BIOLOGICAL STUDIES ON SOME SOUTH AFRICAN *CULICOIDES* SPECIES (DIPTERA: CERATOPOGONIDAE) AND THE MORPHOLOGY OF THEIR IMMATURE STAGES

By

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2. MATERIAL AND METHODS

(a) The Light Trap.

Most of the investigations depended upon a constant supply of wild-caught Culicoides midges. A suction-type light trap of similar design to the modified New Jersey light trap used by Du Toit (1944) was operated nightly over the last three years, 1963 to 1966 (Fig. 1). Insects were attracted to a 100 watt electric globe fixed beneath the 44 inch long cylindrical trap. A strong upward draught of air was created by a large extractor fan mounted in the top of this cylinder and all insects were caught in an organdie-covered cage interposed between the light and the fan. This cage had a wooden frame and was fitted on one side with glass or perspex through which the catch could be clearly seen and, using an aspirator, all Culicoides midges were removed via a sleeve over the inlet.

The trap was suspended three feet above the ground under a large "wag-n-bietjie" tree (Zizyphus sp.), the tree standing about 10 yards from a long open stable normally housing 35 mules and 11 horses at night. This position was found by Prof. R.M. du Toit (personal communication) to be the best site at Onderstepoort for trapping Culicoides. A similar trap situated only 50 yards from this position was found by the writer to catch only one third as many midges.

(b) Laboratory Studies on Life-cycle.

(1) Oviposition.

Live wild-caught Culicoides were fed on the shaven ear of a rabbit. The cage holding the midges was darkened except for a small area of light on the rabbit's ear. Since midges are attracted to light under these conditions, this served to concentrate their numbers in the vicinity of the ear. In this way approximately 50% would feed after two hours although many died, presumably due to desiccation and injury caused when large numbers are concentrated in a small area.

The living midges were then removed with an aspirator, anaesthetized with carbon dioxide (CO_2), and transferred to a sorting-chamber made of perspex and glass (Fig. 2). Here they could be kept anaesthetized for up to half an hour by a slow flow of CO_2 through the

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chamber. This chamber was designed to fit under a dissecting microscope so that the various species of Culicoides could be identified while alive. Engorged specimens of different species were then removed by aspirator to separate tubes for egg-laying.

These tubes were 2 x 1½ inches in size with a 3/4 inch layer of tamped-down moist cotton wool. Two layers of filter paper, exactly fitting these tubes, were placed on the cotton wool. This provided an even surface on which eggs could be laid and reduced the danger of wings becoming stuck to wet surfaces. The tubes were then held in a horizontal position and from one to twenty-five anaesthetized females of a single species were placed on the dry glass sides. This prevented their wings from adhering to the moist filter paper during their subsequent struggles on recovering from anaesthetization. At the same time the tubes were stoppered with cotton wool, and after recovery of the adults, about five minutes later, the tubes were placed erect and held in a room at 72°F for oviposition.

(2) Larvae and pupae.

Glass needle-boxes 3 1/4 inches in diameter by 2 1/4 inches deep with a loose glass top were used to rear the larvae of each species. Following the method of Jones (1960), sifted soil rich in humus and fresh bovine dung were thoroughly mixed in the ratio 2:1 to half-fill the container and was tamped down to provide a slope of about 45°. Tap water was added slowly until the medium was thoroughly wet and free water extended half way up the slope. In this way larvae could choose the correct amount of moisture to suit their requirements.

Eggs laid on the filter paper in the egg laying tubes were added to the larval medium by placing the filter paper discs with one edge submerged so that the paper remained very moist and thus assisted the newly emerged larvae in gaining the larval medium proper. It was seldom necessary to add more water as little evaporation took place. Sometimes an "oily" film would form on the surface of the water and this was removed with tissue or filter paper, immediately it was noticed.

Pupae were recovered from the medium by flooding which caused them to wriggle out of their positions in the medium and to float to the surface where they were

removed by means of a suction tube. Many larvae were also recovered in this way but for almost complete recovery of larvae it was necessary to make use of the method originally described by Ladell (1936) (cited by Kettle & Lawson, 1952). In this method the larval medium was first washed through 10 and 20 mesh sieves to remove very large particles and then through a 100 mesh sieve which retained all larvae and pupae. These were subsequently floated in a saturated solution of magnesium sulphate, removed to clean water using a suction tube, killed in hot water and preserved in 70 per cent ethyl alcohol for later morphological studies.

Observations on the life-cycle made while rearing the various Culicoides species, as well as certain related minor experiments, will be discussed later, but it must be emphasized that a thorough study of the life-cycle of the various Culicoides species was not the aim of this investigation.

(c) Breeding Sites.

To determine the situation of breeding sites, two methods were adopted. These consisted of the use of emergence cages placed over the suspected breeding area, and the removal of breeding medium to cages for emergence in the laboratory.

The emergence cage most commonly used was designed and used by Dr. C.G.H. Fiedler and Dr. R.H. du Toit in 1949 (unpublished data). It consisted of a wooden framework 14 x 9 x 9 inches covered with organdie. Two sheets of glass converged internally so that they formed a non-return route to the organdie-covered section of the cage. Midges were removed from this section through a sleeve at one end of the cage. The lower edge of the cage was fitted with a three inch metal "skirt" which could be pushed into the soil of the suspected breeding site. This anchored the cage and also prevented escape beneath the edges.

Experience with these cages has shown that they are not ideal as the glass non-return section is not altogether proof against return of the midges to the soil, and they are expensive and cumbersome. It is suggested therefore that future investigations on breeding sites should make use of the trap recently described by Davies (1966). Emergence cages placed in situ have the

advantage of disturbing the breeding medium to the least possible extent but the disadvantage of having to be visited regularly. Furthermore, in winter few or no adults emerge so that it became essential to remove breeding medium to the controlled temperature of the laboratory. In the laboratory the medium was placed in a metal or glass container and covered with an organdie cage with perspex viewing window and sleeve for midge removal.

On occasions when a rapid check was required to determine whether a suspected breeding medium contained larvae, the method used by Bidlingmayer (1957) was adopted. This involved covering the surface of a sample of the medium with presifted washed sand to a depth of about one inch, adding water until the sand was just flooded, then leaving overnight for the larvae to make their way to the surface. The following morning the sand layer was quickly scooped out onto a sieve of a size sufficient to retain the sand but not larvae, and the larvae were washed out of the sand. The filtrate was then passed through a 100 mesh sieve and the contents of the sieve emptied into clean water. In this way the presence and approximate number of Culicoides larvae could be determined and the remainder of the sample could either be discarded or kept to determine the species present by adult emergence and identification.

(d) Hours of Activity.

Culicoides are seldom if ever seen during the daytime and appear to be active only at night as evidenced by large and regular light trap catches. To determine the period during the night when Culicoides are active, the cage in the light trap was replaced every two hours between 7 p.m. and 7 a.m. This was repeated on five nights in January, 1963. Each of the six two-hourly catches was counted in the laboratory the next morning.

On the nights analysed by this means, a thermohygrograph was operated a few feet from the light trap so that catches could be compared with temperature and relative humidity. Unfortunately it was not possible to record wind speed during these nights.

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To determine these times it was necessary to take regular samples of the light trap catches and to identify all the species present. A sample of between 500 and 1,000 specimens was preferred but if less than 500 then the entire catch was identified. The Onderstepoort catch was analysed at least twice a week between August, 1965 and May, 1966 (June and July having nil catches) and similar analyses were made of the "Kaalplaas" catches from November, 1965 onwards.

Identifications were made using the key drawn up by Fiedler (1951) and Caeiro's (1959) description of C. gulbenkiani.

3. DISCUSSION OF RESULTS

(a) Laboratory Studies on Life-cycle.

(1) Preoviposition period.

On numerous occasions when large numbers of eggs were required for attempts to start a colony, a thousand or more wild-caught midges, mostly C. pallidipennis, were fed on a rabbit's ear and provided with moist filter paper on which to lay eggs. At 72°F and about 45 per cent R.H. most eggs were laid three to four days after the blood-meal, while at 80°F eggs were laid after two days. This period was not determined for individual species.

(2) Eggs.

One batch of eggs is matured for each blood-meal taken (Kettle, 1962). By studying follicular relicts, Gluchova (1958) (cited by Kettle, 1962) has shown that up to four batches of eggs may be laid in nature by C. grisescens Edwards. The isolated specimens used in the present studies never laid more than one batch of eggs, however, since they either died immediately after egg laying or would not feed again.

Eggs of the various species varied in colour from light to very dark brown, but this colour difference was not consistent in every species. The eggs are normally laid in a double row resembling footprints. They are "sausage-shaped" being about 400 µ long and 50 µ wide.

To determine the number of eggs each female is capable of laying in one batch, the eggs were dissected from females three days after engorging on blood, or the number of eggs oviposited by a female were counted and to these were added unlaid eggs removed by dissection in distilled water.

The number of eggs varied considerably between species but also between individuals of the same species as can be seen in Table 1.

Table 1. Number of eggs laid in a single batch by Culicoides spp.

Species	Number of females	Number of eggs	Average /female
C. pallidipennis	9	41-86	69
C. nivosus	9	126-264	162
C. distinctipennis	6	106-236	140
C. pycnostictus	3	91-126	110
C. milnei	1	93	93
C. schultzei	1	92	92
C. bedfordi	1	122	122
C. ravus	1	119	119
C. babrius	1	70	70
C. magnus	1	142	142
C. gulbenkiani	1	55	55

At a temperature of 70 to 75°F the incubation period for C. pallidipennis, C. distinctipennis, C. pycnostictus, C. nivosus and C. milnei was three days and four days for C. magnus. Most of these periods were determined for a batch of about 100 eggs laid by a single female except in the case of C. pallidipennis where thousands of eggs from hundreds of females were observed.

To test the effect of desiccation on Culicoides eggs a mixed population of wild-caught Culicoides were allowed to lay eggs on moist filter paper over a period of three days so that eggs of all ages from newly laid to those about to hatch were present. Initially very slow desiccation was attempted by slowly drying out a moist pad of cotton wool on which the filter paper with the eggs rested. This was done at 80°F and 85 per cent R.H. It was found, however, that eggs do not gradually collapse as the paper dries out, but remain turgid and

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normal until the paper is completely dry when they then collapse. Slow desiccation thus seems impossible as eggs apparently only require 100 per cent R.H. in order to remain turgid. Thereafter only the filter paper discs of eggs were dried at 85 per cent R.H. After drying for a few hours most of the eggs had collapsed. About one per cent were still turgid and these did not collapse on further drying. On immersion in water these turgid eggs hatched while those which collapsed never recovered. This suggests that in nature most eggs will survive desiccation while they are in a saturated atmosphere and should further drying take place a few eggs may still survive to give rise to a new generation when suitable conditions return.

It is appreciated that more detailed and exact studies such as those by Parker (1950) on the eggs of Scottish midges, are possible, but this was not the aim of the present study. Parker found that most species could not survive 48 hours desiccation and that the age of the egg also affected its ability to survive desiccation. Parker, however, made use of a calcium chloride desiccator which cannot be compared with the conditions of the above experiments.

Thousands of eggs laid over three days by wild-caught Culicoides spp., mostly C. pallidipennis, were immersed in water. Hatching started soon after immersion and continued until most of the eggs had hatched. Inundation of eggs in nature should therefore not adversely affect hatching.

Hundreds of similar eggs were kept on moist cotton wool in a closed petri-dish in the refrigerator at 44°F (6.5°C). Eggs were removed to room temperature at intervals and the numbers hatching noted. After seven days all eggs hatched, after 14 days only 43 per cent were still viable and after 37 days no eggs hatched. The exact time needed to prevent all eggs from hatching was not determined.

(3) Larvae.

The mixture of soil and bovine dung used as a breeding medium was not equally acceptable to all species of Culicoides. The four species, C. nivosus, C. distinctipennis, C. pycnostictus and C. bedfordi, appeared to find this medium suitable for development as many reached the pupal stage within 10 to 20 days of the eggs being placed on the medium.

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Only a few C. schultzei and C. milnei developed to the adult stage, while much difficulty was experienced in rearing C. magnus and C. pallidipennis to the pupal stage, only a few specimens being successful in reaching this stage.

Culicoides larvae are normally found in the surface layer of the medium where they "snake" in and out, or emerge and retreat into burrows as described by Linley (1966). All the species studied except C. bedfordi appeared to have this habit, and on studying the medium under a dissecting microscope with incident light, the larvae of C. distinctipennis remained exposed for from 10 to 20 seconds, appearing to bask in the heat of the light before reversing below the surface. Larvae of C. bedfordi on the other hand were never seen during their entire development and were only found on washing the medium through sieves and floating in $MgSO_4$ solution.

It is normally difficult to decide what the larvae are feeding on. Kettle (1962) reviewed the literature and suggested that those larvae with heavily built pharynges feed on algae, fungi and bacterial films, while those with light pharynges may feed on algae or detritus or be carnivorous. Thomsen (1937) states that Culicoides larvae are carnivorous and occasionally cannibalistic which agrees with her earlier statement that "the carnivorous larvae have a long narrow head, with the mouth-parts directed anteriorly, a weakly sclerotized labium, and a single-toothed mandible". During the present study fourth stage C. milnei larvae were twice seen devouring, tail first, live second instar larvae of the same species, and a number of alcohol preserved C. nivosus fourth stage larvae were found with half-eaten smaller larvae protruding from their mouths. Cannibalism does therefore definitely occur amongst the species studied.

To determine whether larvae can withstand periods of immersion, fourth instar larvae of all the species studied except C. schultzei were placed in petri-dishes of water and observed. Larvae of C. pycnostictus, C. nivosus and C. magnus survived immersion at 72°F for six days and longer, while those of C. distinctipennis, C. pallidipennis, C. milnei and C. bedfordi were still alive after 13 days and longer. Since these tests were not repeated the differences in the periods of survival

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could possibly be due to the physiological state of the larvae or some other factor. The main point illustrated by these tests, however, is that the fourth stage larvae and possibly other stages are capable of surviving long periods of immersion which are likely to occur in nature after periods of heavy rains. Kettle (1962) states that pupation does not occur if larvae are kept flooded. In the present study the odd larva of both C. pallidipennis and C. bedfordi pupated while submerged but died soon afterwards.

Media containing fourth instar larvae of C. nivosus, C. pycnostictus and C. milnei were placed in a refrigerator at 44°F (6.5°C) for from 10 to 14 days. On returning the media to room temperature development was completed in the normal time. No dead larvae were noticed and it would appear that the low temperature did not adversely affect the larvae but temporarily brought development to a complete standstill. Periods of refrigeration longer than 14 days were never attempted. This was found to be a very useful method of delaying development and can be compared with winter conditions in nature where over-wintering takes place in the larval stage. Overwintering appears to take the form of retarded development rather than a diapause since a sample of breeding medium brought into the laboratory at any time of the year will almost immediately give rise to adults.

At 70 to 75°F the period from egg-laying to the first adults averaged 20 days, with emergence extending from 11 to 66 days after oviposition. Larval periods for six species varied between seven and 25 days and the pupal period averaged four days. Males were normally the first to emerge. Under laboratory conditions therefore the minimum period for a generation to be completed is about 25 days (i.e. the period egg to egg). These times are very similar to those found by Jones (1964) for his colony of C. variipennis sonorensis Wirth & Jones. This life-cycle took about 24 days i.e. egg two, larva 15 (12 days and longer), pupa three, period prior to blood ingestion one, and preoviposition period three days.

(4) Pupae.

Pupation takes place in mud at the surface of the medium, the pupa manoeuvering itself into an upright position with the two respiratory horns protruding from

the surface. On immersion most pupae wriggle free of the medium and float to the surface of the water where they hang from the horns. C. pallidipennis pupae, however, were the only ones which did not float to the surface, but after freeing themselves from the immersed substrate they lay loosely on its surface where they died within two days at room temperature. Immersed in water at 6.5°C they remained alive for more than six days but died within one day after removal to room temperature.

Dyce & Murray (1966) described three distinct patterns of pupal behaviour on immersion. Some species float to the surface and are unable to submerge. This is the case in all the South African species studied except C. pallidipennis. Other species repeatedly rise to the surface and then sink. In the third type of behaviour the pupae do not float but on immersion they actively burrow into the substratum. The behaviour of C. pallidipennis warrants it being placed in a fourth group since it does not float or burrow but lies loosely on the substratum until it drowns.

Cannon & Reye (1966) have found a similar type of behaviour in the Australian species C. brevitarsis Kieffer, but do not state whether immersion adversely affects this species. It is of interest to note that the larvae of this species were found in the moist lower regions of fairly dry cow pats. This type of pupal behaviour may therefore indicate a more terrestrial type of development.

(5) Adults.

Adults emerge from the pupal case by pushing forward the "operculum" and splitting the thorax dorsally for part of its length. Emergence can take place from floating pupae (except in C. pallidipennis) or from the surface of the larval medium. The adults are able to walk on water but wing surfaces must not touch the water surface as they appear to adhere to wet surfaces by surface tension. The body surface of the adults is rendered water repellent by a covering of fine hairs.

The life span of the adult in the field is unknown but newly emerged adults of the species studied survive from two to three weeks in the laboratory if provided with 10 per cent honey water and a relative humidity above 50 per cent. Laboratory reared adults would not

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feed on rabbits' ears or mate, however, so that it has been impossible to establish a colony. To date only Megahed (1956), Jones (1964) and Hair & Turner (1966) have managed to maintain Culicoides colonies. Megahed maintained a strain of C. nubeculosus (Meigen) for six years before it weakened and died out, while Jones has maintained a strain of C. variipennis sonorensis since 1957. The colony of C. guttipennis (Coquillet) established by Hair and Turner is only one year old. Establishment of a colony apparently depends upon the selection of a suitable strain from the offspring of many thousands of wild-caught midges.

(6) Conclusions.

These laboratory observations on the life-cycle of some of our Culicoides species may provide some information as to the ecology of these species.

The most important observation is the fact that all larvae are good swimmers and that only C. pallidipennis pupae do not float and soon drown. This means that the inundation by heavy rains of a breeding site common to a few species will affect them differently. In the larval stage they will all survive and will be able to swim to drier parts. If in the pupal stage, however, C. pallidipennis will drown while the pupae of the other species will float and give rise to adults. These adults will then be in a position to mate and lay eggs in the new breeding grounds created by the heavy rains. Inundated C. pallidipennis larvae will be forced to wait for the breeding site to dry out somewhat before further development, pupation and adult emergence can take place. This probably explains the low catches of C. pallidipennis during periods of good rain and their increase in numbers during drier periods which follow. The correlation between Culicoides catches and rainfall will be discussed later.

Another important conclusion which can be made from the study of the life-cycle is that a number of generations can be completed during the summer. Theoretically, according to developmental periods in the laboratory, this should amount to about one generation a month.

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(b) Breeding Sites

Culicoides breed in a variety of situations ranging from rot-holes in trees to manure and manure-polluted mud at the edge of pools. Kettle (1962) reviewed larval habitats for Culicoides in the world and concluded that "the larvae are neither genuinely aquatic nor terrestrial, but occupy the ecotone between, occurring in very wet soil". Very little is known of the breeding sites of Culicoides in the Ethiopian region. Carter et al. (1920) found Culicoides breeding in the following situations :-

C. accraensis - rot-holes in flamboyant tree, Cynometra sp., Eriodendron sp. and other trees.
C. inornatipennis Carter, Ingram and Macfie - rot-holes in Eriodendron sp. and in the stumps of banana plants.
C. clarkei Carter, Ingram and Macfie - rot-holes in Cynometra sp. and Eriodendron sp.
C. similis - bottom of waterlogged canoes.
C. schultzei - bottom of waterlogged canoes, in mud around puddles at a "stand-pipe" or tap and at washing place in the backwaters of a river.
C. eriodendroni - rot-holes in stump of Eriodendron sp. and mango tree.
C. punctithorax - as for C. eriodendroni
C. confusus Carter, Ingram and Macfie - rot-hole in Eriodendron sp.
C. nigripennis - rot-hole in mango tree.

De Meillon (1936, 1937) found C. neoserellus (syn. C. pycnostictus) in a rot-hole in a papaw tree; C. nivosus, C. pycnostictus, C. cornutus and C. schultzei in the mud around rain water pools; and C. engubandei in a rock pool. Fiedler (1951) found C. engubandei and C. onderstepoortensis in the mud at the edge of pools.

Since the early 1940's when Du Toit showed Culicoides to be the transmitters of bluetongue and horsesickness, searches have been conducted at Onderstepoort to discover the breeding places of these midges especially C. pallidipennis, which accounts for up to 90% of the light trap catches in summer. A thorough search was undertaken by Dr. R.H. du Toit and Dr. O.G.H. Fiedler in 1949-1950 (unpublished data). They started by taking samples of suspected breeding areas and recording emergence in the laboratory. Between 16th March and 10th October, 1949 they collected

36 samples of mud from the edge of a small water course (Newtown spruit) leading into Bon Accord dam near Onderstepoort, from around the dam itself and from numerous other water furrows. Their best results are summarized in Table 2.

Table 2. Summary of Culicoides emerging from mud samples from Newtown spruit.

Species	Date						Total
	16.3.49	18.3.49	7.6.49	7.6.49	11.7.49	11.7.49	
C. pycnostictus	43	7	43	431	137	273	934
C. cornutus	33	-	3	8	28	51	123
C. nivosus	56	-	4	29	20	9	118
C. schultzei	6	2	6	25	19	10	68
C. distinctipennis	-	-	-	20	19	-	39
C. ravus	15	2	-	18	-	2	37
C. pallidipennis	-	3	-	-	-	-	3
C. similis	-	2	-	-	-	-	2
Total	153	16	56	531	223	345	1,324

Table 2 shows C. pycnostictus to be predominant with C. pallidipennis and C. similis very rare. The table also shows that larvae are present in July; further evidence that Culicoides overwinter in the larval stage.

During the first three months of 1950 Du Toit and Fiedler used emergence traps. They showed that small numbers of C. pycnostictus were breeding in moist ground covered by short grass on the eastern side of Bon Accord dam when 23 adults were caught in 23 trap nights. On 27th January, 1950 one trap caught 33 C. pallidipennis over 8 nights from a swampy kikuyu grass-covered area at a leaking cement dam at "Kaalplaas". Thereafter 9 to 12 traps were used per night on a large kikuyu grass-covered area at Onderstepoort, part of the area being under water. Over 13 nights from 24th February to 20th March very good catches of C. pallidipennis were made, the catch being as follows :-

C. pallidipennis	661
C. nivosus	182
C. distinctipennis	58
C. pycnostictus	33
C. schultzei	24

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It is important to note that only the traps placed over swampy kikuyu grass-covered areas yielded C. pallidipennis.

The writer repeatedly tested similar situations between 1963 and 1965 but was never able to trap more than the odd adult of this species. These odd specimens were also recovered from very moist areas covered with kikuyu grass and from the banks of the Apies River. The other species mentioned in Table 2 were also found except C. cornutus which has never again been seen although so abundant in 1949. Every conceivable breeding site was investigated ranging from various types and ages of manure, algae in water troughs, compost, stagnant bogs, edges of rivers, lucerne fields, etc. Those samples which yielded fair numbers of adults are given in Table 3. Adults emerged from these samples over a period of from 13 to 22 days at ± 75°F.

It is a mystery why C. pallidipennis which is so abundant in the light trap (Table 7) cannot be found breeding in large numbers in nature. Jones (1961) has suggested that "A preponderance of one species in light traps is often a result of low-density breeding over an extensive area". This may well be the case with C. pallidipennis. Evidence was forwarded earlier that C. pallidipennis may develop in much drier habitats than commonly expected for Culicoides midges. A thorough examination of such sites may eventually reveal the breeding place of C. pallidipennis.

(c) Hours of Activity.

Kettle (1962) reviewed observations throughout the world on the time of flight of Culicoides species and concluded : "Most Culicoides are crepuscular, showing great activity at dawn and dusk. Most crepuscular species, except C. grahamii Austen, continue to be active throughout the night, although they are less abundant after midnight, and they also bite during the day on calm, dull days".

Reuben (1963) studying C. impunctatus Goetghebuer in Scotland used a suction trap and showed these midges to be active throughout the day but most active at night. Kitaoka & Morii (1964a) compared meteorological conditions with light trap catches of six Japanese Culicoides species. The different species varied in their time of flight, some being more abundant just after sunset and near sunrise, while others were present

Table 3. Summary of Culicoides emerging from mud samples from Onderstepoort area.

Date	Suspected breeding site	Culicoides species							
		nivosus	pycnostictus	distinctipennis	schultzei	pallidipennis	milnei	neavei	similis
16.7.63	Stagnant mud at bog near transport stable	6	83						
22.7.63	Stagnant mud in kikuyu field	20	9						
29.1.65	Mud from edge of slime-covered pool in dung-polluted paddock	630	21	2	1				
25.3.65	Dung-polluted mud at leaking water trough	1	44	3	1				
1.7.65	Mud from water-level of Apies river	13	51	11		3			
1.7.65	Mud from swampy kikuyu-covered area	4	7	21		2	2	1	
1.7.65	Mud from swampy veld-grass covered area near river	74	200	45	1	3	2	12	11
Total		798	415	82	3	8	4	13	11

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