Seasonal abundance and parity of stock-associated *Culicoides* species (Diptera: Ceratopogonidae) in different climatic regions in southern Africa in relation to their viral vector potential

G.J. VENTER[1], E.M. NEVILL[1] and T.C. DE K. VAN DER LINDE[2]

ABSTRACT

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Seasonal abundance and parity in *Culicoides* populations, in the vicinity of livestock, were determined at seven sites in five different climatic regions with 220 V down-draught blacklight-traps. In 418 collections made between October 1983 and December 1986, a total of 2 134 171 *Culicoides*, of which 342 571 were identified to species level and sexed, were collected; 267 of these collections (182 321 *Culicoides*) were graded for parity.

In the frost-free summer rainfall area, *Culicoides* were collected in large numbers in light-traps throughout the year; this implies breeding and possible virus transmission throughout the winter in certain parts of South Africa. However, where frost occurred, *Culicoides* numbers usually peaked in late summer and dropped sharply after the first frost. In the latter areas, small *Culicoides* collections during winter may be due to low winter temperatures and rainfall; low temperatures negatively affect adult activity and reduce the rate of development of larvae and pupae; low rainfall would lead to a reduction of available larval habitats. Relatively large numbers of *Culicoides* were collected in winter in the temperate frost-free winter rainfall area.

In each of the four summer rainfall areas, one *Culicoides* species remained dominant throughout the year: at two of these areas this species was *C. imicola*. Other abundant species in some of these summer rainfall areas were *C. schultzei* s.l. and *C. zuluensis*. In the winter rainfall area, *C. zuluensis, C. magnus, C. gulbenkiani* and *C. imicola* shared abundance.

It was established that abdominal pigmentation is an indicator of parity in *C. imicola* in South Africa. With the increase in *Culicoides* numbers towards the end of summer, there was also a rise in the proportion of parous (pigmented) females in most *Culicoides* species, which signifies a higher vector potential for African horsesickness and bluetongue towards the end of summer. This coincides with the seasonal occurrence of viral diseases transmitted by *Culicoides* species. Nulliparous (unpigmented) females of all *Culicoides* species were present throughout the year at all sites where *Culicoides* were continuously collected, confirming uninterrupted breeding in these areas.

Keywords: African horsesickness, age-grading, bluetongue, Ceratopogonidae, *Culicoides*, light-traps, livestock, parity, seasonal abundance, vector potential

INTRODUCTION

African horsesickness (AHS) and bluetongue (BT) occur annually, towards the end of summer, in most parts of South Africa and cause severe disease in horses and sheep, respectively (Verwoerd & Erasmus 1994; Coetzer & Erasmus 1994). The many serotypes of the orbiviruses responsible for these

[1] Entomology Division, Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110 South Africa. E-mail GERT@ moon.ovi.ac.za and ERROLN@moon.ovi.ac.za

[2] UOFS, Department of Zoology/Entomology, Faculty of Science, P.O. Box 339, Bloemfontein, 9300 South Africa. E-mail DVDL@rs.uovs.ac.za

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diseases are transmitted between their respective vertebrate hosts almost exclusively by haematophagous midges of the genus *Culicoides*, which are true biological vectors (Du Toit 1944; Tabachnick, Mellor & Standfast 1992). An initial step in the elucidation of the epidemiology of a viral disease is the identification of all potential vectors. To be eligible, a candidate's distribution must overlap that of the disease it is supposed to transmit, and it must also be common at the time of the year when virus transmission takes place (Standfast & Dyce 1972).

There is no evidence of transovarial arbovirus transmission in *Culicoides* (Nelson & Scrivani 1972; Allingham & Standfast 1990). Adult females become infected only after a bloodmeal has been taken from an infective host. In turn, it can transmit the virus to a susceptible host only after the completion of at least one or more gonotrophic cycles. If blood-feeding is interrupted, and only a partial bloodmeal is taken, the female will not recommence feeding on another host (Walker 1977). Possible mechanical transmission and resulting epidemiological implications are hereby eliminated (Davies 1990). The ratio of parous or pigmented females that have already completed at least one gonotrophic cycle, to nulliparous or unpigmented females that have not completed a gonotrophic cycle, can therefore be used to give an indication of the vector potential of a *Culicoides* population.

The only long-term study published on the seasonal abundance of *Culicoides* species in South Africa, was that of Nevill (1971) conducted over a period of 7 years at the Onderstepoort Veterinary Institute (OVI) between 1963 and 1971. No long-term parity studies have been conducted on any species of South African *Culicoides*. In the present paper, the light-trap collections at seven of the collection sites used for studies on the geographical distribution and relative abundance of *Culicoides* (Venter, Nevill & Van der Linde 1996a) were analysed further to determine and compare the seasonal abundance and parity of *Culicoides* species over a period of more than 2 years in five representative areas in different climatic regions of southern Africa.

MATERIALS AND METHODS

Light-trap collections and age-grading

Down-draught light-traps (220 V) equipped with 8 W blacklight tubes were used to collect *Culicoides* near livestock. Details of the collection method appear in Venter *et al.* (1996a).

The number of *Culicoides* per collection varied from zero to more than 100 000. An attempt was made to identify all the *Culicoides* in a collection, but if this was not possible, a random subsample of 1 000–2 000 insects was identified and sexed with the aid of a slide reference collection, preliminary keys and a wing picture atlas of Afrotropical *Culicoides* (R. Meiswinkel, OVI, unpublished data 1994). Catches that were not identified immediately were stored in 80 % ethanol.

For age-grading, the females of the most abundant *Culicoides* species in the collections at six of the seven light-trap sites were classified according to the abdominal pigmentation method of Dyce (1969) into the following categories:

- Nulliparous or unpigmented females
- Parous or pigmented females
- Gravid females with eggs visible in the abdomen
- Freshly bloodfed females
- To complete the analyses, males were also counted.

Study area

Seasonal abundance and parity in *Culicoides* populations, collected near livestock, were determined at seven sites in five different climatic regions. The location of these seven sites appears in Fig. 1 and Table 1 in Venter *et al.* (1996a). The most important climatic conditions in each of the five areas are summarized in Table 1 (Weather Bureau 1986) and are also included in Table 2 of Venter *et al.* (1996a). Table 1 shows the vegetation-zone classification for each area (Acocks 1988). The number of collections analysed for seasonal abundance and age-grading at each of the seven light-trap sites, and the period covered, are shown in Table 2.

Confirmation of the reliability of abdominal pigmentation as indicator of parity in *C. imicola*

Dyce (1969) showed that the red pigment, deposited in the wall of the abdomen during the first gonotrophic cycle, is a reliable indicator of parity in many Australian *Culicoides* species. The nature and origin of the pigment is unknown but it does not appear to be associated with blood digestion as it also develops in some autogenous species (Birley & Boorman 1982). The pigment is concentrated in the anterodorsal part and is paler towards the posterior and the central axis of the abdomen (Dyce 1969). Abdominal pigmentation is also a reliable indicator of parity in Kenyan species such as *C. pallidipennis* (= *C. imicola*), *C. schultzei* and *C. cornutus*, and in these species this method is favoured as the ovarial relics easily break on examination (Walker 1977).

Nulliparous (unpigmented) *C. imicola* females, collected live at the OVI, were artificially bloodfed according to the method described by Venter, Hill, Pajor & Nevill (1991). Engorged females were sorted on a chill-table. To maximize survival, they were kept in the dark at 23,5 °C, 60% RH and fed a 10% sucrose solution on a cotton-wool pledget. An oviposition substrate, consisting of a petri-dish with damp cotton wool with a double layer of filter paper on top, was provided. Various groups, fed on different days, were examined for pigmentation on days 1–10 after blood-feeding.

Of 354 artificially fed *C. imicola* 216 survived long enough for a pigment evaluation to be made. Fresh blood was visible in the abdomen for 1–2 d after feeding. Pigment was already visible in the abdomen from the second day after blood-feeding and oviposition started as early as 2 d after blood-feeding. However, there were females in which eggs were still visible in the abdomen after 10 d.

Non-bloodfed nulliparous *C. imicola* females, kept under the same conditions as the engorged females, were still unpigmented after 10 d.

Sixteen females that fed on blood, did not develop eggs and were still nulliparous (unpigmented) 7–10 d after they had been fed. In all cases where eggs were developed after the midges had fed, pigment was laid down in their abdomens. It was therefore confirmed that pigmentation can be coupled with parity in the dominant stock-associated *Culicoides* species in South Africa, namely *C. imicola*.

Although it has not been experimentally established that pigmentation is a reliable indicator of parity in *C. zuluensis*, *C. magnus*, and *C. gulbenkiani*, the validity of the technique for various species worldwide has encouraged us to examine these species also. The results obtained must, however, be regarded as tentative.

RESULTS AND DISCUSSION

A total of 2 134 171 *Culicoides,* of which 342 571 were identified to species level and sexed, were trapped in 418 collections made at seven sites between October 1983 and December 1986 (Table 2). In 267 of these collections (774 810 *Culicoides*), the most abundant dominant *Culicoides* species and *C. imicola* were graded for parity (Table 2).

The number of collections made at each site was dependent on the collector present and most of the collections were made during summer. At Upington (Veekos and Karakul), only two collections were made in winter. At Roma only one collection was made in winter. This made reliable statistical comparison of the data difficult.

The monthly variation in *Culicoides* numbers and the seasonal abundance of the most abundant species (and *C. imicola* when it was not the most abundant species) trapped at each site are shown in Fig. 1 and Table 3, respectively. At each of the four summer rainfall areas, namely Eiland, Onderstepoort, Upington and Roma, one *Culicoides* species remained dominant throughout the year (Table 3). The seasonal variation in the four most abundant species at Stellenbosch (winter rainfall area) can be seen in Table 3.

The number of light-trap collections identified each month, the monthly average number of parous *Culicoides* of the most abundant species collected per night and the percentage parous females at each site, are shown in Table 4 for six of the seven study sites.

TABLE 1 Details of the five representative *Culicoides* light-trap collection areas (Weather Bureau 1986)*

Collection site	Grid reference	Height above sea level) (m)	Average annual daily		Lowest ave. daily min. temp. (°C)	Average total of days with min. temp.		Rainfall (mm)			Vegetation zone (Acocks 1988)
			Max. temp. (°C)	Min. temp. (°C)		< 0 °C	< 5 °C	Summer Oct. to March	Winter April to Sept.	Total	
Eiland, N. Province	23°40′S, 30°45′E	400	28,9	14,2	1,9	0,4	22,5	449	79	528	North-eastern mountain sourveld
Onderstepoort, Gauteng Province	25°39′S 28°11′E	1 219	26,3	9,3	–3,7	32,8	103,6	604	102	706	Other turf thornveld
Roma, Lesotho	29°27′S, 27°45′E	1 690	22,0	7,6	–4,4	56,8	135,1	599	167	766	*Cymbopogon-Themeda* veld (sandy)
Upington, N. Cape Province	28°28′S, 21°20′E	793	28,3	12,1	–3,2	16,4	69,9	147	48	195	Orange River broken veld
Stellenbosch, W. Cape	33°56′S, 18°52′E	119	22,5	10,6	2,7	0,1	46,3	146	473	619	Renoster bushveld; coastal region

* These data were included in Tables 1 and 2 of Venter *et al.* (1996a)

TABLE 2 Details of total *Culicoides* light-trap collections identified in seasonal abundance study and age-graded at seven sites in the vicinity of livestock in five representative climatic areas in southern Africa

Site	*Culicoides* identified*					*Culicoides* age-graded						
	Time span	No. of collections	No. of *Culicoides* collected	Average catch size	No. of *Culicoides* identified	Time span	No. of collections graded	Total no. *Culicoides* collected	Average catch size	Species graded	No. collected	No. graded
Eiland	March 1984– June 1986	39	1 730 191	44 364	90 752	Sept. 1985– July 1986	21	516 558	24 598	*C. imicola*	448 835	41 135
Onderstepoort												
Stable 3	Oct. 1984– Aug. 1986	146	104 720	717	88 358	Oct.1984– Aug. 1986	144	104 541	726	*C. imicola*	102 433	86 314
Camp 168	March 1984– Dec. 1986	82	92 540	1 129	68 202	Oct. 1984– Aug. 1986						
Roma	Sept. 1985– Sept. 1986	32	32 778	1 024	31 897	Sept. 1985– Sept. 1986	32	32 778	1 024	*C. zuluensis* *C. imicola*	24 321 551	23 729 547
Upington												
Karakul	Dec. 1983– May 1986	35	4 646	133	4 646 6 678	Sept. 1985– May 1986	23	3 272	142	*C. schultzei* s.l. *C. imicola*	1 704 455	1 704 455
Veekos	Oct. 1983– June 1986	22	23 999	1 091		Sept. 1985– June 1986	14	8 907	636	*C. schultzei* s.l. *C. imicola*	8 332 281	3 652 176
Stellenbosch	Jan 1984– Nov. 1986	62	145 297	2 344	52 039	July 1985– Nov. 1986	33	108 754	3 296	*C. magnus* *C. zuluensis* *C. gulbenkiani* *C. imicola*	34 746 32 374 18 336 14 039	6 904 5 148 5 810 6 747
Totals and means		418	2 134 171	5 106	342 571		267	774 810	2 902		686 407	182 321

* These data were included in Table 3 of Venter *et al.* (1996a)

Eiland, Northern Province

Eiland, a mineral spring and holiday resort in the Hans Merensky Nature Reserve, is situated in the subtropical Lowveld of the Northern Province. As long-term weather records for Eiland were not available, those for nearby Chester (23°47′S, 30°36′E), where the climate closely resembles that of Eiland, were used for a description of the general climate of this area (Table 1). With annual average maximum and minimum temperatures of respectively 28,9 °C and 14,2 °C, this was the hottest area in the survey, and on average there are 32,1 d annually when the maximum temperature is above 35 °C. This is a medium-high summer rainfall area (528 mm) with no or only light frost occurring in winter. There are, on average, only 0,4 d annually when the minimum temperature is below freezing point and a further 22,5 d when the minimum temperature is below 5 °C (Table 1).

The light-trap was situated at a horse stable that housed about 30 horses each night and that was surrounded by irrigated kikuyu (*Pennisetum clandestinum*) lawn. Except for the horses, there were no other stock animals or farming activities in the light-trap vicinity in this predominantly cattle-ranching region. It was, however, shown that this light-trap is representative of *Culicoides* found in the vicinity of livestock in the Eiland area (Venter *et al.* 1996a).

Seasonal abundance of Culicoides *species at Eiland*

A total of 1 730 191 *Culicoides*, of which 90 752 were identified to species level, were collected in 39 light-trap collections ($\bar{x}$ = 44 364) made from March 1984 to July 1986 at Eiland for studies on seasonal abundance (Table 2).

C. imicola, the only proven vector of BT and AHS virus in South Africa (Du Toit 1944; Du Toit, unpublished data cited in Wetzel, Nevill & Erasmus 1970), accounted for 93% of the *Culicoides* species collected at Eiland. The proportion of *C. imicola* in the catches was never lower than 82% in any season (Table 3). The seasonal abundance of *Culicoides* as demonstrated in Fig. 1a is therefore dictated by *C. imicola* numbers. From these results it is clear that even if *C. imicola* were to have a low vector capacity for virus transmission, its superabundance would make it the strongest vector candidate in this area. The second most abundant group, *C. schultzei* s.l., which can consist of up to five different species (Cornet & Brunhes 1994), accounted for less than 6% of all *Culicoides* species collected (Venter *et al.* 1996a).

From the light-trap results it can be seen that at Eiland *Culicoides* occur throughout the year and can be collected in large numbers even in winter (Fig. 1a; Table 3). This is probably because of the relatively high winter temperatures and the absence of frost (Table 1). Nulliparous *C. imicola* females collected in winter at Eiland confirms continuous breeding (Table 4). This finding as well as the large *Culicoides* collections which can be made as early as October (Fig. 1a), suggest that the sharp decline in light-trap collections of *Culicoides* in July and August is possibly the result of reduced activity rather than an actual reduction in the size of the *Culicoides* population. This supports the findings by Nevill (1971) in 1967/68 at Onderstepoort, when large collections in autumn gave rise to large collections in the following spring.

Parity of Culicoides *species at Eiland*

Of the 39 light-trap collections made at Eiland, 21 were graded for parity in *C. imicola* (Table 2). Blood-feds, gravid females and males together made up less than 4% of the collections. This was characteristic of nearly all collections made in this survey and of light-trap collections in general (Kettle 1962; Walker & Boreham 1976).

The large percentage parous females (Table 4), together with the large numbers of *Culicoides* collected in the autumn and winter at Eiland (Fig. 1a), suggests a high vector potential for *C. imicola.* The infection rate of this species for BT virus, serotypes 3 and 6, was established at 31 and 24%, respectively (Venter *et al.* 1991). Results of studies on zebra in the adjacent Kruger National Park showed that many young zebra became infected with AHS and that they circulate the virus in winter (Barnard 1993). Furthermore, results of a virus survey of 25 sites in different climatic zones throughout South Africa, conducted from 1979–1985 by Nevill, Erasmus & Venter (1992) showed that the highest virus-isolation rates were from *Culicoides* light-trap catches from Eiland. When these facts are combined, they indicate that apart from Eiland being suitable for continuous virus transmission throughout the year, it can also be regarded as an enzootic zone for AHS, and probably for BT as well.

Onderstepoort, Gauteng Province

The OVI is 1 219 m above sea level and consequently the winters are much more severe than at Eiland. There are, on average, 32,8 d annually, when the minimum temperature is below freezing point, and a further 70,8 d when it is below 5 °C (Table 1). Rainfall (706 mm) is higher than at Eiland (Table 1).

Collections were made at two sites at the OVI, namely stable 3 and camp 168. The light-trap at stable 3 was surrounded by buildings which restricted the visibility of the light-trap, with no natural vegetation or possible *Culicoides* breeding sites in sight. Livestock at the light-trap included a variable number of cattle and sheep.

TABLE 3 Seasonal abundance of the most abundant *Culicoides* species (and *C. imicola* if it was not the most abundant species) collected at each of seven collection sites, expressed as a percentage of the total number of *Culicoides* collected per night. The number of collections at each site as well as the average catch size per night for each season are also shown. Collections were made between October 1983 and December 1986

Site	Spring Sept. to Nov.	Summer Dec. to Feb.	Autumn March to May	Winter June to Aug.
Eiland				
C. imicola	86,4	82,6	98,0	97,2
No. of collections	16,0	2,0	13,0	8,0
Average catch size	41 634,4	25 010,0	71 304,4	10 883,0
Onderstepoort				
Stable 3				
C. imicola	96,3	98,2	96,1	97,4
No. of collections	35,0	25,0	26,0	60,0
Average catch size	454,0	1 522,3	1 784,6	91,4
Camp 168				
C. imicola	74,3	77,4	85,6	70,9
No. of collections	26,0	10,0	23,0	23,0
Average catch size	871,7	2 913,9	1 690,3	80,9
Roma				
C. zuluensis	55,1	75,7	82,3	80,0
C. imicola	0,1	1,6	2,9	0,0
No. of collections	13,0	10,0	8,0	1,0
Average catch size	395,2	1 905,5	1 073,3	5,0
Upington				
Karakul				
C. schultzei s.l.	55,0	46,8	35,5	37,6
C. imicola	8,3	9,6	15,8	0,9
No. of collections	11,0	10,0	12,0	2,0
Average catch size	232,0	129,9	57,1	54,5
Veekos				
C. schultzei s.l.	62,1	88,7	83,6	75,9
C. imicola	4,4	5,1	9,9	13,8
No. of collections	11,0	6,0	3,0	2,0
Average catch size	1 610,3	957,4	171,0	14,5
Stellenbosch				
C. magnus	36,6	12,3	4,8	44,1
C. gulbenkiani	20,8	27,0	38,3	40,9
C. zuluensis	31,7	7,9	17,9	24,2
C. imicola	3,5	31,6	28,6	4,0
No. of collections	18,0	8,0	13,0	23,0
Average catch size	4 750,3	2 068,6	2 011,9	743,0

At camp 168 the light-trap was situated in more natural surroundings and only 10 m from a ground-drainage canal, which was overgrown with natural (pioneer) vegetation. Livestock in the vicinity included horses and cattle. It was shown that this light-trap is representative of *Culicoides* found at livestock in the wider Onderstepoort area (Venter, Meiswinkel, Nevill & Edwardes 1996b).

Seasonal abundance of Culicoides *species at Onderstepoort*

At stable 3 a total of 104 720 *Culicoides* were collected in 146 light-trap collections ($\bar{x}$ = 717) from August 1984 to October 1986 for seasonal abundance studies (Table 2). At camp 168 a total of 92 540 *Culicoides* were collected in 82 light-trap collections

($\bar{x}$ = 1 129) from March 1984 to December 1986 (Table 2). The latter catches were analysed for seasonal abundance only.

If the species composition and seasonal abundance as determined at stable 3 and camp 168 are compared, a similar pattern is found (Fig. 1b; Table 3). As at Eiland, these light-trap collections were also dominated by *C. imicola* and the variation in total *Culicoides* numbers as demonstrated in Fig. 1b are therefore also dictated by *C. imicola* numbers (Table 3). Also here it is clear that even if *C. imicola* were to have a low vector capacity for virus transmission, its superabundance would make it the strongest vector candidate in this area (Venter *et al.* 1996b).

At Onderstepoort *Culicoides* numbers started to rise from December onwards and the largest collections were made in February/March (Fig. 1b). This was followed by a decline in *Culicoides* numbers, which coincided with the decline in minimum temperatures, and only very small numbers were collected from June to August (Fig. 1b). Much smaller numbers of *Culicoides* were collected in winter at Onderstepoort ($\bar{x}$ < 100) than at Eiland ($\bar{x}$ > 10 000) (Table 3). *Culicoides* numbers at Onderstepoort took longer to build up to a peak (February/March) than at Eiland, where numbers peaked as early as October. The peak in *Culicoides* numbers at Onderstepoort was also much lower than at Eiland (Fig. 1).

Parity of Culicoides *species at Onderstepoort*

The number of light-trap collections analysed for parity at stable 3 are shown in Table 2. The weekly variation (17 point running mean) in parous/nulliparous numbers of *C. imicola* is shown in Fig. 2.

Nulliparous, unpigmented ($\bar{x}$ = 63,5%) and parous, pigmented *C. imicola* females without a bloodmeal or eggs ($\bar{x}$ = 32,2%) formed the most important part of the collections, which indicates that mainly females seeking a bloodmeal were captured (Fig. 2).

The presence, throughout the year, of more nulliparous than parous females is an indication that breeding takes place continuously (Fig. 2; Table 4).

The fluctuation in the parous rate is the same as found for *C. imicola* in Kenya, and can be an indication of overlapping generations (Walker 1977). The influence of rainfall on the breeding sites of *Culicoides* can influence the fluctuation in the parous/nulliparous numbers. High rainfall can lead to the drowning of pupae of *C. imicola* and to larvae delaying pupation until suitable conditions return (Nevill 1967). This will lead to a decrease in the number of nulliparous females, but at the same time rainfall will increase the number of breeding sites, which in turn will lead to an increase in the numbers of nulliparous females a few weeks after the rainfall. High rainfall in December 1984 could therefore have been the reason for the sharp decline in numbers of *C. imicola* in January 1985 (Fig. 2) and for the rise in numbers a month later. To establish a correlation between rainfall and parous/nulliparous levels, however, will require a much more intensive study over a longer period.

Because only parous females transmit virus, the vector potential of a *Culicoides* population will be at its maximum when large numbers of parous females are present. The average number of parous females per trap-night collected during the study period was 325 (Fig. 2). From October 1984 to April 1985 and from December 1985 to May 1986 the number of parous females was above the 325 average and it can be argued that this will be the best time for virus transmission (Fig. 2). This coincides with the seasonal occurrence of BT (Nevill 1971; Verwoerd & Erasmus 1994) and AHS (Coetzer & Erasmus 1994) outbreaks in the Onderstepoort area.

Roma, Lesotho

Light-trap collections were made at St. Mary's High School in Roma, western Lesotho (Venter & Sweatman 1989). As long-term weather records were not available for Roma, those for nearby Ladybrand (29°10´S, 27°45´E, height above sea level = 1 612 m) and Modderpoort (29°06´S, 27°27´E, height above sea level = 1 610 m) of which the climates closely resemble that of Roma, were used for a description of the general climate for this area (Table 1). With annual average maximum and minimum temperatures of 22,0 °C and 7,6 °C, respectively, this was the coldest area in this survey. It is a high-lying summer rainfall area with very cold winters with severe frost and high rainfall (766 mm). There are, on average, 56,8 d annually when the minimum temperature is below freezing point and for a further 78,3 d the minimum temperature is below 5 °C (Table 1).

The light-trap was permanently sited under the eaves of an open barn which nightly housed 12 cattle. Within 300 m of the trap were a few sheep, chickens and pigs, but no horses (Venter & Sweatman 1989).

Seasonal abundance of Culicoides *species at Roma*

A total of 32 778 *Culicoides* were collected in 32 light-trap collections ($\bar{x}$ = 1 024) between September 1985 and September 1986 (Table 2). The monthly variation in *Culicoides* numbers at Roma is shown in Fig. 1c. Contrary to the first two areas, *C. imicola* numbers were small and never accounted for more than 3% of the *Culicoides* collected (Table 3). *C. zuluensis* was the most abundant species throughout the year and its proportion in the collections varied from

TABLE 4 Seasonal changes in parity of the most abundant *Culicoides* species at each of six collection sites. Included are the number of collections graded each month, the average number of parous females per collection, and the percentage parous females at each site (in brackets)

Site and species		Spring			Summer			Autumn			Winter		
		Sept.	Oct.	Nov.	Dec.	Jan.	Feb.	March	April	May	June	July	Aug.
Eiland													
C. imicola:	1985/86	5	6	1	–	–	–	2	3	1	2	1	–
		5 485,6	15 346,2	66,0	–	–	–	6 377,0	11 340,0	10 525,0	17 109,5	215,0	–
		(23,4)	(55,1)	(42,9)	–	–	–	(49,7)	(64,0)	(54,3)	(56,8)	(37,9)	–
Onderstepoort: Stable 3													
C. imicola:	1984	–	–	–	–	–	–	–	–	–	–	–	1
		–	–	–	–	–	–	–	–	–	–	–	20,0
		–	–	–	–	–	–	–	–	–	–	–	(23,0)
	1984/85	1	6	5	4	5	3	5	4	4	9	12	8
		521,0	181,7	424,8	993,0	315,4	390,7	811,8	909,3	148,8	48,2	9,0	28,3
		(33,4)	(29,5)	(36,8)	(39,0)	(41,9)	(30,5)	(38,1)	(41,8)	(40,7)	(26,1)	(12,6)	(14,4)
	1985/86	5	7	5	4	5	4	4	4	5	8	11	9
		145,4	79,6	87,8	192,8	596,8	653,3	938,5	370,8	383,8	21,7	6,0	23,8
		(42,5)	(37,8)	(36,0)	(30,8)	(32,6)	(34,1)	(34,6)	(34,8)	(44,2)	(41,6)	(26,6)	(31,3)
	1986	5	1	–	–	–	–	–	–	–	–	–	–
		88,0	91,0	–	–	–	–	–	–	–	–	–	–
		(43,9)	(35,0)	–	–	–	–	–	–	–	–	–	–
Roma													
C. zuluensis:	1985/86	2	2	6	3	4	3	4	3	1	1	–	–
		34,5	34,5	112,5	144,7	437,1	1 229,7	478,0	182,7	0,0	0,0	–	–
		(22,8)	(19,3)	(35,7)	(29,2)	(37,9)	(42,1)	(33,3)	(39,0)	–	–	–	–
	1986	3	–	–	–	–	–	–	–	–	–	–	–
		17,0	–	–	–	–	–	–	–	–	–	–	–
		(26,2)	–	–	–	–	–	–	–	–	–	–	–
C. imicola:	1985/86	1,5	–	–	0,7	3,9	23,3	13,3	6,7	0,0	0,0	–	–
		(75,0)	–	–	(66,7)	(43,0)	(28,6)	(20,8)	(25,7)	–	–	–	–
	1986	–	–	–	–	–	–	–	–	–	–	–	–
Upington, Karakul													
C. schultzei s.l.:	1985/86	4	2	4	1	4	1	3	3	1	–	–	–
		2,0	3,5	17,3	2,0	9,0	0,0	1,0	0,0	0,0	–	–	–
		(4,5)	(7,1)	(8,7)	(5,9)	(24,0)	–	(11,1)	–	–	–	–	–
C. imicola:	1985/86	0,0	0,0	3,8	2,0	0,8	0,0	1,3	0,0	0,0	–	–	–
		–	–	(7,4)	(16,7)	(10,7)	–	(16,7)	–	–	–	–	–
Upington, Veekos:													
C. schultzei s.l.:	1985/86	3	2	3	1	–	1	–	–	2	2	–	–
		7,0	24,0	86,5	776,0	–	8,0	–	–	38,5	2,5	–	–
		(34,2)	(8,4)	(9,8)	(20,1)	–	(17,4)	–	–	(28,0)	(17,2)	–	–
C. imicola:	1985/86	1,7	3,0	2,3	36,0	–	2,0	–	–	4,0	0,5	–	–
		(17,2)	(14,3)	(12,6)	(40,9)	–	(28,6)	–	–	(45,3)	(50,0)	–	–
Stellenbosch													
C. magnus:	1985	–	–	–	–	–	–	–	–	–	–	1	4
		–	–	–	–	–	–	–	–	–	–	36,0	32, 8
		–	–	–	–	–	–	–	–	–	–	(14,5)	(11,2)
	1985/86	4	3	4	1	2	–	1	2	–	2	3	2
		725,0	905,3	65,8	38,0	165,5	–	60,0	616,0	–	20,0	7,3	168,0
		(12,6)	(34,8)	(37,1)	(25,2)	(27,8)	–	(36,8)	(38,9)	–	(31,5)	(11,0)	(23,7)
	1986	2	1	1	–	–	–	–	–	–	–	–	–
		99,0	738,0	83,0	–	–	–	–	–	–	–	–	–
		(28,5)	(11,0)	(21,3)	–	–	–	–	–	–	–	–	–
C. zuluensis:	1985	–	–	–	–	–	–	–	–	–	–	18,0	10,0
		–	–	–	–	–	–	–	–	–	–	(11,3)	(3,2)
	1985/86	288,0	336,0	55,8	49,0	192,0	–	15,0	360,5	–	54,5	1,7	66,0
		(9,5)	(41,3)	(34,0)	(33,6)	(40,0)	–	(26,3)	(34,9)	–	(15,1)	(5,7)	(17,1)
	1986	29,5	1 140,0	75,0	–	–	–	–	–	–	–	–	–
		(11,1)	(41,7)	(25,5)	–	–	–	–	–	–	–	–	–
C. gulbenkiani:	1985	–	–	–	–	–	–	–	–	–	–	44,0	36,3
		–	–	–	–	–	–	–	–	–	–	(44,0)	(26,9)
	1985/86	181,0	611,3	58,3	85,0	246,0	–	15,0	688,0	–	187,0	3,3	133,5
		(24,6)	(45,0)	(31,2)	(37,4)	(44,2)	–	(83,3)	(44,2)	–	(38,1)	(29,2)	(28,8)
	1986	103,0	1 128,0	30,0	–	–	–	–	–	–	–	–	–
		(16,0)	(34,5)	(33,3)	–	–	–	–	–	–	–	–	–
C. imicola:	1985	–	–	–	–	–	–	–	–	–	–	0,0	3,3
		–	–	–	–	–	–	–	–	–	–	–	(20,2)
	1985/86	75,3	2,0	37,3	191,0	607,5	–	168,0	854,5	–	6,0	0,3	0,0
		(36,9)	(27,8)	(39,8)	(38,4)	(30,0)	–	(35,9)	(34,2)	–	(19,1)	(5,6)	–
	1986	0,0	68,0	14,0	–	–	–	–	–	–	–	–	–
		–	(17,2)	(23,7)	–	–	–	–	–	–	–	–	–

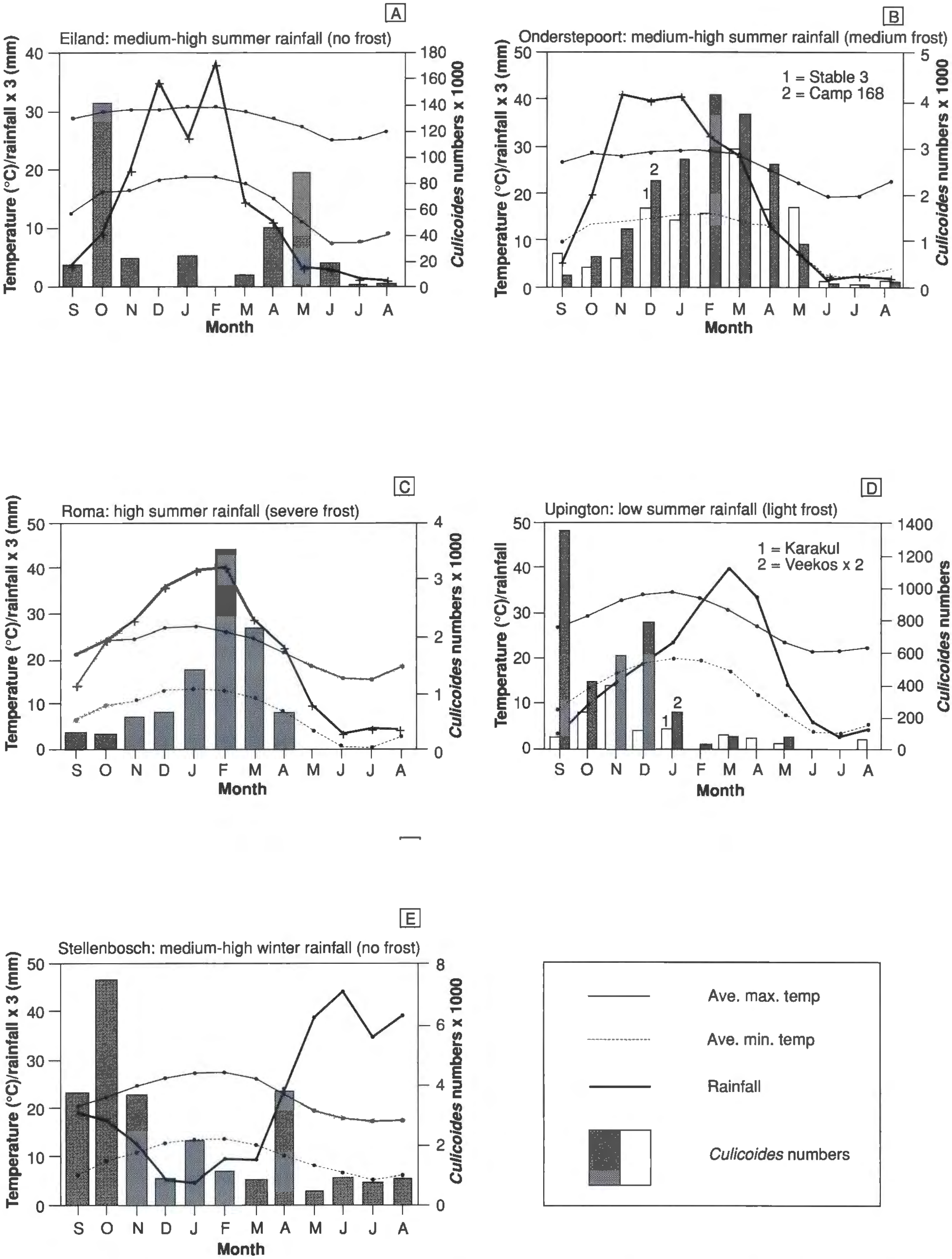

FIG. 1 Average monthly variation in *Culicoides* numbers, calculated over a 12–32-month period (1983–1986) at (A) Eiland, (B) Onderstepoort (stable 3 and camp 168), (C) Roma, (D) Upington (Karakul and Veekos) and (E) Stellenbosch, together with minimum and maximum temperature and rainfall at each location

55,1 % in spring to 82,3% in autumn (Table 3). Variation in *Culicoides* numbers at Roma is therefore due mainly to variation in the numbers of *C. zuluensis*. Other abundant *Culicoides* species in the spring collections were *C. pycnostictus, C. magnus* and *C.* sp. 35, a member of the *C. schultzei* group (Venter & Sweatman 1989).

Culicoides numbers increased from January onwards and peaked towards the end of summer in February (Fig. 1c). This was followed by a decline in March, which coincided with the drop in minimum and maximum temperatures. Only very small collections were made in May ($\bar{x}$ = 3) and June ($\bar{x}$ = 5), while no *Culicoides* were collected in July and August (Venter & Sweatman 1989).

Parity of Culicoides *species at Roma*

Age-grading of the most abundant species (*C. zuluensis*) and *C. imicola* was done on all 32 collections made at Roma. The number of light-trap collections made and the monthly average number of parous *C. zuluensis* and *C. imicola* collected per night, are shown in Table 4.

If pigmentation is a reliable indicator of parity in *C. zuluensis*, the results indicate that the vector potential is highest in February (Table 4), which is the time of year when the highest *Culicoides* numbers are found (Fig. 1c). The lower percentage of parous females (Table 4) as well as the smaller number of *Culicoides* found in spring (Fig. 1c) indicate a low vector potential of *C. zuluensis* in spring (Table 4). The largest average number of *C. imicola* (23,3) per collection was also collected in February. This coincides with the time of occurrence of BT, AHS and ephemeral fever outbreaks in most of the temperate parts of South Africa (Verwoerd & Erasmus 1994; Coetzer & Erasmus 1994; St. George 1994).

Upington, Northern Cape Province

With an annual rainfall of only 195 mm, Upington was the most arid area in this survey. It is an area with hot summers and cold winters with light frost. There are, on average, 69,1 d annually when the maximum temperature is above 35 °C and 16,4 d when the minimum temperature is below freezing point, and a further 53,5 d when it is below 5 °C (Table 1).

Collections were made at two sites in the Upington area, namely Karakul and Veekos. Karakul is a research station and experimental farm near Upington. It is 10 km from the Orange River and has no irrigation. For comparison, light-trap collections were also made at Veekos, an experimental farm situated on the banks of the Orange River and which is irrigated from the river. Sheep were the dominant stock species in the vicinity of the light-trap at both these sites.

Seasonal abundance of Culicoides *species at Upington*

The total number of *Culicoides* collected and identified for the seasonal abundance study as well as the time span over which collections were made at Karakul Research Station and at Veekos Experimental Farm are shown in Table 2.

Far more *Culicoides* were collected at Veekos ($\bar{x}$ = 1 091) than at Karakul ($\bar{x}$ = 133) (Table 2; Fig 1d) (Venter *et al.* 1996a). This is understandable as local conditions at each of these two sites differ greatly. Veekos is far more suitable for *Culicoides*, as the availability of water increases the likelihood of breeding sites, while vegetation flanking the river and irrigated areas provides food and shelter for a greater variety of possible hosts. Added to this, a more suitable micro-climate aids survival of *Culicoides* adults.

The representation of *C. schultzei* s.l., the most abundant species, varied from 55,0% in spring to 35,5% in autumn at Karakul, and from 62,1 % in spring to 88,7 % in summer at Veekos (Table 3). Other abundant species at both these sites were *C. nivosus* (16,8 %), *C. bedfordi* (14,5%) and *C. imicola* (9,8%) (Venter *et al.* 1996a). The monthly variations in *Culicoides* numbers at Karakul and Veekos are shown in Fig. 1d. Rainfall is relatively low and apparently has no influence on the seasonal variation in *Culicoides* numbers at either Karakul or Veekos. Although *Culicoides* collections were small throughout the year, noticeably more insects were collected in October/November before the peaks in rainfall and the extremely high summer temperatures (Fig. 1d). *Culicoides* numbers were much smaller at Karakul than at any other collection site (Fig. 1, Table 2). As at Roma, no *Culicoides* were collected during the greater part of winter. *Culicoides* were collected in only two collections which were made in June (Table 4). Low winter temperatures together with hot dry summers, apparently inhibited *Culicoides* numbers at Karakul.

Parity of Culicoides *species at Upington*

The number of *Culicoides* collected and graded for parity as well as the time span during which collections were made at Karakul and Veekos, are shown in Table 2. The number of light-trap collections made each month and the monthly average number of parous *C. schultzei* s.l. and *C. imicola* collected per night at Karakul and Veekos are shown in Table 4.

At Karakul parous females of both *C. schultzei* s.l. and *C. imicola* were poorly represented in the collections (Table 4). This suggests that survival of *Culicoides* in this, at times, harsh climate is poor and that the vector potential of both these species is therefore very low and the possibility of virus transmission very slight. Virus isolation attempts from 51

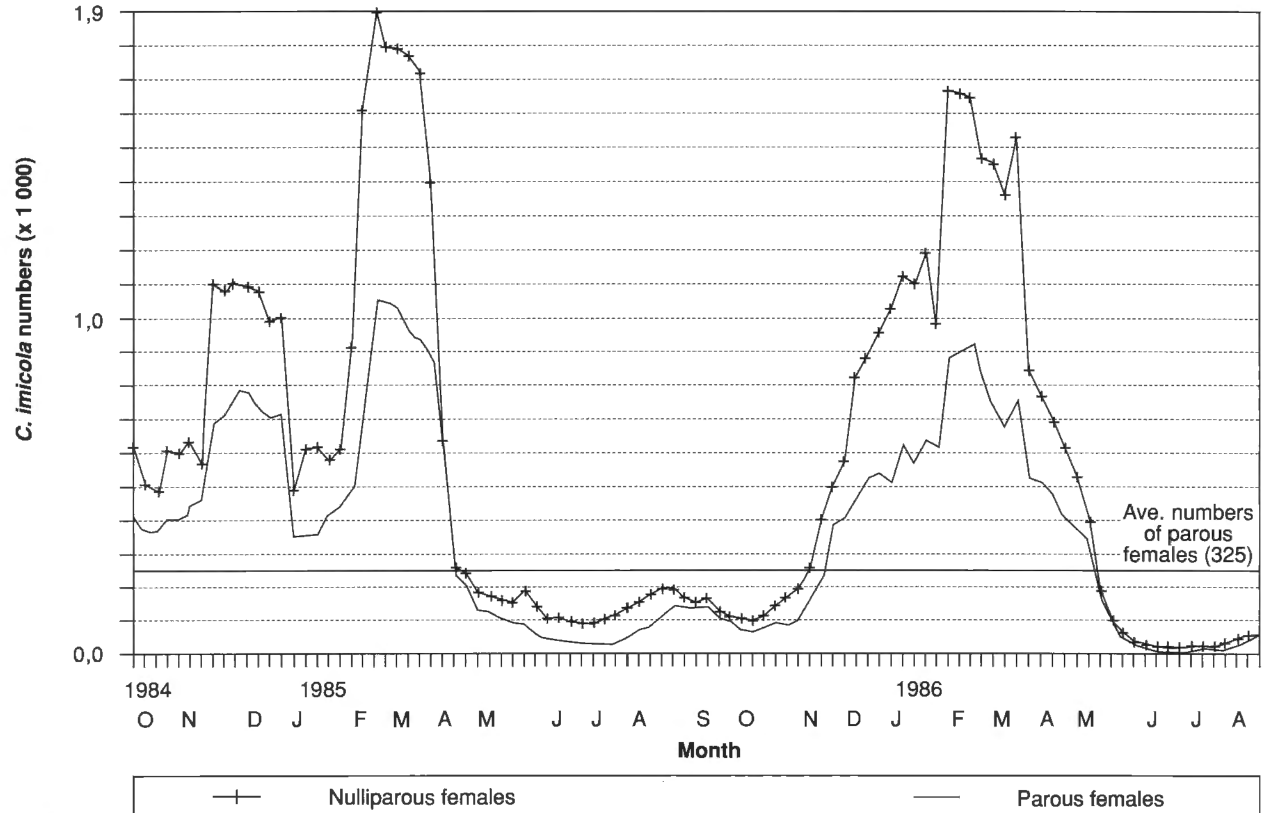

FIG. 2 Weekly variation (17 point running mean) in the ratio of nulliparous and parous *C. imicola* females at Onderstepoort (stable 3) from October 1984 to August 1986. For comparison, a line representing the average number of parous *C. imicola* females (325) is included

Culicoides light-trap collections from 1979–1985 from this site produced only one unidentified virus (Nevill *et al.* 1992).

Irrigation may also create *Culicoides* breeding sites in an area. The representation and the number of parous females of both *C. schultzei* s.l. and *C. imicola* collected at Veekos were noticeably greater than at Karakul (Table 4). The chances for virus transmission should therefore be higher at Veekos.

Contrary to what was found at the other collection sites, *C. schultzei* s.l. males, on average, accounted for 31,6% of the light-trap collections made at Karakul and 32,7% of the collections made at Veekos. This could also indicate that harsh climate prevents a build-up in the number of females and is supported by the relatively low parous rate.

Stellenbosch, Western Cape Province

Stellenbosch is situated in a winter rainfall region with warm dry summers, mild relatively frost-free winters and medium rainfall (619 mm). There are, on average, 0,1 d annually when the minimum temperature drops below freezing point and a further 46,2 d when it is below 5 °C (Table 1).

The trap was situated at the Welgevallen Experimental Farm at Stellenbosch. It was shown that the light-trap at Welgevallen gave a true reflection of the *Culicoides* found at livestock in the Stellenbosch area (Nevill, Venter, Edwardes, Pajor, Meiswinkel & Van Gas 1988). Cattle were the dominant stock species in the vicinity of the light-trap in this intensive farming region.

Seasonal abundance of Culicoides *species at Stellenbosch*

The number of *Culicoides* collected and identified in the seasonal abundance study, as well as the time span over which collections were made at Stellenbosch, are shown in Table 2. Here, as opposed to the sites in the summer rainfall region, collections were not dominated by one species. Abundant *Culicoides* species at Stellenbosch were *C. magnus* (29,0%), *C. gulbenkiani* (27,0%), *C. zuluensis* (25,7 %), *C. imicola* (11,3%) and *C. bolitinos* (4,6%) (Venter *et al.* 1996a). The seasonal variation of the first four species can be seen in Table 3.

Stellenbosch has a mild winter with good rainfall, and relatively large *Culicoides* numbers were found throughout the year. While above-average collections were made in April, the largest collections were made from September to November (Fig. 1e). As at Eiland, large *Culicoides* populations can survive the winter and be responsible for large collections made as early as October (Fig. 1e).

Parity of Culicoides *species at Stellenbosch*

The number of each of the abundant *Culicoides* species collected and graded for parity as well as the time span over which these collections were made at Stellenbosch are shown in Table 2.

Nulliparous females of all four abundant species were collected over the whole collection period, which confirms uninterrupted breeding (Table 4).

Since only parous individuals can transmit virus, consideration of *Culicoides* numbers alone, in identifying the best potential vector, can be misleading. This is the case in the Stellenbosch situation where up to four *Culicoides* species can be co-dominant (Table 3). If only parous individuals of each species are considered, the best potential vectors for e.g. 1985/86, were *C. imicola* for the period December to April, and *C. magnus* for August to October, while *C. gulbenkiani* was the best vector candidate in June (Table 4). In November, collections were smaller and the parity rates of each species were similar (Table 4). In a survey conducted from 1979 to 1985, the largest number of virus isolations from *Culicoides* was made in January and February (Erasmus & Nevill, unpublished data 1992). This finding coincides with the period in the present study, i.e. December to April when *C. imicola* was found to be the best potential vector.

CONCLUSION

In the frost-free summer rainfall area of South Africa (Eiland), large numbers of *Culicoides* were collected in light-traps throughout the year. This shows that breeding is continuous and that viruses could possibly be transmitted in winter. This area can therefore be regarded as a potential enzootic zone for AHS (and BT). *C. imicola* is the dominant species in this area and reaches exceptionally high numbers in the vicinity of stock animals.

As altitude increases towards the central plateau, there is a corresponding drop in winter temperatures. In the summer rainfall areas where frost occurs, *Culicoides* disappear from light-trap collections after the first frost; this is followed in spring by a slow build-up in populations until peak numbers are reached in the second half of summer. Together with the rise in *Culicoides* numbers towards the end of summer, there was also a rise in the ratio of parous (pigmented) females of most *Culicoides* species, which signifies a higher vector potential towards the end of summer. This coincides with the time of occurrence of BT, AHS and ephemeral fever outbreaks in most of the temperate parts of South Africa (Verwoerd & Erasmus 1994; Coetzer & Erasmus 1994; St. George 1994).

The small *Culicoides* numbers in winter are probably due to reduced adult activity and slower development of the immature stages as a result of low temperatures. It may also be due to lower rainfall and the consequent reduction in the semi-aquatic larval habitats. In the Onderstepoort area the numbers of *C. imicola* increase as the season progresses, as long as normal summer rains fall frequently enough to soak the soil to produce new larval habitats (Nevill 1971). Irrigation can also play a role in artificially maintaining large populations, especially of *C. imicola* (Venter *et al.* 1996b).

Relatively large numbers of *Culicoides* were also collected during winter in the temperate frost-free winter rainfall area of the Western Cape Province. The implication is that here, depending on the availability of circulating virus and susceptible hosts, virus transmission may also take place throughout the year. Results of the 1979–1985 survey by Nevill & Erasmus (unpublished data 1992) in which orbiviruses were isolated from *Culicoides* each month of the year, supports this observation.

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REFERENCES

ACOCKS, J.P.H. 1988. Veld types of South Africa. (*Memoirs of the Botanical Survey of South Africa;* no. 57).

ALLINGHAM, P.G. & STANDFAST, H.A. 1990. An investigation of transovarial transmission of Akabane virus in *Culicoides brevitarsis. Australian Veterinary Journal*, 67:273–274.

BARNARD, B.J.H. 1993. Circulation of African horsesickness virus in Zebra (*Equus burchelli*) in the Kruger National Park, South Africa, as measured by the prevalence of type specific antibodies. *Onderstepoort Journal of Veterinary Research*, 60:111–117.

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

COETZER, J.A.W. & ERASMUS, B.J. 1994. African horsesickness, in *Infectious diseases of livestock with special reference to southern Africa*, edited by J.A.W. Coetzer, G.R. Thomson & R.C. Tustin. Cape Town: Oxford University Press, 1:460–475.

CORNET, M. & BRUNHES, J. 1994. Revision des especes de *Culicoides* apparentees a *C. schultzei* (Enderlein, 1908) dans la region afrotropicale (Diptera, Ceratopogonidae). *Bulletin de la Societé Entomologique de France*, 99:149–164.

DAVIES, C.R. 1990. Interrupted feeding of blood-sucking insects: Causes and effects. *Parasitology Today*, 6:19–22.

DU TOIT, R.M. 1944. The transmission of bluetongue and horse-sickness by *Culicoides. Onderstepoort Journal of Veterinary Science and Animal Industry*, 19:7–16.

DYCE, A.L. 1969. The recognition of nulliparous and parous *Culicoides* (Diptera: Ceratopogonidae) without dissection. *Journal of the Australian Entomological Society*, 8:11–15.

KETTLE, D.S. 1962. The bionomics and control of *Culicoides* and *Leptoconops* (Diptera, Ceratopogonidae = Heleidae). *Annual Review of Entomology*, 15:203–209.

NELSON, B.A. & SCRIVANI, R.P. 1972. Isolation of arboviruses from parous midges of the *Culicoides variipennis* complex and parous rates in biting populations. *Journal of Medical Entomology*, 4:277–281.

NEVILL, E.M. 1967. Biological studies on some South African *Culicoides* species (Diptera: Ceratopogonidae) and the morphology of their immature stages. M.Sc. (Agric.) thesis, University of Pretoria, Pretoria.

NEVILL, E.M. 1971. Cattle and *Culicoides* biting midges as possible overwintering hosts of bluetongue virus. *Onderstepoort Journal of Veterinary Research*, 38:65–72.

NEVILL, E.M., VENTER, G.J., EDWARDES, M., PAJOR, I.T.P., MEISWINKEL, R. & VAN GAS, J.H. 1988. *Culicoides* species associated with livestock in the Stellenbosch area of the Western Cape Province, Republic of South Africa (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 55:101–106.

NEVILL, E.M., ERASMUS, B.J. & VENTER, G.J. 1992. A six-year survey of viruses associated with *Culicoides* biting midges throughout South Africa (Diptera: Ceratopogonidae), in *Bluetongue, African horse sickness and related orbiviruses*, edited by T.E. Walton & B.I. Osburn. Boca Raton: CRC Press.

STANDFAST, H.A. & DYCE, A.L. 1972. Potential vectors of arboviruses of cattle and buffalo in Australia. *Australian Veterinary Journal*, 48:224–227.

ST GEORGE, T.D. 1994. Bovine ephemeral fever, in *Infectious diseases of livestock with special reference to southern Africa*, edited by J.A.W. Coetzer, G.R. Thomson & R.C. Tustin. Cape Town: Oxford University Press, 1:553–562.

TABACHNICK, W.J., MELLOR, P.S. & STANDFAST, H.A. 1992. Working team report on vectors: Recommendations for research on *Culicoides* vector biology, in *Bluetongue, African horse sickness and related orbiviruses*, edited by T.E Walton & B.I. Osburn. Boca Raton: CRC Press.

VENTER, G.J. & SWEATMAN, G.K. 1989. Seasonal abundance and parity of *Culicoides* biting midges associated with livestock at Roma, Lesotho (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, 56:173–177.

VENTER, G.J., HILL, ELAINE, PAJOR, I.T.P. & NEVILL, E.M. 1991. The use of a membrane feeding technique to determine the infection rate of *C. imicola* (Diptera, Ceratopogonidae) for 2 bluetongue virus serotypes in South Africa. *Onderstepoort Journal of Veterinary Research,* 58:5–9.

VENTER, G.J., NEVILL, E.M. & VAN DER LINDE, T.C. DE K. 1996a. Geographical distribution, relative abundance and vector potential of stock-associated *Culicoides* (Diptera: Ceratopogonidae) in various climatic regions of southern Africa as determined with light-trap collections. *Onderstepoort Journal of Veterinary Research,* 63:25–38.

VENTER, G.J., MEISWINKEL, R., NEVILL, E.M. & EDWARDES, M. 1996b. *Culicoides* (Diptera: Ceratopogonidae) associated with livestock in the Onderstepoort area, Gauteng Province, South Africa as determined by light-trap collections. *Onderstepoort Journal of Veterinary Research,* 63:315–325.

VERWOERD, D.W. & ERASMUS, B.J. 1994. Bluetongue, in *Infectious diseases of livestock with special reference to southern Africa*, edited by J.A.W. Coetzer, G.R. Thomson & R.C. Tustin. Cape Town: Oxford University Press, 1:443–459.

WALKER, A.R. & BOREHAM, P.F.L. 1976. Saline as a collecting medium for *Culicoides* (Diptera, Ceratopogonidae) in blood-feeding and other studies. *Mosquito News*, 36:18–20.

WALKER, A.R. 1977. Adult lifespan and reproductive status of *Culicoides* species (Diptera: Ceratopogonidae) in Kenya, with reference to virus transmission. *Bulletin of Entomological Research*, 67:205–215.

WEATHER BUREAU 1986. *Climate of South Africa, WB 40.* Weather Bureau, Pretoria: Department of Environmental Affairs.

WETZEL, H., NEVILL, E.M. & ERASMUS, B.J. 1970. Studies on the transmission of African horsesickness. *Onderstepoort Journal of Veterinary Research*, 37:165–168.