached. This selective feeding habit may explain why some non-target Simulium species survive temephos treatments that are lethal to S. damnosum s.l.

The general principles of larviciding as applied against S. damnosum s.l. are shown in Fig. 8.2, where a hypothetical system of three rivers (R_1 - R_3) flow into a lake formed by a dam.

In the wet season when water discharges and velocity are high, S. damnosum s.l. breeds in the rapids b_1 to b_5. Sites b_2 and b_4 can be treated by insecticide applied at the road bridges at T_1 and T_2, but because no insecticide can traverse the lake, the large site at b_5 must be treated from the dam T_3, where the sluices make an ideal point of access. Because b_1 is upstream of the road a special track will have to be cut to T_4. The combined effect of treatments at T_2 and T_4 will be sufficient to eliminate b_3.

SEE CORRIGENDUM

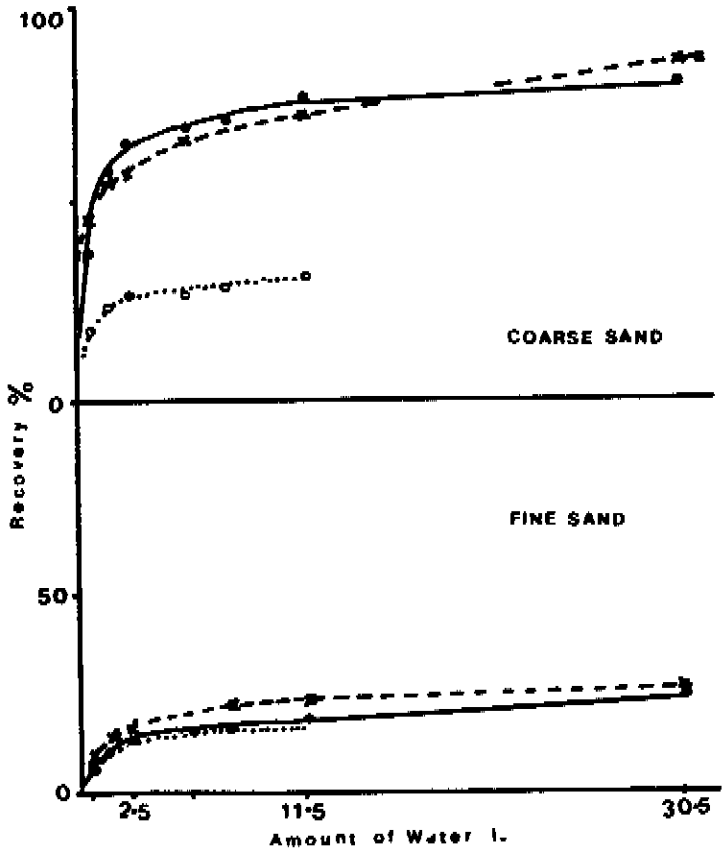

Figure 8.1 Cumulative percentage recovery of temephos in water after being passed through columns packed with coarse or find sand.

o____o 5% wdw quick flow.
x----o 50% wdp quick flow.
o....o 5% wdp slow flow.

[After Suzaki, T (1983)]

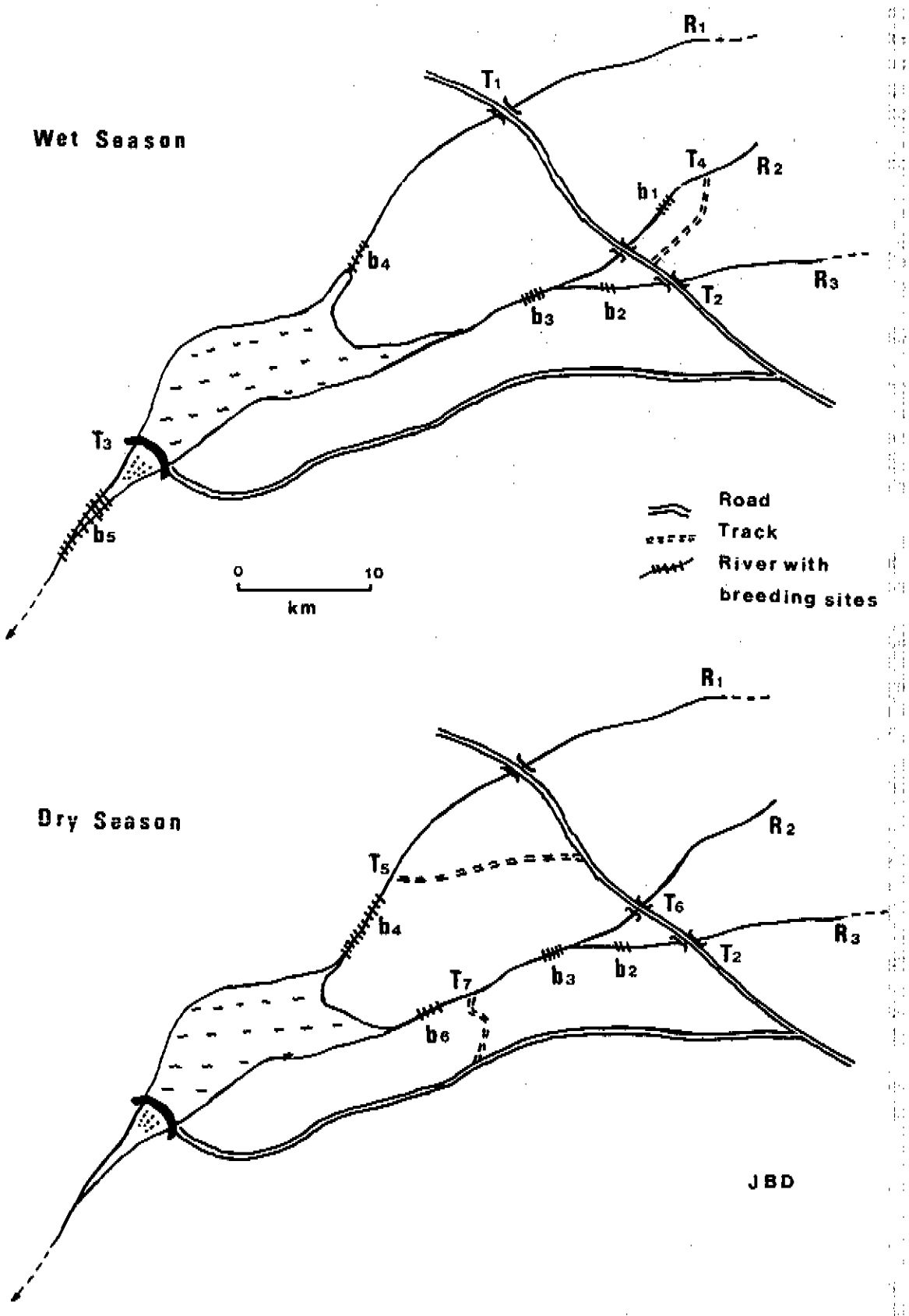

Figure 8.2 A hypothetical system of three rivers (R_1, R_2 and R_3) feeding into a lake formed by a dam at T_3 and showing the changes in S. damnosum s.l. breeding sites (b_1-b_6) according to season. (For further explanation see text.)

In the dry season, when discharges are low and the dam sluices are closed to conserve water, breeding sites b_1 and b_5 no longer function. However, due to rocks becoming exposed by falling water levels in the lake, a new site b_6 has appeared and b_4 has increased in length, replacing b_5 as the major source of flies. Sites b_3 and b_2 can still be treated from the road bridges, but, because of intervening stretches of still water, special access tracks are required to treat b_4 and b_6 at T_5 and T_7.

This example illustrates the dynamic nature of larviciding where constantly changing river profiles require a flexible treatment strategy. When insecticides are applied by air, as in O.C.P., some access roads may still be required for monitoring and surveillance purposes.

In Central America, where S. ochraceum s.l. breeds along the whole length of very small streams whose discharge may be less than 3 litres/sec, seasonal variations tend to determine the number of streams that are flowing, rather than the distribution of breeding sites along them. The carry of insecticide along these streams is measured in metres rather than kilometres, and here slow release formulations such as briquettes* have been found to be the most practical means of application. They may need to be placed every 20 to 50 metres.

The methods of measuring river discharge and calculating dosages are discussed in Section X.7.

Frequency of larviciding is determined by the length of the larval development cycle, as the insecticides currently in use are not persistent and do not kill either eggs or pupae. Consequently, following each application there remains in the river a stock of eggs from which the larval population can be immediately replenished. In practice, it is advisable to treat at one or two days less than the larval duration to allow some logistic flexibility. It is also easier to organize treatment cycles based on multiples of a week.

The length of the larval stages has been discussed in Sections III.1 and IV.1. In West Africa, against S. damnosum s.l. with normal development times of 7-12 days, treatments are usually made at weekly intervals. Other vectors such as S. ochraceum s.l. and S. metallicum s.l. could possibly be treated at 10 or 14 day intervals depending on the ambient temperature and altitude, while the S. neavei group have the potential for even less frequent applications. There are definite financial, ecological and logistic advantages to extending the treatment cycles as long as possible, as less staff and equipment are required, and less insecticide is used in fewer applications per year.

Where control is to be achieved by environmental means, such as interrupting the discharge from dam sluices, all aquatic stages would be killed, so the interval between successive interventions could be longer. For example, interrupting the flow for one day in ten would probably be sufficient to kill each generation of S. damnosum s.l.

VIII.5 Brief review of control programmes

About 50 onchocerciasis vector control schemes have been attempted since 1950. With few exceptions they were not successful, because the areas under control were too small or the projects were not sustained for long enough to have much impact on the disease. However, a few projects have been extremely successful and, in some cases, have even led to the eradication of the vector.

Eradication of S. neavei has been achieved in five schemes, four in Kenya and one in Uganda. Success was probably due to the long life-cycle, the very specialized larval habitat of this species and the relatively small and isolated nature of the foci.

On the Victoria Nile at Jinja, Uganda and on the Congo river at Kinshasa (then Leopoldville) and Inga, Zaire, control campaigns using DDT were strikingly successful. The precise reasons for these lasting successes remain unclear.

In West and Central Africa several control projects were carried out from 1955 in Burkina Faso, Chad, Côte d'Ivoire, Ghana, Mali, Nigeria and Sierra Leone. The tactics and strategy developed from some of these projects permitted the successful launching of the Onchocerciasis Control Programme in the Volta River Basin Area which is described in Section IX.

A vector control project was started in 1979 in the small focus of San Vicente Pacaya, Guatemala. Initially, temephos in a solid, slow-release formulation was applied every two weeks. From 1982 this procedure was changed to fixed dosing with 24 g of 5% water-dispersible powder in every 50-100 m stretch of breeding stream, irrespective of water discharge. The area under control was gradually increased, eventually covering about 90 km^2. Thanks to very careful planning and dedicated field-work this control strategy has resulted in epidemiologically significant reductions in biting rates.

VIII.6 Evaluation

In the first few years of control the only indicators of success will be reductions in vector density and the number of Onchocerca larvae of all stages carried by them. Techniques to monitor these are given in Section VII. However, the final assessment of the success of vector control must be to measure the impact on the incidence of O. volvulus infection. Information on the incidence of infection comes from epidemiological evaluation, the objectives of which are to study the regression of the reservoir of infection in man and to assess the decrease in the incidence of severe ocular pathology. This is beyond the scope of this Manual.

IX. THE ONCHOCERCIASIS CONTROL PROGRAMME IN WEST AFRICA

No account of the biology and control of the vectors of onchocerciasis can be complete without an overall description of the Onchocerciasis Control Programme in West Africa (OCP) because the techniques of control and evaluation are based on the knowledge of Simulium bionomics described earlier in this Manual and serve to illustrate how this knowledge is applied in a practical way.

The following account is adapted from the 3rd Report of the WHO Expert Committee on Onchocerciasis 1987, with some later updates and amendments.

IX.1 General description

The Onchocerciasis Control Programme in West Africa (OCP) is currently operating in Benin, Burkina Faso, Côte d'Ivoire, Ghana, Guinea, Guinea-Bissau, Mali, Niger, Senegal, Sierra Leone and Togo, all countries that had extensive areas of hyperendemic savanna onchocerciasis when field activities began in

1974 (Fig. 9.1). The programme is a corporate undertaking of 11 participating countries in West Africa, the Control Programme donor community (countries, foundation, and development banks) and four United Nations agencies (UNDP, FAO, the World Bank, and WHO) which together make up its governing body, the Joint Programme Committee (JPC). WHO is the executing agency with the World Bank as the financial trustee; scientific and technical advice is provided by an independent Expert Advisory Committee (EAC), and a Committee of the Sponsoring Agencies (CSA) provides managerial support.

The objective is to eliminate onchocerciasis as a disease of public health importance, and as an obstacle to socioeconomic development, throughout the Programme area, and to ensure that the Participating Countries are in a position to maintain this achievement, thus allowing the repopulation and development of those valleys previously almost deserted because of onchocerciasis.

The control strategy of the Programme is to interrupt the transmission of O. volvulus by the weekly application of rapidly biodegradable larvicides delivered by aircraft to all breeding sites of the savanna species of the S. damnosum complex; to carry this out over a sufficiently large area so as to counteract reinvasion by flies from outside; and to continue until such time as the human reservoir of microfilariae is eliminated. The results of the vector control are monitored by measuring the annual biting rates and annual transmission potentials, and in terms of changes in the intensity, prevalence, and morbidity of onchocerciasis as assessed by epidemiological evaluation of sample populations.

The rationale behind the control strategy is the belief that there are different forms of O. volvulus transmitted by different members of the S. damnosum vector complex, with only the savanna form being of real importance in blinding onchocerciasis. On this basis, control is targeted against S. damnosum s.s., S. sirbanum, and S. squamosum, as well as against the S. sanctipauli subcomplex in areas where it is associated with the savanna species, or where there are special epidemiological considerations, e.g. S. soubrense B transmitting blinding onchocerciasis in southern Sierra Leone.

Control activities have continued without interruption since February 1975 and including the 1985 expansion to southern and western extension areas, now covers an area of 1 325 000 km^2. Almost all the larvicide has been applied to breeding sites from helicopters and fixed-wing aircraft. During the wet season a fleet of ten helicopters and two fixed-wing aircraft operates over 23 000 km of rivers. Temephos is at present the most cost effective larvicide, and is environmentally safe. Following the development of resistance to this insecticide in some parts of the area, first chlorphoxim and then Bacillus thuringiensis H-14 were introduced at certain seasons on some rivers. Bacillus thuringiensis H-14 is the least damaging of all.

To evaluate the efficiency of the control measures, a network of fly catching points is visited at weekly or fortnightly intervals by teams of two vector collectors, who work alternate hours from 07h00 to 18h00. Most of the catch is dissected and the effectiveness of control is judged by comparing daily, monthly and annual biting rates and transmission potentials.

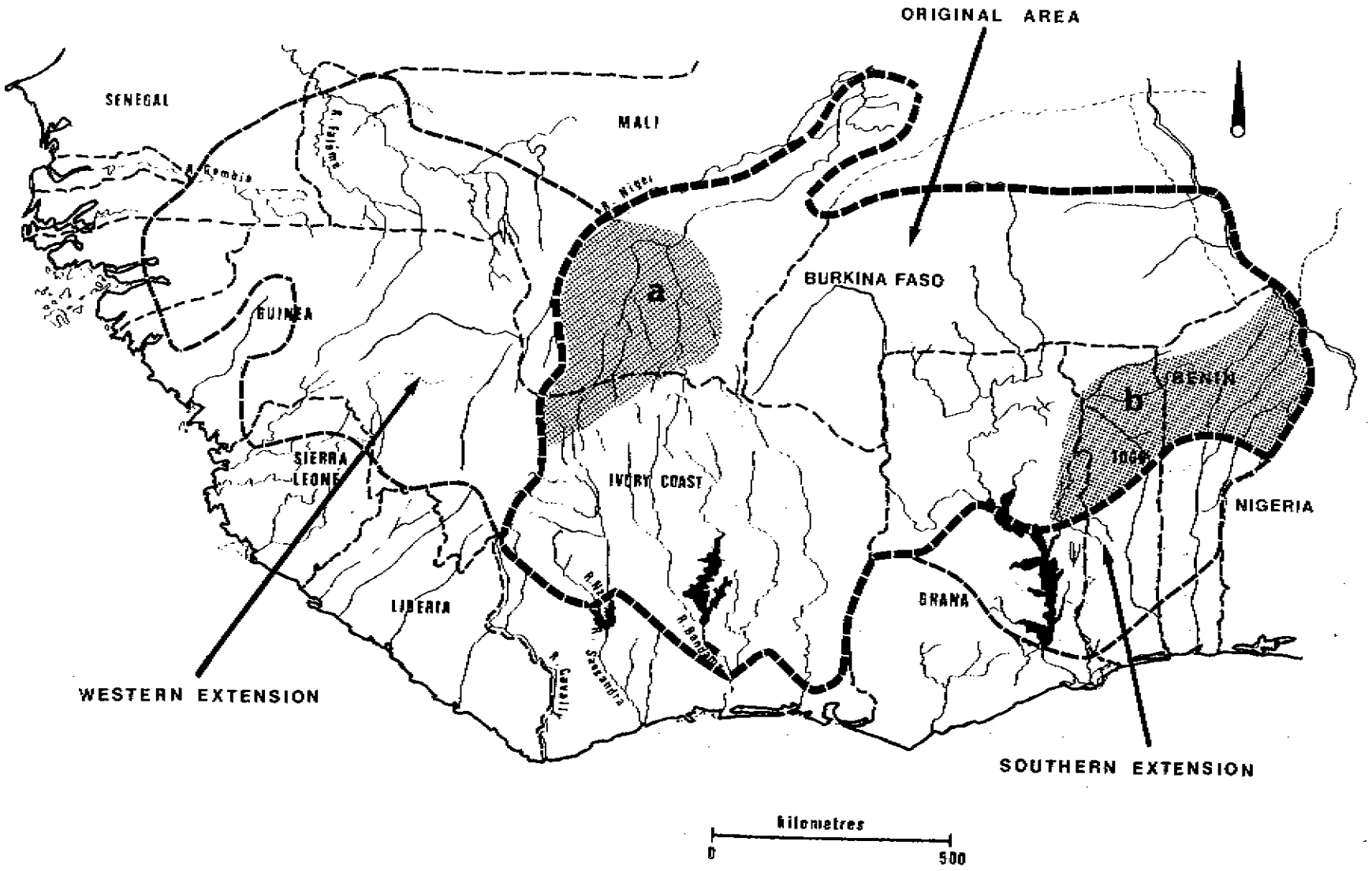

Figure 9.1 Outline map of the Onchocerciasis Control Programme in West Africa showing the original area and the two main extensions. The shaded areas 'a' and 'b' indicate the main areas that were reinvaded in 1985/86.

The programme is also active in the field of socioeconomic evaluation in onchocherciasis controlled zones, in manpower training, and in a wide range of fundamental and applied research aimed at solving operational problems

IX.2 Problems encountered

The Simulium damnosum reinvasion phenomenon

It has long been known that S. damnosum s.l. is capable of travelling long distances. With this in mind, the boundaries of the original Programme were set so that a large central area would be protected. When, in 1975, the Phase I area of the OCP (Fig. 9.1) was brought under control, the full extent of the migratory abilities of S. damnosum s.l. became apparent. Initially, throughout the whole area in which breeding sites were treated there was a spectacular reduction in the number of biting flies. However, this success was short-lived. At the end of the dry season (March and April) of that year biting rates again rose considerably in large parts of the area, particularly in those river valleys located in the southwestern sector, and they stayed high until the end of the rainy season. It soon became evident that the flies were immigrants originating from breeding places outside the treated zone. The same phenomenon recurred every year and is referred to as "reinvasion". To better understand the threat posed by reinvasion, the OCP made an exhaustive study of the phenomenon.

Extensive study showed that the majority of invading flies belonged to the two "savanna" cytospecies S. damnosum s.s. and S. sirbanum; and practically all of those caught deep inside the area were parous. There was also evidence that the proportion of old parous flies in the biting population rose with increasing distance from the boundary of the control area. Many of the invading flies carried infective larvae indistinguishable from O.volvulus.

Because of the concentration of fly populations in certain areas it was thought that reinvasion was wind-supported and governed by the movements of the Inter-Tropical Convergence Zone (ITCZ)*. The main direction of movement of migrating flies was from the south-west, the same direction as the prevailing monsoon winds.

The origin of the flies invading the western parts of the OCP area therefore had to be in regions to the south and west of that area where S. damnosum s.s. and S. sirbanum were breeding. In 1979, the Control Programme was extended into southern Côte d'Ivoire to include all areas where the savanna cytospecies were known to breed, reinvasion was considerably reduced. It was then clearly demonstrated that by extending the Programme area and treating the appropriate breeding sources, the problem of reinvasion could be solved, even though the distances covered by the invading flies were more than 400 km from their presumed points of departure.

The areas that are still reinvaded annually are located in the western part of Mali ((a) in Fig. 9.1) and in the northern part of Togo and Benin (b). For the reinvasion in the west, the sources of migration are suspected to be in Guinea and Sierra Leone. In the east, the sources are thought to be in the southern extension area and possibly also in Nigeria. In Togo the migrating flies include S. squamosum, a species that breeds profusely in the mountainous country along the Togo-Ghana boundary.

In the original Onchocerciasis Control Programme area of 654 000 km^2, about 247 000 km^2 located in the north and north-east are completely without flowing water for at least four months a year, but the long-range migratory behaviour of the savanna cytospecies, S. damnosum s.s. and S. sirbanum, enables them each year to recolonize large areas that are without permanent breeding sites.

There is good evidence that at least one member of the S. sanctipauli subcomplex has a tendency to extend it range northwards in the rainy season, but it does not make long-range movements. The species that has the least tendency to migrate, S. yahense, is rarely found more than a few kilometres from its breeding sites.

Insecticide resistance

From the beginning of larviciding early in 1975 until 1980, temephos was the only insecticide used in the Programme area. In 1978/1979, larvicide treatments were extended further south in Côte d'Ivoire, and included breeding sites of the S. sanctipauli subcomplex. This was followed in 1980 by a series of treatment failures on the lower Bandama river, which was shown to be due to resistance to temephos and was found to be limited to the S. sanctipauli subcomplex. The resistance spread rapidly to include all the previously known distribution of the subcomplex in the treated part of Côte d'Ivoire, southern Burkina Faso (mid 1981), and subsequently into western Ghana (early 1982), eastern Mali, and eastern Guinea (early 1986).

The first response to this resistance was the use of another organophosphate, chlorphoxim, which was at that time the only operationally tested alternative. In less than one year, the same vector population developed full resistance to this compound as well. Chlorphoxim was then replaced by B. thuringiensis H-14. In 1982, it was found that the chlorphoxim-resistant population had reverted to normal susceptibility some months after the withdrawal of the insecticide. This allowed year-round control of the S. sanctipauli subcomplex by alternating the use of B. thuringiensis H-14 with chlorphoxim.

During the dry season of 1982/83 an isolated population of S. damnosum s.s. on the lower Bandama river in southern Côte d'Ivoire, became resistant to temephos. In order to eliminate this resistant population, blanket B. thuringiensis H-14 treatments were carried out in this zone and continued until the end of 1984. The treatments were effective and when, after four months suspension of larviciding savanna species reappeared in April 1985 they were found to have a normal level of susceptibility to temephos. Subsequently, temephos resistance in savanna flies has been detected in a few other foci and has been successfully dealt with in a similar manner. After 1986 there has been a rapid spread of lowered susceptibility throughout the programme area.

Development of resistance in savanna species is a serious threat to the Onchocerciasis Control Programme. Even though replacement compounds are available they are more difficult and more expensive to use than temephos. A strong network of resistance monitoring, conducted by specialized mobile teams has been established and must remain part of the Control Programme activities until vector control ceases.

IX.3 Environmental impact of larviciding

Because the continued use of larvicides for 20 years over a large area may have serious ecological implications, a monitoring protocol was developed to evaluate the impact of the larviciding on the aquatic fauna. An environmental monitoring network has been established in several countries. In addition, ecological studies are undertaken on all new larvicides. An independent advisory body, the Ecological Group, assesses the data obtained and conveys its conclusions to the Programme.

They have found that after an initial application of temephos there is a very marked fall in the density of aquatic invertebrates. This modification of the fauna lasts for about a year. It is followed by repopulation from untreated rivers, that make up more than 35% of the total flowing waters within the Programme area, so that a new balance is established. The various taxa are not affected in the same way. But most of those species present before the start of operations are still to be found after treatment, though a few have disappeared from large areas. In general, the effect of temephos after eight years of weekly applications, has been a 30% reduction in the invertebrate biomass. Nevertheless, a wide variety of organisms persists in all treated river basins.

The dynamics of fish populations do not operate on the same time-scale as invertebrates and must be studied for many years before meaningful results can be obtained, but so far, the proper use of temephos seems to have no detrimental effect on fish.

All larvicides have been carefully screened by hydrobiologists for their effects on non-target organisms and have been approved by the Ecological Group who have placed certain restrictions on the use of some of them (e.g., permethrin).

IX.4 Results of the of *S. damnosum* control in the Onchocerciasis Control Programme

Entomological evaluation

An area under vector control in the West African savanna may be considered safe for resettlement, if the annual biting rates are less than 1 000 and the annual transmission potentials less than 100 for two consecutive years. However, the present strategy of the Control Programme is to reduce transmission to insignificant levels by means of vector control and to maintain this control for a sufficiently long period to allow the initial reservoir of infection to fall so low that vector control can be safely interrupted.

For most catching points in the Control Programme area the entomological evaluation started only shortly before the start of control operations, so the lack of sufficient pre-control data complicates the general assessment of the changes in the entomological pattern that have been brought about by vector control. However some data collected before the Programme started provide important clues about the impact of the large-scale control. Annual biting rates in the White Volta basin, along the Bougouriba, the upper Comoe, the Léraba, and the Upper Bandama rivers reached levels of 50 000-250 000 in the 1960s and the early 1970s. Local vector control along the latter three rivers from 1967 to 1975 reduced these figures to about 25 000 bites per man per

year. More limited data on transmission suggest that the annual transmission potentials usually more than 2 500 and could reach levels as high as 10 000 along the White Volta and 18 000 along the River Léraba. Most of the data from the 1960s was collected in years when the rains were heavier than they have been since the Programme started, but the information that is available for the early 1970s does not suggest that the intensity of transmission was much lower along these rivers during the droughts in those years. Against the background of these figures the achievements of OCP are impressive.

In the central well-controlled area covering about 85% of the original Control Programme area, and where the vectors are almost exclusively S. damnosum s.s. and S. sirbanum, control has been very effective.

In 50% of this central area, S. damnosum s.l. has been virtually eliminated. Annual biting rates are usually zero and rarely exceed 100, while annual tranmission potentials are zero throughout the area. This zone includes the dry, northern sectors of the Programme area where most rivers flow only for a few months during the rainy season. It also includes former notorious onchocerciasis areas, such as the middle parts of the White and the Red Volta River basins and the lower River Koulpeolgo basin in Burkina Faso, all of which had extremely high intensities of infection and high blindness rates before control began.

Further south, S. damnosum s.l. has not been eliminated but is usually only present at low densities with annual biting rates normally below 500 and rarely exceeding 1000. At most of the catching points infective flies are detected only on very rare occasions. Such points are classified as having annual transmission potentials that are below the measurable threshold using the present sampling methods and are probably close to zero. The results for the White Volta Basin in Ghana, including the Red Volta, Sissili, and Kulpawn rivers were less satisfactory because of localized treatment failures in 1981 and 1985.

In reinvaded areas, where immigrant flies usually bite close to their oviposition sites and where there is satisfactory control of local breeding, transmission is virtually confined to the vicinity of breeding rivers, where transmission potentials may remain unacceptably high. For example, reinvasion is especially heavy along the Baoulé and Bagoe rivers in Mali, along the Mô, Kara, and Keran rivers in Togo, and the Alibori and Sota systems in Benin. In all these areas there are places where ATPs up to 1000 have been registered in some years.

It has been clearly shown that extension of control to source areas can prevent reinvasion. This has been demonstrated for the Bandama, Bougouriba, Léraba and Sassandra river systems.

In southern Côte d'Ivoire results are complicated by the development of resistance in the S. sanctipauli subcomplex that occurred within one year of the extension of control into this area. In some years, Annual Biting Rates and Annual Transmission Potentials due to this subcomplex have been high, although the savanna species have usually been effectively controlled. The epidemiological significance of this remains uncertain.

IX.5 Recent developments and conclusions from the period 1975-1991

During 1986 and the beginnning of 1987, lowered susceptibility of the savanna species of the blackfly to temephos spread to several river systems throughout the Original area. It also appeared in rivers in the Western Extension area where control activities had not yet started and there was a real risk that resistance to temephos would eventually encompass the entire area. However, the situation was brought under control both in the Original and in the Extension area by rotational application of the replacement larvicides then available: Bacillus thuringiensis (B.t. H-14), chlorphoxim, permethrin and carbosulfan, but there were substantial cost implications.

Both the entomological and epidemiological evaluations have confirmed that larvicidal vector control has effectively interrupted transmission of O. volvulus in the centre of the area. To achieve the objective of the Programme this control will have to be maintained until the residual microfilariae have died out in the human population. The most recent estimate for the average lifespan of the adult female worm is 12 years, to which must be added a further three years for the life expectancy of the last microfilariae produced, thus making a total of 15 years. However, some of the adult worms will live longer than 12 years. The significance of these long-lived worms has to be taken into account when planning the future of the Programme.

Since the start of vector control, the target savanna species of the blackfly, S. damnosum s.s. and S. sirbanum, have in most of the Original Programme area been maintained at a density so low that transmission has been virtually interrupted. In the remaining, essentially the reinvaded zones, the Annual Biting Rates and Annual Transmission Potentials have, until recently, been unacceptably high but the progressive larviciding of sources of reinvasion in the Extension areas (since 1989 in the Western and since 1987 in the Southern Extension area) is to a large extent protecting the reinvaded zones.

The suppression of the vector populations is intended to last for fourteen years, the time needed for the human reservoir of the parasite (Onchocerca volvulus) to die out. After that the blackfly will be allowed to re-enter the area in question. This situation has already been reached in most of the Original OCP area where larviciding has come to an end.

Expressed in epidemiological terms, the Community Microfilarial Load (CMFL)[1], the overall prevalence of the disease and the CMFL in the anterior chamber of the eye (CMFL/AC (an important risk factor for the development of ocular lesions) have been reduced to practically nil in most of the Original Programme area. The results in the reinvaded zones are less satisfactory.

Since large-scale field trials with ivermectin commenced in 1987, and following the findings of the January 1989 session of the TDR/OCP/OCT Subcommittee for Monitoring that the drug could safely be used on a large scale provided that a 36-hours period of observation for side-effect was allowed for, OCP conducts a programme of community-wide distribution of ivermectin

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[1]The geometric mean microfilarial load per skin snip for a cohort of adults aged 20 years or more (including those with negative count).

essentially in communities with a high risk of onchocercal blindness (CMFL: 10 or more). This activity is carried out in close collaboration with the national health administrations concerned and with a strong national participation. So far more than 250 000 persons have started their annual treatment schedule which will be continued for several years to come.

The direct effect of vector control in terms of re-settlement in fertile, riverine areas, previously deserted in fear of onchocercal infection, has been slow and difficult to measure. The PAG Mission estimated in 1973 that more than 65 000 km^2 of land in the river valleys (close to 10% of the Original Programme area, and the most fertile) had been abandoned and that around one million people could eventually be resettled in these valleys once under control. Studies in Burkina Faso have shown a three to six- fold increase in cultivated lands in river valleys under vector control since the start of Programme operations. Elsewhere, there has been a development of large-scale agroindustrial complexes where sugar, cotton, rice and tea are grown, often using irrigation.

It is estimated that 15 million hectars of tillable land along 18 000 km of rivers in the Original Programme area is ready for resettlement. It is expected that this figure will increase to 25 million by the end of the 1990s. 3.3 million tons of foodstuff valued at US$ 340 million will be grown on the resettled land, enough to feed 17 million people.

The search for new larvicides has been successful In addition to the two organophosphorous compounds, temephos and chlorphoxim, three larvicides are now available to OCP for rotational use in temephos/chlorphoxim resistant areas. These are B.t. H-14 which is low-cost, target specific and unlikely to develop resistance in the vector; permethrin, a pyrethroid, used for limited durations of continuous application; carbosulfan which is rather expensive and whose use is restricted to periods of high water flow; and pyraclofos, an organophosphate and potential replacement for chlorphoxim which is being withdrawn from production.

Considerable progress has also been made in such fields as identification and behaviour of blackfly species, case diagnosis including sero- and immunological testing, DNA probing to distinguish the blinding form of the parasite from that giving rise to less severe manifestations, and the determination of the longevity of the parasite. Thus, examination of excised nodules, supported by a statistical/epidemiological trend analysis, has shown the reproductive life of the female worm to be in the order of 11 to 12 years with another two to three years of potential infectivity before the last microfilariae die out, a finding of great operational significance to the Programme.

The Onchocerciasis Chemotherapy Project (OCT) collaborated with the Special Programme for Research and Training in Tropical Diseases (TDR) and the pharmaceutical industry in the development of ivermectin for use in humans. The drug is taken orally and rapidly brings the microfilarial load to a very low level lasting a few months before rising rather steeply to approach the pretreatment level within a year. It is recommended to be given once or twice annually for extended periods excluding pregnant women, mothers during the first week of lactation, children less than five years old, and patients suffering from a few specified diseases. The clinical effects include immediate alleviation of discomforts such as itching, and a significant reduction in the risk of developing eye lesions.

Preliminary results of the first studies indicated that ivermectin treatment had a considerable effect on transmission when distributed to a sufficiently large proportion of the target population but more recent investigations concluded that the impact of community-wide application of the drug was insufficient to be of value in sustained control of transmission.

As regards the search for a macrofilaricide which would, in principle, be given once only, OCT and TDR are supporting clinical trials of one candidate with several back-up compounds further down the development pipeline.

Operational research concerned with such activities as aerial larviciding and entomological surveillance, as well as the organization of large-scale ivermectin distribution, is a permanent feature of the Programme. The results have contributed to an enhanced cost-efficiency of operations as has the annual Staff seminar instituted by the Programme Director.

The construction of a transmission model and its application to OCP operations have been of crucial importance for such issues as the determination of the length of the reproductive life of the female worm and the strategy of future OCP operations including decision on withdrawal of larviciding from areas exposed to control during 14 years or more.

Between 1974 and 1990, 338 candidates essentially from the Participating Countries have been awarded OCP fellowships as follows: 180 in entomology, 32 in hydrobiology, 32 in epidemiology, 42 in parasitology, 30 in ophthalmology, 12 in health economics, 6 in administration and 4 in language training. To this should be added in-service training from which a large proportion of the 600 OCP staff, of whom 96% are Africans, have benefitted.

Devolution plans have so far been prepared by Burkina Faso (presented to JPC in 1988), by Mali and Niger (presented to JPC at its December 1989 session) and by Benin, Côte d'Ivoire, Ghana and Togo (presented to JPC in 1990). The Burkina Faso and Mali plans both foresee onchocerciasis surveillance combined with that of trypanosomiasis, the Niger plan combines onchocerciasis and leprosy surveillance, the Ghana plan includes the control of yaws, leprosy and guinea worm, the Togo plan combines surveillance of onchocerciasis with control of mycobacterial diseases while the plans for Côte d'Ivoire and Benin situate onchocerciasis surveillance in the context of general epidemiological surveillance and control.

The OCP Expert Advisory Committee recommended at its June 1989 session that larviciding should remain the exclusive means of transmission control, that vector control be intensified in the Extension areas and that larviciding be maintained for fourteen years.

The Committee further recommended that ivermectin (supplied free of cost by the manufacturer) be used exclusively for the purpose of reducing morbidity and be distributed widely for the treatment of infected cases.

IX.6 The future of the OCP

With five larvicides at its disposal and the capability to utilize them effectively to overcome future instances of resistance, the Programme can now implement vector control in the Extension areas in full confidence that aerial larviciding there will achieve the desired effect on the transmission of the disease.

At the same time, the emergence of ivermectin has brought a new dimension to the Programme. With community-wide application of the drug to infested foci on a long-term basis, the clinical effect and ocular manifestations of the disease will rapidly become a saga of yesterday, while vector control eliminates the human reservoir of the parasite within the OCP area.

In the meantime, larviciding has ceased in most of the non-reinvaded zones within the Original Programme area. After cessation, entomological surveillance will continue for another two years and larviciding will be confined to limited operations in two circumscribed zones where transmission had only been partly controlled due to operational difficulties.

Such limited vector control should be completed in 1994 by which time OCP will have withdrawn entirely from the non-reinvaded 90% of the Original Programme area, well within the originally allocated 20 year period.

Vector control operations in the Southern and Western Extension areas will continue on the lines of the control strategy recommended by the Expert Advisory Committee.

Finally, the Programme will intensify its efforts in the field of devolution, work out guidelines and support the Participating Countries in preparing for the eventual assumption of the surveillance of onchocerciasis and the control of possible instances of its recrudescence by means of ivermectin treatment and containment. This will be done in close collaboration with the WHO Regional Office for Africa.

A Plan of Operations for the fourth Financial Phase (1992-1997) will be considered by the Joint Programme Committee in December 1991.

Given that full coverage of vector control was only attained during the last few years in the Extension areas, larviciding there will need to continue until, or about, the end of the present decade, thus allowing for uninterrupted control during 14 years, the period required to virtually eliminate the human reservoir of the parasite. Entomological surveillance will continue for two years beyond that date in order to confirm that transmission has definitively ceased.

However, there will be a gradual reduction in the extend and intensity of control activities, and this in particular during the last two years of the Programme's life when larviciding will have stopped and entomological surveillance will remain the only vector control activity.

During the "phasing out" period the inter-country arrangement for the coordination of, and support to, the devolution process after the closure of OCP will be put in place.

IX.7 Further Reading

A more detailed account of the activities of Onchocerciasis Control Programme up to 1985 can be found in the document "10 Years of Onchocerciasis Control" published by WHO (OCP/GVA/85.1B) in 1985, and in the Annual Progress Reports of the Onchocerciasis Control Programme.

X. TECHNIQUES

X.1 Haemalum staining

Used to detect Onchocerca larvae in alcohol preserved flies, staining is best done on batches of 20 to 50 flies in a small vial. Drain off the alcohol and replace with tap water. After about half an hour replace with fresh water. One hour later drain off the water and cover the flies with Mayer's Haemalum and leave for at least three days to stain. If there is danger of fungus growing in the stain as often happens in the humid tropics, a few crystals of Nipagin (methyl 4-hydroxy-benzoate) added to the stain will reduce its growth. To dissect, remove three flies from the stain, place briefly on absorbent paper to remove surface stain and arrange them on a single slide. Place a drop of 20% acetic acid on each fly and allow to stand for a few minutes to allow the acid to begin softening the muscles and neutralize the stain. This time can be spent preparing a second batch of three flies. Dissection then continues as described in Section VII.1. The strongly red stained Onchocerca larvae can be seen contrasting with the rapidly destaining pink muscle fibres. This is Nelson's (1958) technique as modified by Garms and Cheke (1985).

The main disadvantage of the technique is that parous flies cannot be detected so it is necessary to dissect all the flies for infections. This can be overcome by combining both techniques, by dissecting living flies and preserving individual parous flies singly in 80% ethanol in the wells of microtitre plates for staining and dissection later.

X.2 Rearing adult flies from pupae

It is often necessary to rear adult flies from pupae for taxonomic purposes or for obtaining nulliparous flies for insecticide testing or other experiments. Single flies may be reared by cutting a small section of the substrate, such as a leaf, to which the pupa is attached and placing it on a small piece of damp filter paper in a vial which should then be closed. The vial should be kept in a cool place, and inspected twice a day until the adult emerges. Once emerged, the fly should be transferred to a dry vial for several hours to allow the wings to harden.

Large numbers may be reared by placing a quantity of vegetation supporting pupae into a large jar of about 1 litre or more capacity. The moisture already on the vegetation will be sufficient to keep the contents moist and no extra water should be added. The mouth of the jar is then inserted into a tube of fine gauze supported by a wire frame, and both wire and gauze covered by a plastic bag to keep everything humid. The glass of the jar should then be covered by a dark cloth, and the mouth and gauze pointed towards a sunlit window or bright light. As the flies emerge they will fly into the gauze bag from which they can be removed by suction tube or pooter. Each day, the contents should be removed and rinsed with clean water, and the jar washed out to remove dead larvae and other decomposing matter.

X.3 Preserving Simulium larvae for cytotaxonomy

When surveying new areas it is important that samples of late instar larvae should be preserved for later cytotaxonomic identification. This requires that they be preserved in Carnoy's fixative using the following technique.

Carnoy's fixative is made up of three parts absolute ethanol and one part glacial acetic acid. It is absolutely essential that new alcohol and acetic acid is used as both substances will absorb water from the atmosphere and become diluted while on the laboratory shelf. The two components should be mixed only a minute or two before the larvae are added, as they react together to form ethyl acetate. The best practice is to prepare a number of small bottles or vials marked approximately at the 15 ml and 20 ml levels. These may be filled to the 15 ml mark with absolute ethanol, and well stoppered. They should then be stored in the refrigerator. A separate bottle of about 50 ml of glacial acetic acid should also be stored at about + 4°C. Remember that acetic acid will destroy rubber and some plastics.

When larvae are ready for preservation take a vial from the refrigerator, and top up to the top mark with cool acetic acid. Large living gill-spot larvae that have been collected in the field and kept cool can then be picked off the vegetation or from the plastic bag and placed directly into the Carnoy's fixative. The best chromosome preparations are usually obtained from larvae on which the gill-spots have reached their full size, but are not yet completely black to the unaided eye. Not more than 50 larvae should be preserved in 20 ml. At the end of the same day, pour off all the original Carnoy's fixative and replace with fresh. Alternatively, the larvae can be preserved at the riverside, in which case the solutions should be kept cool and mixed immediately before use. Once in Carnoy's fixative the larvae should be kept refrigerated until required for cytological examination.

X.4 Rearing and colonization

It has been mentioned earlier that our knowledge of the biology of the Simuliidae has been limited by the difficulties of rearing most species through from egg to adult, mating, feeding and subsequently to laying more eggs.

The difficulties mostly lie in three areas.

(a) Providing suitable flowing water conditions for the aquatic stages, particularly larvae; [One basic difficulty in onchocerciasis areas lies in maintaining a constant electrical supply for the circulating pumps, Raybould and coworkers (1982)]; (b) persuading emerged adults to mate and feed; and (c) in the laboratory egg-laying usually leads to the death of the female, and many eggs may be infertile.

The subject has been extensively reviewed, with summaries of equipment and systems, by Edman and Simmonds (1985) and updated by Edman and Simmonds (1987).

Most of the species that have been colonized for two or more generations have been temperate species, but a few examples of colonization of onchocerciasis vectors do exist, namely, one report of S. neavei group and a few successful attempts with the S. damnosum complex. With the last, there is evidence that some cytospecies are more amenable to colonization (mating and feeding more easily in captivity) than others. However no easily colonized vector species has yet been found.

It is surprising that there are no published attempts to colonize the South American vector species. In view of their higher degree of zoophily, and preference for slower moving waters, some of them may prove to be good

candidates for colonization.

X.5 Traps

A requirement of all traps is that they should be cheap to construct, have little intrinsic value to reduce the chance of theft, and should not require much effort or labour to service.

(a) Adult Simulium

Methods for sampling adult simuliidae (including using human bait) have been extensively reviewed by Service (1977, 1981, 1987) with photographs and diagrams of many different techniques. An extensive literature has accumulated on this subject due to a desire to find a replacement for human bait for sampling females of the vector species. So far no technique has been found that is as easy to operate, stores the insects alive in good condition or is as selective (light traps, for example may catch Simulium at dusk, but they also collect countless specimens of unwanted insects that need to be sorted and discarded). Promising traps for special situations are the aluminium plaque, for gravid females (Bellec, 1976), the vehicle mounted traps for free-flying adults (Roberts and Irving-Bell, 1985) and the biconical Challier and Larveissière (1973) tsetse trap as modified and used by Cheke and Garms (1987), although the attractiveness of this trap appears to decline with time and washing.

An interesting use of the aluminium plaque has been the collection of presumably zoophilic S. damnosum s.l. deep inside the Onchocerciasis Control Programme area where no man-biting females could be found (Bellec et al, 1984).

(b) Aquatic stages

Larvae of vector species are often required for cytotaxonomic identification, and pupae for rearing into adults. In rivers where natural removable substrates such as leaves or grasses are few, or larvae may be too deep to be collected normally or occur on large immovable boulders, the only way to collect undamaged larvae is to employ artificial substrates.

In many situations branches or palm fronds jammed between rocks will provide excellent attachment sites for larvae. However, if more standardized collecting is required, strips of plastic cut from bags and sacks can be tied in the fastest flowing section of the river. The advantage of plastic (polyethylene) strips is that they tend to float near the surface and so may also provide oviposition sites. They also rise and fall with changes in river level. Artificial substrates are usually left in place and inspected every 3-4 days.

(c) Crabs

Sampling rivers for S. neavei group larvae entails collecting the crabs on which they are attached. In small rivers this may be most easily achieved by employing children to overturn rocks and collect crabs by hand, but this also causes considerable mortality to young crabs and disrupts the environment. A variety of crab traps have been described by McMahon and coworkers (1958), Raybould and White (1979) and Raybould (1969). They are essential for wider and deeper rivers; most of them operate on the principle of the crab-pot used by

marine fishermen where the crabs enter a baited cage via a funnel. Crabs may also be caught by using fish or meat bait wrapped in pieces of old fish net attached to lengths of fishing line (Davies, J.B. personal observation). The crabs attracted to the bait become entangled in the meshes of the net, and have difficulty in escaping when the line is pulled up.

X.6 Determining the susceptibility or resistance of Simulium larvae to insecticides

Before any insecticide control is contemplated, it will be necessary to determine the susceptibility of the vector species to the proposed insecticides, as cases have been known where resistance has already been provoked by the agricultural use of the same or similar insecticides. Tests will also be required from time to time during the course of an insecticidal programme to confirm that the target species is still susceptible, particularly in cases where control has broken down for one reason or another.

The techniques deployed by the Onchocerciasis Control Programme and adopted by WHO are based on the test published by Mouchet and coworkers (1977) which uses larvae and a three-hour exposure time. This is similar to the tests described by Jamnback (1976) which used a 24 h exposure time. The Mouchet test has been formalized by the WHO mimeograph publication WHO/VBC/81.811 of 1981 (Section X.111 Annex 2). Pipettes and test solutions of the most common insecticides may be obtained free from the Division of Control of Tropical Diseases, WHO, Geneva.

Except for the S. neavei group, the test should be suitable for all vector species whose larvae can be visually distinguished from other species in the same habitat. Tests for S. neavei group species were devised by Raybould (1966), and by Raybould and Clark (1974). The last also appears as Annex 2 in Jamnback (1976).

For testing larvae against B. thuringensis H. 14 the series of four papers by Guillet and coworkers (1985 a-d) should be consulted.

X.7 Measuring river discharges and calculating dosages

The quantity of insecticide to be applied at any dosing point depends on the discharge of the river at that point (or better, in the target area further downstream). The discharge is the volume of water passing a point on the bank in unit time, and is usually measured in m^3/sec (cumsecs) (an older unit is ft^3/sec (cusecs)). This is calculated by multiplying the cross-section area of the river below the water level by the mean velocity of the water.

Since onchocerciasis control is a long term undertaking, it is advisable to have river discharge curves (discharge related to depth of water) compiled by professional hydrologists, for each appropriate stretch of river, and river depth guages installed so that at each site the equivalent discharge can be obtained from the depth using a graph or table. In the absence of discharge curves it will be necessary to measure and calculate the discharge at each visit, and over a year or more sufficient data can be accumulated to allow the curves to be drawn. Several methods for measuring discharges are given by McMahon (1957) and Hill (1959). The following simple method is suitable for medium-sized rivers. Discharges in small streams are best measured by timing how long it takes to fill a bucket or other container of known volume.

First it is necessary to obtain a profile of the river bottom. If this has not already been done in an earlier dry season, it must be done by measuring the depth at at least five sites across the width of the river, using a marked and weighted cord (larger rivers should be plumbed at about 10 m intervals). Calculate the average depth (H), and measure the width of the surface of the river (W). Now measure the velocity of the water at the same positions (with a current meter at two-thirds of the depth at each position, (without a meter take two-thirds of the surface velocity obtained by timing a float over a measured distance). Take the average of all the velocity readings (V).

$$\text{Estimated Discharge } D = H \times W \times V \quad m^3/sec$$

To calculate the quantity of insecticide to be used, the following information is required:

Concentration of active ingredient in the formulation. C%
Target dosage of insecticide. mg/l
Time over which dosage is calculated M min

$$\text{Quantity of formulation required} = \frac{D \times mg/l \times M \times 6}{C\%}$$

Therefore, quantity required to treat 1 m^3/sec at a target dose of 0.05 mg/l over 10 min with a formulation containing 25% active ingredient.

$$1 \times 0.05 \times 10 \times 6) - 25 = 0.12 \text{ litres.}$$

X.8 Target dosages

The 'correct' dosage for each insecticide depends on the manufacturer (insecticides with the same specifications obtained from different manufacturers will not necessarily be equally effective at the same concentrations), formulation, and concentration, as well as the nature of the rivers in which it is to be used. Therefore in each new area the most suitable dosage to be used should be determined by trial.

The following target dosages have been used, or are currently in use:

For S. damnosum s.l. in West Africa

Temephos EC	0.1 mg/l/10 min
Chlorphoxim EC	0.005 mg/l/10 min
Permethrin EC	0.02 mg/l/10 min
B. thuringiensis (H14)	0.1 - 1.0 mg/l/10 min (basis of calculation unknown)

For S. ochraceum s.l. in Guatemala

Temephos WDP	0.1 mg/l/60 min as 5% briquettes

EC = Emulsifiable Concentrate WDP = Water Dispersible Powder).

X.9 Preparation of reference collections

In any study, particularly if control measures are being used, it is important to make a collection of reference (voucher)specimens* from representative sites within the area, and at different seasons of the year. This collection should be added to from time to time as surplus material becomes available. This is important because knowledge of taxonomy and techniques for study are constantly improving. For example, it may be necessary to refer back to early material to confirm the identity of local species after a taxonomic review following the discovery of a new diagnostic character.

Larvae and pupae are best preserved in 80% ethyl alcohol (ethanol). Although 70% ethanol is often used, it may become diluted by water on and in the specimens, to a level where its preservative action is lost. Specimens can be placed directly into the alcohol in the field ('Bijou' or 'Universal' thick glass specimen bottles are excellent containers for field work), but back at the laboratory, they should be transferred to fresh alcohol, usually after sorting, and placed in glass vials, labelled with the date and place of collection, identity and reference number, if catalogued. Labels should be written in pencil on white paper and placed inside the vial. The vials should be closed with cotton-wool, cork, or screw caps and placed in larger glass containers half-filled with the same alcohol. Half-litre 'jam-jars' with tight fitting screw lids or 'Kilner' or 'Mason' preserving jars are best. Metal lids must have a complete layer of plastic on the inside to prevent rusting. A cork or cardboard insert to the lid is not sufficient. A point that is often forgotten is that reference collection jars must be inspected every few months, and the alcohol topped-up if necessary.

Adult flies should be preserved dry for taxonomic purposes, otherwise they may be preserved in 80% ethanol as for larvae and pupae. Dry flies need to be mounted.

Glue-mounting: The fly is glued to the tip of a tapered strip of card, or is attached by a dab of shellac to the shaft of a long mounting pin. This technique is not ideal as the glue may obscure much of the specimen, especially in the smaller species, and specimens easily become detached during handling and mailing.

Micro-pinning: A fine stainless steel micro-pin (Minuten) about 12.5 mm long is pushed through the thorax of each freshly-killed fly until the fly is centred on the pin which is then inserted into a short stage of polyporus and the whole carried on a long strong pin. The long pin should also carry card labels giving the date, site, identity of the specimen and any other relevent information (Fig. 10.1). Where the fly has been reared from a pupa, the pupal skin and cocoon can be lightly glued to another strip of card and mounted on the same pin. Flies should be pinned through the side of the thorax soon after killing and while they are still soft. Pinned specimens should be kept in cork or polythene lined air-tight boxes, that will protect them from damp, ants and mites. Avoid putting freshly mounted specimens directly into storage boxes before they have had an opportunity to dry thoroughly. The inside of the box should be treated with merthiolate fungicide, and have a container for naphthalene. In the field the whole box should be tightly wrapped in a plastic bag containing silica gel.

Drying and pinning alcohol-preserved flies: Flies that have been preserved in 80% alcohol may be dried and pinned using the following technique (given to J. B. Davies by B. V.Peterson). However it must be emphasised that specimens prepared in this way are not as good as freshly pinned material.

Impale each fly on a micro-pin as described above, while it is in a dish of 80% alcohol, then holding the fly by the pin, transfer it to two or more changes of absolute alcohol, for 30 min each. When satisfied that all water has been removed, place the fly into xylene (xylol) for at least 10 min. Remove the fly from the xylene and touch it very gently onto absorbent paper to remove any surplus liquid. Mount onto a larger pin as above, and allow to dry thoroughly.

X.10 Mailing specimens

Many specimens reach their destination in broken or irretrievably damaged condition. This is nearly always because of poor packing before despatch.

Specimens in fluid. These should be mailed in leak-proof screw-cap vials. Corks and press-in caps are not suitable as they may become dislodged by the changes in pressure caused by air transport. Each vial should be lightly plugged within with tissue or cotton to prevent the specimens from slopping back and forth in transit, and air bubbles should be rigorously excluded as they can do great damage. Each vial should be individually wrapped in tissue or cotton-wool, and all vials packed in a strong container such as a wooden box or tin (instant coffee tins are a good example). The container should in turn be packed in a larger carton containing shock-absorbent material such as wood-wool, or polystyrene flakes or balls.

Pinned flies. These should be securely pinned into a rigid box of wood, cardboard or plastic. This container should be packed into a much larger carton so that there is a minimum of 70 mm of shock-absorbent material over each face of the container. If the specimes are staged, they should be held rigid with extra pins to stop the stages from swinging. The specimen-box should contain a loose ball of cotton-wool in one corner to trap any specimen that might break loose. Any openings should be covered with cling-film.

Marking packages. Packages should carry "Fragile" stickers and labels indicating the contents as "Specimens for scientific study: no commercial value".

Advising specialists Specialists should be advised in advance if specimens are being mailed to them. If identification is likely to be a major task, then the prior agreement of the specialist should be sought before despatch. If specimens are to be returned, remember that, by convention, a specialist is considered free to keep up to one-third of all material unless other arrangements have been made. A list of WHO collaborating centres is given at the end of this Section (X.14).

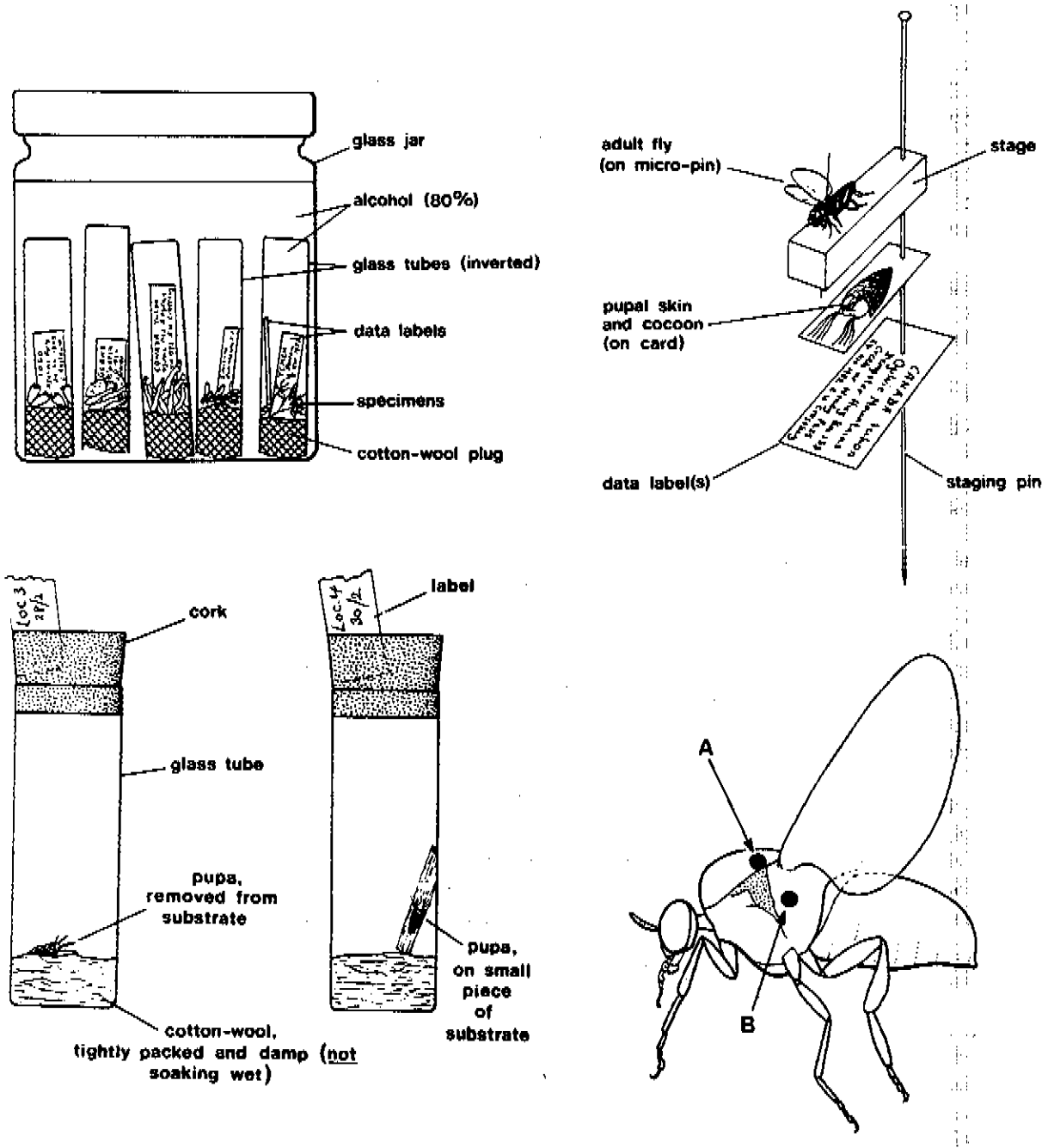

Figure 10.1 Methods for preparing and storing reference specimens

Top left: Storing larvae, pupae and adults in alcohol.
Top right: Pin mount of an adult with associated pupal skin and cocoon.
Bottom left: Rearing adults from individual pupae.
Bottom right: Alternative positions for pinning adult flies to avoid damaging important diagnostic features.
A. diagonal mount; B. straight mount

(Original R. W. Crosskey)

X.11 Preservation for special techniques

Dry preservation: Some identification techniques such as the cuticular hydrocarbon* and some DNA methods require the flies to be stored dry. This is best achieved by killing several flies together by placing their collecting tube in the sun, and then transferring them to a specially prepared storage vial. The vial should contain about 1 cm. of silica gel at the bottom, held in place by a wad of cotton-wool that has been firmly compressed by tamping with the end of a pencil. A small loose wad of cotton-wool should be gently pushed in on top of the flies to stop them shaking about in the tube and an air-tight plastic or screw cover is essential. An information label can be included with the flies.

Preservation for isoenzyme* electrophoresis: requires that the specimens (larvae and pupae also) should be alive at the time of testing. Flies may be kept alive at 4°C in a domestic refrigerator for a few days, but any longer storage requires special facilities. Freezing at -20°C in a domestic freezer is not suitable for more than a day or two, as the enzymes will still degrade. For longer storage, temperatures below -40°C are necessary, and liquid nitogen storage is the best.

X.12 Hair tufts used in identifying some species of the S. damnosum complex

The identification of some species within the S. damnosum complex can be assisted by examining the colour of certain tufts of hairs or setae. The stem vein of the wing bears a conspicuous tuft of long hairs on the dorsal surface. This tuft is sometimes called the 'wing tuft' or 'basal wing tuft', terms which suggest erroneously that the tuft may be located on the basal section of the radial vein. Care should be taken to avoid confusing this tuft with two other adjacent tufts of hairs at the base of the wing on the second auxillary sclerite and basicosta (Fig. 10.2).

In West Africa, between Ghana and Sierra Leone, if all the hairs on the stem vein tuft are white, and the fore coxa is also pale, then the specimen most likely belongs to either S. damnosum s.s. or S. sirbanum. In Sierra Leone and Liberia, if all the hairs are black, and the hairs on the ninth abdominal tergite are long and black, then S. yahense is strongly indicated.

X.13 Preservation of onchocerca larvae

It may be necessary to preserve filarial larvae dissected from flies for later identification or morphological study. Their very small size presents problems in that during storage in alcohol in vials, some specimens may be lost because they stick to the walls of the container. Even so, for studies involving DNA probes, larvae should be stored in 80% propanol (not ethanol), unless requested otherwise.

Some filarial larvae can be distinguished from those of O. volvulus by size. This is particularly so with the third stage (L_3) (Garms & Voelker, 1969; Bain & Chabaud, 1986). To make a semi-permanent preparation, the larva should be removed from the slide on which the fly is dissected using the point of a fine needle, placed into distilled water on another slide, and killed by heating (if possible it should be measured). Now transfer the larva to a small drop of glycerol (glycerine) and cover with a cover-slip. The quantity of glycerol should be just sufficient to fill the space under the cover-slip. The slide may now be sealed with nail varnish. The larva may shrink on contact with the glycerol, but it should revert to its original size and shape within about a day.

Microfilariae in a blood meal may be preserved by dissecting out the entire blood mass from the stomach, spreading it on a clean slide, and drying with gentle heat. The slide may then be stored. To stain, dehaemoglobinize in distilled water, and stain with Mayer's Haemalum (or fix with methanol and stain with Giemsa or a similar stain as for a blood slide).

Microfilariae can be extracted from the hard pellet of blood in alcohol-preserved flies by soaking the blood pellet in 20% acetic acid on a slide foe at least half an hour.

X.14 WHO Collaborating Centre for specimens of Simulium:

Department of Entomology, The Natural History Museum, Cromwell Road, London, SW7 5BD, United Kingdom

SEE CORRIGENDUM

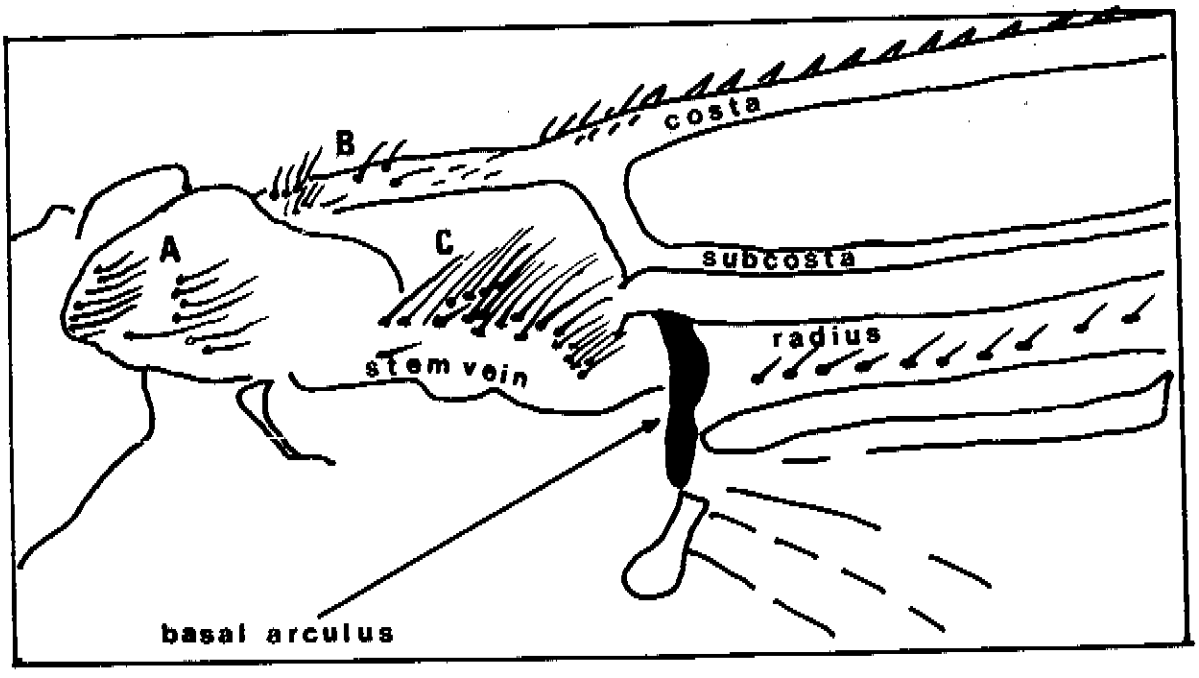

Figure 10.2 Basal portion of the wing showing the hair tufts on the dorsal surface, which may be confused in the identification of some members of the S. damnosum complex.

A. Tuft on the second axillary selerite (not used).
B. Basicostal tuft (not used).
C. Large tuft on the stem vein (used).

(Redrawn by J. B. Davies from an original by D. Kurtakl.)

XI GLOSSARY OF TERMS

ANTERIOR	The front end.
ANTERODORSAL	The front of the upper surface or back.
ANTEROVENTRAL	The front of the under surface.
ANTHROPOPHILIC	Man-biting (literally: liking man).
ANTHROPOTROPHIC	Prefers to feed on man even when other hosts are available.
ANTICOAGULANT	Stops blood from clotting.
APICAL	At the tip or end of (APEX).
ATROPHIED	Wasted.
BASITARSUS	Fifth segment from the end of the leg.
BIMODAL	Having two peaks. A bimodal activity pattern would show two periods of high activity.
BIOTYPE	A small topographic unit, including the biotic community.
BRIQUETTES	An insecticide mixed with clay, plaster-of-paris (gypsum) or other substance that will allow it to be moulded and hardened into brick-like blocks that will slowly dissolve in water. Thereby releasing the insecticide over an extended period.
CARAPACE	The chitinous dorsal shield of some Crustacea.
CHROMOSOME	A linear structure within the nucleus of a cell composed of proteins and DNA which holds the genetic material or genes of an organism, the arrangement of which is unique to each organism.
COCOON	A woven silk covering to a pupa.
COPULATION	Physical union of male and female during mating.
CUTICULAR HYDRO-CARBONS	Hydrocarbon chemicals in the surface layer of the cuticle which can be used to separate sibling species. Examination requires expensive and sophisticated apparatus, but has the advantage of using dried material.
CYTOSPECIES	Species which because of their morphological similarity, have been identified primarily by cytological characters, such as the banding patterns of the chromosomes*.

CYTOTAXONOMY	The description of species based on a study of the banding patterns of the chromosomes*.
DEC	Diethylcarbamazine, a microfilaricidal* drug.
DERMIS	The true skin or elastic vascular layer lying below the outer protective layer of the skin (the epidermis).
DICHOPTIC	Having the eyes separated at the top of the head. Compared to them meeting at the mid-line (holoptic*).
DIURNAL	Daytime - usually relating to a cycle of activity. The opposite of nocturnal*.
DIVERTICULUM	A long sac-like pocket, closed at the far end.
DORSAL	Upper surface or back.
EMBRYOGENESIS	The formation or development of eggs or offspring.
ENZYMATICALLY	Separation of isoenzymes* to identify some sibling species of Simulium complexes.
EVERSIBLE	Can be turned inside-out like a glove or sock.
FACETS	The hexagonal shaped divisions on the surface of the eyes.
FILAMENTOUS	Thin and thread-like.
FRONS	The space between the eyes at the top of the head or 'forehead'.
GONADS	Internal sex organs.
GONOTROPHIC CONCORDANCE	The production of an egg-batch immediately following a blood meal, so that blood-feeding and egg-laying follow each other in a regular sequence.
GONOTROPHIC CYCLE	The events between successive egg-layings, or the time interval between them.
GRAVID	Swollen and full of mature or nearly mature eggs.
HCH	Hexochlorcyclohexance, a chlorinated hydrocarbon insecticide; also known as BHC.
HETEROGENEITY	The occurrence of different mutations that determine the same phenotype.
HISTOBLAST	A pocket in the cuticle containing developing organs.
HOLOPTIC	Having the eyes meeting at the top of the head; see dichoptic*.

HOMOGENEOUS	Of the same or similar shape, constant in shape.
HUMERI	The anterior corners of the scutum (singular: humerus).
HYPERENDEMIC	Heavily endemic. In onchocerciasis usually applied to a prevalence in excess of 60%.
INVAGINATION	A small tuck or pocket formed by the folding of the wall of an organ.
ISOENZYME	An enzyme that occurs in several forms because of minute differences in molecular structure which are often controlled genetically (alloenzyme is a more accurate term). Separation of these enzymes by electrophoresis has been used to identify some sibling species of the S. damnosum complex.
ITCZ	Intertropical Convergence Zone. The imaginary line where the humid SW winds from the Atlantic meet the dry NE winds from the Sahara and Europe.
IVERMECTIN	A drug which has been used in the veterinary field for many years, and has now been approved for limited use against human onchocerciasis. Marketed under the brand name 'Mectizan'. So far appears to act as a microfilaricide* only.
LATEROVENTRAL	At the side of the under or front surface.
MACROFILARICIDE	A drug which kills the adult filaria worm (see microfilaricide*).
MECONIUM	A green granular substance found in the stomach of newly emerged flies. Generally considered to be the remains of the larval gut contents.
MEMBRANE	A thin sheet of tissue. Adj. MEMBRANOUS.
MERMITHIID	Nematode parasites of the Order Mermithidae. The Genus Isomermis, the commonest parasite of the S. damnosum complex has an aquatic adult. Similium are infected in the aquatic stages, and the larvae are often carried over into the adult fly.
MESO-AMERICA	'Middle America'. Usually refers to the states including and between Mexico and Panama.
MESONOTUM	The notum (tergum) of the mesothorax.
METAMORPHOSIS	Development through the life-cycle, proceeding from eggs through larval and pupal stages to the adult (imago). Each stage different in shape from the others (complete metamorphosis).
MICROFILARIA(E)	The first larval stage of O. volvulus shed by the female worm and found in the skin of the human host.

MICROFILARICIDE	A drug which kills microfilariae but not the adult worms. The most common drugs are DEC* and ivermectin*.
NEOTROPICS	The tropical areas of the North and South American continents.
NOCTURNAL	Night time, usually applied to an activity cycle. Opposite of diurnal*.
NODULECTOMY	The surgical removal of Onchocerca nodules.
NULLIPAR	A fly that has never matured and deposited eggs, and therefore is unlikely to have taken blood. By inference a nullipar is most unlikely to be infected with Onchocerca. Adj. NULLIPAROUS.
OVARIOLE	Each individual egg in its tube, including the ovarian follicle and oocyte, many of which make up an ovary.
PAROUS	Having matured and laid eggs. Therefore by inference having taken at least one blood meal. UNIPAR = one gonotrophic cycle; MULTIPAR = more than one gonotrophic cycle.
PERITROPHIC MEMBRANE	A specialized proteinous membrane which is secreted by the stomach wall to enclose the blood meal. Its formation is apparently stimulated by the stretching of the gut wall and it gets progressively thicker over a period of 2 to 3 hours. Microfilariae* can only penetrate it in its early thin stage.
PHARATE	Hidden within the skin of a previous stage.
PHENOTYPE	An identifiable or functional characteristic of an organism.
PHORETIC	Living attached to another animal, but not parasitic upon it.
POLLENOSE	Covered with a fine pollen-like powder such as is often found on the skin of a plum.
POLYTENE CHROMOSOMES	Chromosomes* which have divided many times but have not separated, thereby forming thick bundles of up to a thousand parallel strands. After staining, their banding pattern can be seen under moderate magnification, and they are therefore convenient for examination. They are found in the nuclei of the cells of a few specialized tissues only.
POOTER	A mouth-operated aspirator or suction tube for collecting small insects.
PRECIPITIN TESTING	A serological technique whereby the source of a blood meal can be identified by testing it with antibodies to blood from known host species.

REFERENCE COLLECTIONS	See voucher specimens.
RELICT EGGS	Fully developed eggs found in an undeveloped ovary and left over from a previous ovulation. A sure indication of parity.
SIBLING	Very closely related. Literally means brothers or sisters. In taxonomy* means the species which make up a single species complex and usually cannot be distinguished by morphological features.
SPERMATOPHORE	A gelatinous capsule containing sperm which is transferred to the female during copulation*.
SUBGLOBULAR	Almost spherical.
SYNCHRONOUS	Simultaneous or taking place at the same time.
TAPERING	Becoming thinner towards one end.
TAXON (TAXA)	The basic taxonomical units. Often refers to species, but can refer to an element of the classification at any level.
TAXONOMY	Classification of simuliid into species and genera by entomological names.
TERGITE	Hard chitinous plate forming part of the cuticle of a segment (dorsal surface).
TERMINALIA	The last two or three visible segments and their appendages at the tip of the abdomen which make up the externally visible sex organs of either sex.
TORSO	The main body of a human excluding the head and limbs.
TRILOBED	Having three lobes or extensions.
TUBERCLE	A small projection or 'bump'.
TURBID	Muddy, carrying a lot of sediment.
VENTER	Underneath surface of the abdomen.
VISCERA	Internal organs of the abdomen.
VIVIPAROUS	Giving birth to live young instead of eggs.
VOUCHER SPECIMENS	Samples of properly preserved and labelled specimens collected during a study, that are available for future examination. Particularly useful when the classification of a group has been revised, or when new techniques have been developed. (Also called reference collections).
ZOOPHILIC	Feeding on animals other than man.
ZOOTROPHIC	Prefers feeding on animals even when man is available.

XII REFERENCES

General reading

The following books cover many aspects of the taxonomy, biology and control of the Simuliidae, and should be consulted as primary sources of further information:

Crosskey, R.W. Natural History of Blackflies. J. Wiley & Sons, Chichester, 1990, 680 pp.

A comprehensive review of the biology of the Simuliidae with considerable coverage of vectors and pests. (Due to be published October 1990.)

Dalmat, H.T. The black flies (Diptera, Simuliidae) of Guatemala and their role as vectors of onchocerciasis. Washington DC. Smithsonian Miscellaneous Collections, Vol. 125, No. 1, 1955, 425pp.

A mine of useful information on all simuliid species found in Guatemala, with large sections on bionomics and behaviour. Unfortunately out of print.

Freeman, P. & de Meillon, B. Simuliidae of the Ethiopian Region. London British Museum (Natural History), Publication No. 194: 1953, vii + 224 pp.

Exhaustive descriptions of the African species with observations on habits. Most of the information is still valid.

Kim, K.C. & Merritt, R.W. (Eds.) Black flies; ecology, population management, and annotated world list. Philadelphia, Pennsylvania State University Press, 1987, xv + 528 pp.

A wide-ranging collection of chapters by 52 authors on all aspects of Simulium biology, vectors and non-vectors.

Laird, M. (Ed.) Blacklies: The future for biological methods in integrated control. London, Academic Press, 1981, ii + 399 pp.

Chapters by 40 authors on simuliid biology related to population management. The state of knowledge in 1980.

Philippon, B. & Séchan, Y. L'onchocercose humaine en Afrique de l'ouest. Paris, Initiations-Documents Techniques ORSTOM, No. 37, 1978, 197 pp.

A French language field handbook on the biology and control of simuliids in West Africa.

Suzuki, T. A guidebook for Guatemalan onchocerciasis (Robles' disease), with special reference to vector control. Guatemala, Guatemala-Japan Cooperative Project on Onchocerciasis Research and Control, 1983, 155 pp.

Yamagata, Y., Araki, J., & Sumiyo, I. (Eds.) Manual of onchocerciasis (Robles' disease) control in Guatemala. Guatemala, Guatemala-Japan Cooperative Project on Onchocerciasis Research and Control, 1983, 162 pp.

These two books give a detailed description of techniques and methods for setting up and carrying out control of Simulium ochraceum) and surveys for onchocerciasis, but are also relevant to other species. They should be read together. Available in Spanish or English (with a Spanish/English/Japanese Glossary) from: Department of Onchocerciasis, SNEM, 5a Ave. 11-40 Zona 11, Guatemala City.

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XIII ANNEX

INSTRUCTIONS FOR DETERMINING THE SUSCEPTIBILITY OR RESISTANCE OF BLACKFLY LARVAE TO INSECTICIDES[1,2]

XIII.1 INTRODUCTION

In order to detect the appearance of an insecticide-resistant strain of blackfly larvae, it is necessary to establish a baseline for the species, either before the wide use of insecticides or with specimens from an untreated area. Where regular operations are undertaken to control blackfly larvae, the normal susceptibility levels of the larvae should be determined as early as possible. To this end, several tests should be performed at various localities and seasons to assess normal biological variation. Tests should then be continued at regular intervals to determine any significant reduction in susceptibility.

If known, the history of the use of insecticides in the area, for both blackfly control and major agricultural uses, should be noted.

It is stressed that this test is not designed to indicate the relative effectiveness of the insecticides in the field. For this purpose, other techniques must be used.

XIII.1.1 Establishing the baseline

Batches of blackfly larvae are exposed to different concentrations of insecticide and the mortality at each level is determined. It is suggested that a preliminary test be made on a wide range of concentrations. On this basis, a series of at least four concentrations should be chosen, some of which will give partial mortalities (i.e. at least one should give 100% mortality and two from 5% to 50% mortality. Tests at these concentrations should be repeated four times with samples from the same population of blackfly larvae. The susceptibility characteristics are established from the regression line based on the average mortalities observed in the four tests at each concentration.

XIII.1.2 Subsequent routine checks by diagnostic concentration

In routine monitoring for resistance, it is not necessary to employ the full range of concentrations used to establish the baseline susceptibility. Use can be made of a diagnostic concentration with a high probability of killing all normally susceptible larvae.

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[1] These instructions, originally reproduced as WHO/VBC/81.811, supersede information given in WHO/VBC/75.591.

[2] This method is not suitable for testing the larvae of S. neavei complex which live on freshwater crabs. For a method suitable for testing this species see: Raybould, J.N. Bulletin of the World Health Organization, 35: 887-892 (1966).

XIII.1.3 Choosing the diagnostic concentration

The diagnostic concentration is chosen on the basis of the baseline data for each insecticide. As explained in Annex 1 "Criteria and meaning of Tests for Determning Susceptibility or Resistance of Insects to Insecticides"[3] the most scientific way of selecting a diagnostic concentration is to plot the baseline data on logarithimic-probability paper and find the concentration corresponding to 99-9% mortality to a given standard concentration of insecticide. As a "rule of the thumb", it is usually adequate to use double the lowest concentration that has consistently given a complete kill in all tests used to establish the baseline.

Monitoring checks are made periodically with the chosen diagnostic concentration and occasional survivors in such checks may be due to normal variation, but the regular occurrence of survivors (e.g. on three successive occasions) constitutes a signal for further investigations. Such investigations should include four tests at each of the concentrations used in establishing the original baseline. In this way, it is possible to detect the presence of physiological resistance and to determine the approximate proportion of resistant individuals in a population.

XIII.2 COMPOSITION OF THE KIT

EQUIPMENT AND/OR INSECTICIDE SHOULD BE ORDERED SEPARATELY
THE ORDER SHOULD SPECIFY THE INSECTICIDES REQUIRED AND THE NUMBER OF 50 ml STANDARD SOLUTION OF EACH

XIII.2.1 Equipment

(a) 4 1-ml pipettes for insecticides, one for ethanol and five rubber suction bulbs;

(b) 3 eyedroppers with rubber suction bulbs; and

(c) instruction sheets, 20 reporting forms and three log-probability papers for plotting regression lines[4].

The users are expected to provide their own collecting and test vessels.

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[3]WHO Technical Report Series, No. 585, 1976 (Report of the twenty-second WHO Expert Committee on Resistance of Vectors and Reservoirs of Disease to Pesticides) pp. 78-83.

[4]Additional report forms and log-probability papers can be ordered separately.

Insecticides

One set of DDT	625 mg/l,[5]	125 mg/l,	25 mg/l,	5 mg/l, 1 mg/l
One set of methoxychlor	"	"	"	"
One set of chlorphoxim	312.5 mg/l,	62.5 mg/l,	12.5 mg/l,	2.5 mg/l, 0.5 mg/l
One set of chloro-pyrifos-methyl	"	"	"	"
One set of pirimiphos-methyl	"	"	"	"
One set of temephos	"	"	"	"

The above alcoholic solutions are supplied in 50 ml bottles. Each set includes one 50 ml bottle of ethanol.

Caution: Ethanol used for solutions and control has been denatured by addition of 2% butanone.

XIII.3 COLLECTION AND TRANSPORT OF LARVAE

Larvae are taken from their breeding places by collecting the trailing vegetation and other submerged objects to which they are attached. Frequently, several species of blackfly occur in the same stream. Knowledge of the characteristics of the breeding sites sometimes makes it possible to collect almost pure samples of a particular group of species, such as Simulium damnosum s.l. However, the composition of the sample tested must be identified accurately in the laboratory after testing.

When transport to the laboratory is necessary, the material with the attached larvae is kept wet in a bucket or plastic bag, but must not be immersed in water. A cooling box or bag is advantageous. In this way, the larvae will remain in good condition for two to three hours and so they should be transferred from the field to the laboratory within this period. For a complete test with one insecticide, 300-600 larvae must be collected. However, the procedure detailed below can be applied in the field.

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[5] mg/l unit in accordance with the International System of Units = ppm.

XIII.4 PROCEDURE

Twenty-five fourth to fifth instar larvae[6] are placed in each of 10 glass or enamelled containers[7] (approximately 15 cm internal diameter, 7 cm high) filled with 50 ml of river water. When all larvae are distributed in the test containers, the river water is gently discarded, avoiding larval detachment, and immediately replaced by 250 ml of insecticide solution. Two of the containers are the controls.

The test concentrations are prepared by pipetting 2 ml of the appropriate standard insecticide solution into 498 ml of distilled water pre-oxygenated by bubbling air and vigorously stirring. This dilution of insecticide is equally distributed into two test containers. The two controls are prepared by the addition of 2 ml ethanol to 498 ml of distilled water prepared as above. The temperature of the water dilutions of insecticide and ethanol should be 19-20°C. However, to obtain intermediate concentrations, 1 ml of any standard solution may be diluted to 499 ml of distilled water, instead of 2 ml in 498 ml.

The test containers are placed in locations where temperature variations are low (i.e. wet sand on the edge of rivers or isothermic cabinets (Fig. 13.1). The temperature of the water solutions should not exceed 25 ^{o} C during the test period. In tropical countries the test should be carried out early in the morning or in the evening. After an exposure period of three hours, the provisional mortality is recorded. Larvae without any spontaneous movements are counted as dead. The living and dead larvae exposed to each insecticide concentration should be preserved separately in 70% ethanol or other appropriate preservative. The final mortality is established after stereomicroscopic examination of each larva to check its specific identify and its development stage (Fig. 13.2) (sixth and seventh instars are discarded).

XIII.5 GENERAL REMARKS

The accuracy of insecticide solutions in ethanol will be affected if the ethanol is allowed to evaporate from the standard solutions. The bottles should, therefore, be tightly stoppered after use. The contents should no longer be used when they have decreased below 5 ml.

Test vessels should be carefully cleaned after use to remove traces of insecticide. They should be thoroughly rinsed, scrubbed with detergent and water (or cleaned with potassium dichromate and sulfuric acid), and rinsed again. Pipettes should be throroughly cleaned with acetone or alcohol.

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[6]The various instars of Simulium larvae are identified by their size and the colour and appearance of their hsitoblasts.

[7]Containers other than glass or enamel vessels can interfere with the action of the pesticides.

XIII.6 RESULTS

To construct the dosage-mortality regression line from the results obtained in quadruplicate tests at the chosen concentration and, in many cases, also from the single preliminary test, the results should be plotted on the logarithmic-probability paper provided. The regression line may be fitted by eye, and the dosages expected to kill various percentages can be read from it. The dosage to kill 50% is known as LC_{50}>; that for 95% kill is LC_{95}, etc. The regression line can be extended to estimate the $LC_{99.9}$ (though it must be realized that this is very approximate). For accurate methods of computing various LC estimates, see Swaroop.[8]

In tests where the control mortality is between 5% and 20%, the percentage mortalities should be corrected by Abbott's formula:

$$\frac{\% \text{ test mortality} - \% \text{ control mortality}}{100 - \% \text{ control mortality}}$$

XIII.7 INTERPRETATION OF RESULTS

See "Criteria and meaning of tests for determining the susceptibility or resistance of insects to insecticides".[9]

XIII.8 DISTRIBUTION OF REPORTS

It is of considerable importance that WHO should receive copies of results obtained from the use of this test kit. It is therefore requested that copies of all reports be sent to the following addresses:

(1) World Health Organization, Operational Research unit, Division of Control of Tropical Diseases, 1211 Geneva, Switzerland; and

(2) the appropriate WHO Regional office[10].

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[8]Swaroop, S. Statistical methods in malaria eradication, Geneva, World Health Organization Monograph Series, No. 51, 1966.

[9]WHO Technical Report Series, No. 585, 1976 (Twenty-second Report of WHO Expert Committee on Resistance of Vectors and Reservoirs of Disease to Pesticides), Annex 1, pp. 78-83.

[10]Addresses of WHO Regional Offices are as follows:

World Health Organization, Regional office for Africa, P.O. Box No. 6, Brazzaville, People's Republic of the Congo; World Health Organization, Regional Office for the Eastern Mediterranean, P.O. Box 1517, Alexandria, Egypt; World Health Organization, Regional Office for South-East Asia, World Health House, Indraprastha Estate, Mahatma Gandhi Road, 120001 New Delhi, India; World Health Organization, Regional Office for the Americas/Pan American Health Organization, 525, 23rd Street, N.W., Washington, D.C. 20037, United States of America; World Health Organization, Regional Office for Europe, 8 Scherfigsvej, Copenhagen Ø, Denmark; World Health Organization, Regional Office for the Western Pacific, P.O. Box 2932, Manila, Philippines.

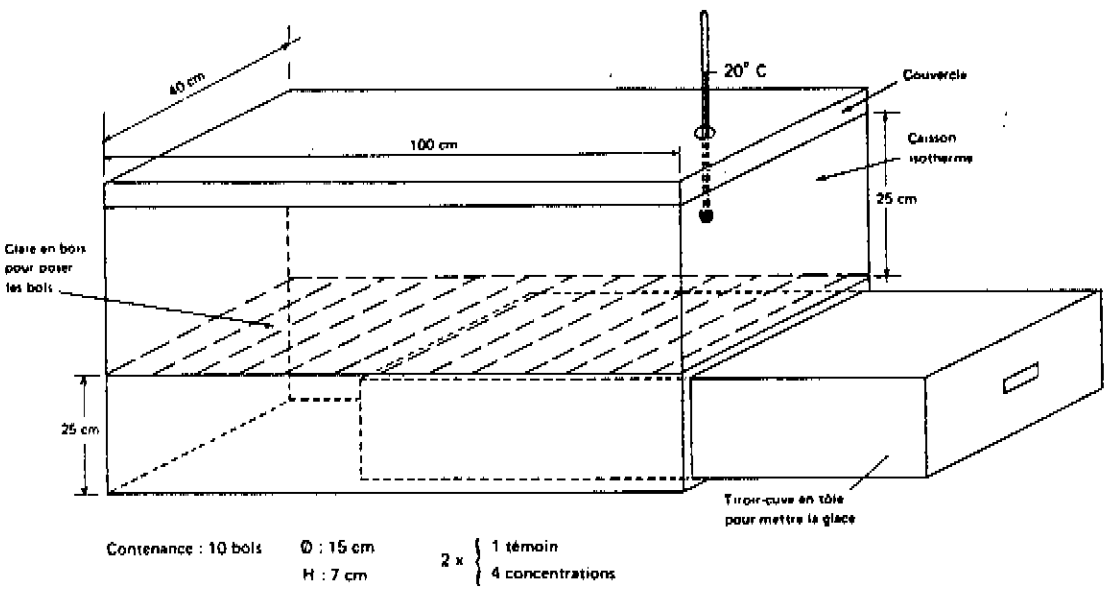

Figure 13.1 Isothermic cabinet at 20°C þ 1°C for the carrying out of insecticide susceptibility tests of short duration

Couvercle = lid.
Caisson isotherme = isothermic cabinet.
Tiroir-cuve en tôle pour mettre la glace = gakvabuzed netak drawer for storing ice.
Claie en bois pour poser les bols = wooden grid to support bowls.
Contenance = capacity.
Bols = bowls.
Témoin = control.

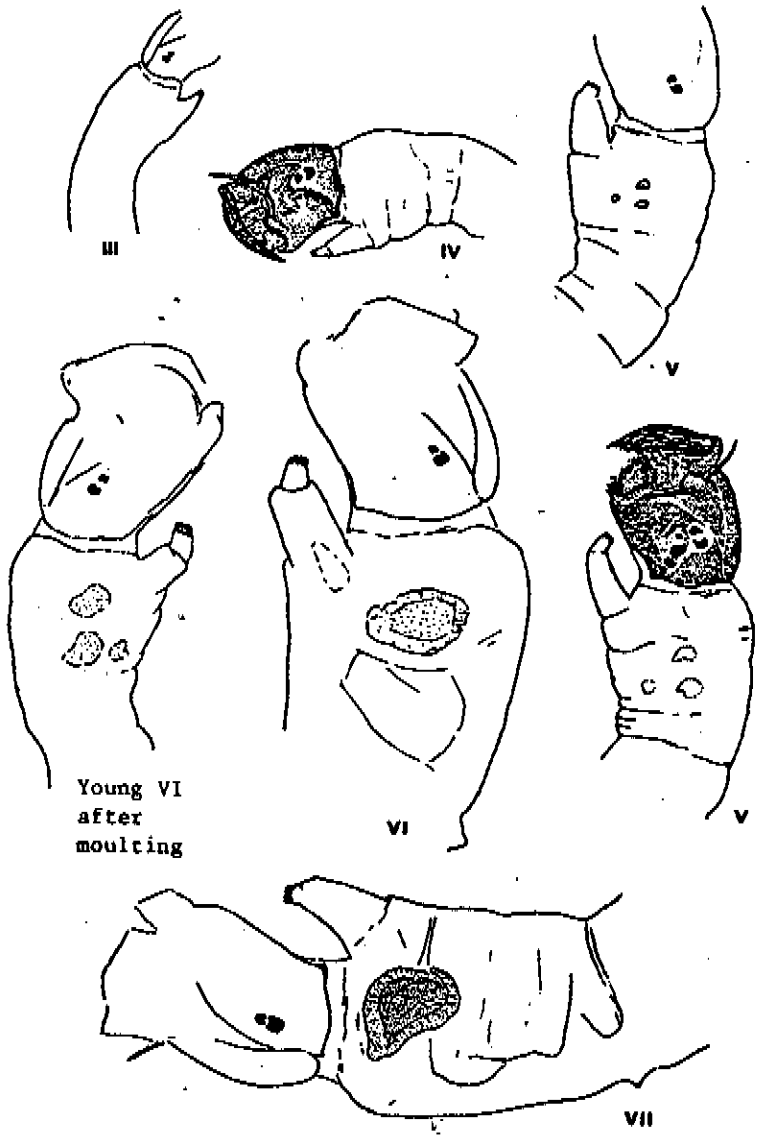

Figure 13.2 Morphological characteristics of larval stage, 3 to 7 of S. Damnosum spp.

(Taken from Mouchet, J. et al. 1977. Méthodologie pour tester la sensibilité aux insecticides des larves de Simulium damnosum s.l. Cah. ORSTOM, sér. Ent. méd. et Parasitol., 15:55-66.)

QUESTIONNAIRE FOR SELF-EVALUATION

1. Name the causative agent of onchocerciasis: (Page 1)

.....

2. What is the geographical distribution of onchocerciasis? (Page 2)

.....

3. Why is onchocerciasis also called "river blindness"? (Page 5)

.....

4. In which host does Onchocerca multiply? (Pages 6 and 7)

.....

5. Describe three effects that Onchocerca have on the human body body (a-c); what is the common name of the first larval stage of Onchocerca (d)? (Pages 6-8)

(a).....

(b).....

(c).....

(d).....

6. Name three primary vectors of onchocerciasis and the country or region in which they are found (a-c): (Page 10)

(a).....

(b).....

(c).....

7. Give the normal development times of the following stages in the life cycle of Simulium damnosum s.l. (a) egg; (b) larva; (c) pupa: (Pages 10, 21 and 26)

(a).....

(b).....

(c).....

8. Where are the eggs, larvae and pupae of most onchocerciasis vectors found? (Pages 19-20)

.....

9. How many larval instars are there in S. damnosum s.l.? (Page 9)

.....

10. What is a pharate pupa? (Page 9)

.....

11. How and when does a Simulium become infected with Onchocerca spp.? (Pages 5, 7 and 8)

.....

12. How long is the Onchocerca development cycle in Simulium at 27-30°C (a)? How many larval stages are there and what are they called (b)? (Page 8)

(a).....

(b).....

.....

13. Compare the length of the Onchocerca development cycle in the vector with the length of the gonotrophic cycle (a). Why are the differences important (b)? (Pages 24, 26)

(a).....

(b).....

14. If a Simulium ingested 100 microfilariae at a single blood meal how many would you expect to develop to the infective stage? (Page 8)

.....

15. What is different about the larval habitat of the S. neavei group (a), and how does this affect their control (b)? (Pages 19, 20, 21, 34, 36, 37)

(a).....

(b).....

16. Describe the condition of three organs which may be used to determine whether a Simulium is parous (Pages 17, 24, 26, 54, 55):

(a).....

(b).....

(c).....

17. List three main approaches to onchocerciasis vector control (a-c). Which do you think is the most practical, and why (d)? (Pages 60, 61, 62)

(a).....

(b).....

(c).....

(d).....

18. What is the objective of onchocerciasis vector control (a)? Assuming that eradication is not possible, for how long would you have to continue before the parasite might be eliminated (b)? (Pages 60, 67, 68)

(a).....

(b).....

19. In a larviciding campaign, what would be the first choice of larvicide (a), how frequently would it need to be applied (b), and what is the principle used to get the larvicide to the larvae (c)? (Pages 62, 63, 66, 72)

(a).....

(b).....

(c).....

20. How would you monitor the effect of larviciding? (Pages 71, 72)

.....

.....

21. Name two biological events that might threaten the success of a control programme (Pages 70, 71, 72):

(a).....

(b).....

22. What is a sibling species (a), and why is it important to be able to separate them (b)? (Pages 29)

(a).....

(b).....

23. Give three techniques that can be used to separate some sibling species of the S. damnosum complex: (Pages 29, 30)

(a).....

(b).....

(c).....

24. Name 5 countries in the WHO Onchocerciasis Control Programme (OCP): (Page 67)

.....

.....

.....

25. In the OCP's control area what percentage of S. damnosum s.l. has been eliminated? (Page 73)

.....

26. In which year did the large-scale ivermectin field trials commence (a)? What were the findings (b)? (Page 74)

(a).....

(b).....

27. What is the most direct effect of the vector control programme of the OCP? (Page 75)

.....

28. In terms of the future of the OCP, what is the concept of devolution (a)? How will the collaboration and eventual assumption of onchocerciasis surveillance and possible control be accomplished (b)? (Pages 76, 77)

(a).....

(b).....

Questionnaire for return to CTD

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