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CULICOIDES SPECIES ASSOCIATED WITH LIVESTOCK IN THE STELLENBOSCH AREA OF THE WESTERN CAPE PROVINCE, REPUBLIC OF SOUTH AFRICA (DIPTERA: CERATOPOGONIDAE)

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ABSTRACT

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 (1988).

A total of 33 564 *Culicoides* midges was collected in 44 light trap collections made at 22 sites in the Stellenbosch area during November 1986. Of the 23 species present in these collections 8 were frequently encountered namely, *C. magnus*, *C. imicola*, *Culicoides* sp. 49, *C. zuluensis*, *C. gulbenkiani*, *C. pycnostictus*, *C. distinctipennis* and *C. nivosus*. Although *C. magnus* was abundant at all trap sites, the prevalence of the other species appeared to be affected by the proximity of the light trap to different host animals and/or larval habitats. Plain-wing species and members of the *C. schultzei* group were rarely collected.

The larval habitats of most of the above species were located by the use of tent-type emergence traps. All these habitats were found on irrigated pastures or where drainage water had accumulated. The difference in the requirements of the various species was associated with certain factors, such as degree of moisture, the type and amount of organic matter present and the particle size of the underlying soil.

The identity of the blood-meals of 69 individual *Culicoides* belonging to 7 species was determined. The 5 commonest species had all fed on cattle and 4 of these on sheep. Two species, *C. pycnostictus* and *C. distinctipennis* were positive for bird blood.

INTRODUCTION

A survey by Venter, Nevill & Meiswinkel (1987) indicated that at 17 light trap sites throughout the Republic of South Africa 10 *Culicoides* species predominated, but not all species were collected at all sites. Of these, *C. imicola* was the dominant species at 11 places. Few of the other 9 species ever dominated a site's catch. The catches made by the light trap on Welgevallen Research Farm at Stellenbosch were a notable exception, however, in that *C. gulbenkiani, C. magnus, C. zuluensis* and *C. imicola* alternated as the dominant species (G. J. Venter, unpublished data, 1987). The question then arose as to whether the collections made by means of this trap represented the true situation for the Stellenbosch area or whether they were merely a reflection of the proximity of larval habitats and/or particular host animals.

To answer these questions, and at the same time learn more about this area's *Culicoides* fauna, a 12-day study trip to the region was undertaken during November 1986.

MATERIALS AND METHODS

Study area

The "Stellenbosch area" referred to in this study covered 540 km^2 from roughly 33° 40′ S to 34° 00′ S and from 18° 40′ E to 19° 00′ E. The south-eastern part is mountainous (Jonkershoek Mountains, 1 494 m; Skurweberg, 914 m). Wide, flat valleys (Groot Drakenstein, 146 m) open to the north-west into gently rolling countryside (Elsenburg, 162 m).

This is a winter rainfall region receiving from 600 to 1 000 mm per annum depending on proximity to the mountains where precipitation is highest (S.A. Weather Bureau, 1986). Most rain falls from April to September, these 6 months receiving about 75 % of the annual total. Summers are relatively hot (Elsenburg mean daily maximum 28,3 °C) and winters are mild (Elsenburg mean daily minimum 7,0 °C). Temperatures below freezing are seldom recorded.

According to Acocks (1975) the natural vegetation of the study area may be classified as Coastal Rhenosterbosveld and Macchia or Fynbos. However, very little of this vegetation remains except in protected areas such as Jonkershoek. Much of the region is intensively farmed especially where water is available for irrigation during the dry summer. Here the production of grapes and deciduous fruits is important as are kikuyu, white clover and other pasture types for intensive livestock farming e.g. Elsenburg and Groot Drakenstein. Where less water is available winter wheat is planted and livestock are raised on natural grazing e.g. Klipheuwel.

Light trap collections

Four 220 volt ultraviolet down-draught suction light traps were used. Collections were made directly into phosphate buffered saline (PBS) to which 0,5 % 'Savlon'[1] antiseptic had been added according to the method of Walker & Boreham (1976). This method not only provided specimens in excellent condition for identification but engorged specimens could also be recovered for later bloodmeal identification. Each day traps were visited and the collections returned to the field laboratory where they were kept at 4 °C until they could be sorted. Because of the volume of work involved most collections were transferred to 80 % ethyl alcohol and were processed at the Veterinary Research Institute, Onderstepoort, on our return from the field. After trapping for 2 consecutive nights at a site, traps were moved to new localities.

Twenty-two trap sites were eventually used, starting at Welgevallen and radiating out in a north-north-west to easterly arc up to 30 km from Stellenbosch. One or more traps were situated in the Stellenbosch, Elsenburg, Mariendal, Groot Drakenstein, Klapmuts, Klipheuwel and Jonkershoek areas. The locations of these sites are given in Table 1.

Light traps were usually hung as close as possible to various livestock species. At 7 sites cattle were the dominant stock species, sheep at 5 and horses at 3 sites. One trap was placed near poultry and 1 near pigs and caged birds. A further trap was located near Angora

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[1] ICI (South Africa) Ltd.

TABLE 1 Identification of the *Culicoides* species recovered from light traps in the Stellenbosch area during November 1986 (percentages)

Culicoides species	Livestock in the vicinity of the light traps																						
	Cattle							Sheep					Horses			Angora goats	Poultry	Pigs & birds	No stock				Means & totals
	Welgevallen Stellenbosch	AM Farms, Groot Drakenstein	Schoongezicht, No. 1 Stellenbosch	Schoongezicht, No. 2 Stellenbosch	Mariendal	Elsenburg	Blaauwblommetjieskloof Klipheuwel	AM Farms, Groot Drakenstein	Elsenburg	Baraboo Stellenbosch	Welgevallen Stellenbosch	San Remo Klipheuwel	Lowanee stud Paardeberg	Mariendal	Bona Vista Klapmuts	Willow Farm Klapmuts	Mariendal	Elsenburg	Amoi, Estates Stellenbosch	Bergplaas Resort Stellenbosch	Jonkershoek Mr Bird's House	Jonkershoek Reserve Gate	
C. magnus	**46,1**	16,0	6,8	32,5	**42,9**	7,3	17,8	14,5	**46,7**	22,4	14,2	**40,0**	**57,7**	8,7	11,5	11,7	26,5	**48,3**	14,3	13,2	24,4	25,2	24,9
C. imicola	15,7	11,9	1,4	0,6	23,8	4,2	20,5	**34,2**	17,2	**61,4**	**58,6**	34,7	9,7	**36,3**	**42,2**	13,1	16,7	13,6	2,6	0,7	0,7	12,0	19,6
Culicoides sp. 49	5,8	13,0	**55,2**	16,9	9,5	2,0	7,8	18,1	6,7	10,8	3,7	16,8	0,9	35,1	38,5	12,2	16,3	3,1	7,8	3,1	**43,2**	7,6	15,2
C. zuluensis	22,5	5,2	22,3	**34,8**	4,8	0,3	1,9	17,4	4,0	2,9	20,3	1,1	9,6	1,9	4,1	6,2	4,2	1,3	13,8	12,8	1,5	8,3	9,1
C. gulbenkiani	9,2	**52,2**	10,3	2,6		**76,4**	0,6	5,0	4,3	0,5	1,4		0,2	3,1	1,3	1,2	3,7	1,6	0,4		0,4	0,1	7,9
C. pycnostictus	0,4	0,7	0,3	1,8	9,5	6,3	10,8	1,0	13,5	0,1	0,3	2,1	11,6	9,0	1,3	**34,5**	**27,0**	17,7	23,2	**35,8**	2,0	3,1	9,6
C. nivosus	0,1	0,2			4,8	1,7	**37,2**	0,8	1,6		0,4	4,2	8,1	3,3	0,1	3,1	0,5	3,4	0,4	0,7	0,2	0,1	3,2
C. distinctipennis	0,2	0,5	0,6	3,4	4,8	1,7	0,5	8,6	5,3	0,1	1,0		0,6	2,1	0,8	15,9	5,1	11,0	**36,2**	32,6	26,2	**41,4**	9,0
C. huambensis	0,1	0,2	3,2	7,1			0,2	0,2	0,3	1,7	0,1		0,2		0,1				0,2		0,1	0,4	0,6
C. similis							0,9				0,1	1,1			0,3	0,6							0,1
C. expectator							0,8						0,5			1,5			0,1				0,1
Culicoides sp. 35						0,2	0,9						0,6	0,2									0,1
C. angolensis		0,1		0,4							0,1								0,7		0,3	0,6	0,1
Culicoides sp. 9																					0,2	0,2	0,1
C. onderstepoortensis																					0,5	0,3	0,1
C. olyslageri																			0,1		0,1	0,2	0,1
C. accraensis group																						0,2	0,1
Other species*				0,1			0,2	0,2	0,5		0,1		0,3	0,2					0,1	1,0	0,3	0,2	0,1
No. of collections	2	1	1	2	3	2	2	2	2	2	2	2	2	3	2	2	3	2	1	2	2	2	44
Total No. of midges	3 713	3 059	710	3 481	21	601	1 291	906	868	1 407	2 576	95	1 308	424	2 144	1 601	215	447	1 647	288	2 645	4 117	33 564

* Includes *C. brucei*, *C. engubandei*, *C. coarctatus*, *C. neavei*, *Culicoides* sp. 3 and unidentified species

goats in a wooded situation where birds could be expected to roost. The remaining 4 traps were placed at wooded sites where there was little or no farming activity (Table 1).

Larval habitat studies

Ten tent-type emergence traps as described by Pajor (1987) were used. They were erected over likely larval habitats at Welgevallen and at Elsenburg Research Station 20 km away. If no *Culicoides* were collected at a site then the trap was relocated. Traps were visited daily and the *Culicoides* species collected, identified and counted.

Soil samples (100 mℓ each) were also taken from sites at which *Culicoides* were present and returned to Onderstepoort for analysis of water content, field capacity, specific mass and general description.

To determine whether any *Culicoides* species were breeding in cow or horse dung, old moist dung was collected from pastures and placed in open cardboard boxes which in turn were kept in fine gauze bags. Emerging *Culicoides* were aspirated from the bags and identified.

Bloodmeal identification

Engorged *Culicoides* were removed from the light trap collections and identified to species. The PBS was absorbed with tissue paper and the dried specimens placed in individual Size 1 gelatine capsules[2]. Each capsule was numbered and stored over silica gel. On our return to Onderstepoort the bloodmeals were identified by means of a cross-over electrophoresis precipitin test (Culliford, 1964). The antisera were restricted to horse, cattle, sheep and chicken and were prepared in rabbits (Boreham & Gill, 1973). Non-specific, cross-reacting antibodies were precipitated out by absorbing the antisera with the heterologous serum. This was particularly important in the case of cattle and sheep which may otherwise cross-react.

Results and Discussion

Light trap collections

An analysis of the light trap collections is summarized in Table 1. The single most abundant species per site appears in bold type.

A total of 33 564 *Culicoides* belonging to 23 species were collected in 44 catches made at 22 sites. The most abundant species at this time of the year were *C. magnus, C. imicola, Culicoides* sp. 49, *C. pycnostictus, C. zuluensis, C. distinctipennis, C. gulbenkiani* and *C. nivosus*. Fifteen other species were also collected, many of these in the Jonkershoek Valley where few livestock are kept. All the species found at Welgevallen were relatively common throughout the Stellenbosch area. Previous results for Welgevallen (Venter *et al.*, 1987) can therefore be accepted as fairly representative for this region.

The following comments can be made for each of the more abundant species:

(1) *C. magnus* was the most abundant midge and was associated with all stock types but was also commonly encountered where stock was not obviously present.

(2) *C. imicola* was the main species collected near sheep and horses.

(3) *Culicoides* sp. 49 was unexpectedly common in the Stellenbosch area and sometimes especially so near cattle and horses. This new species, which breeds in cow dung, was incorrectly identified as *C. pallidipennis* (= *C. imicola*) by Nevill (1968) but is now referred to as *Culicoides* sp. 49 by R. Meiswinkel (unpublished data, 1987). In the study by Venter *et al.* (1987) no attempt was made to identify *Culicoides* sp. 49 separately from its near identical sibling *C. imicola*. However, greater familiarity with the wing pattern of *Culicoides* sp. 49 permitted it to be identified as such in the present study.

(4) *C. zuluensis* accounted for a high proportion of the catches especially near cattle and sometimes sheep.

(5) *C. gulbenkiani* was common at only 2 sites. Both were similar to the Welgevallen site in that the dominant stock species was cattle, and the sites were on intensively farmed irrigated areas.

(6) *C. pycnostictus* and *C. distinctipennis* were never abundant near livestock but were more common near poultry and in wooded situations where birds could be expected to roost.

(7) *C. nivosus* was only abundant at 1 site, a dairy in the drier Klipheuwel area.

Only 1 "plain-wing" *Culicoides* species was collected *(C. engubandei)*, and only a few specimens of the *C. schultzei* group (*Culicoides* spp. 3, 9 & 35 of R. Meiswinkel). These groups are more common in the arid regions of South Africa.

Larval habitat studies

Over a period of 11 days a total of 1 601 *Culicoides* were collected in 10 emergence traps set mostly on irrigated pastures at Welgevallen and Elsenburg. These collections comprised the following species: *C. gulbenkiani* 43,8 %, *C. distinctipennis* 23,5 %, *C. magnus* 18,2 %, *C. zuluensis* 10,0 %, *C. pycnostictus* 2,7 %, *C. imicola* 1,1 %, and 4 other species each accounting for less than 0,2 %.

These results are not representative for all *Culicoides* species which occur in the Stellenbosch area, as a special effort was made to pinpoint the larval habitats of *C. gulbenkiani, C. magnus* and *C. zuluensis* which, until this study, were poorly known. An analysis of the main *Culicoides* larval habitats located during this survey appears in Table 2.

The old, moist horse and cow dung which was collected and held for *Culicoides* emergence yielded 247 *C. gulbenkiani*, almost equal numbers from both types of dung. The following conclusions could be drawn from these dung emergence results and from the emergence trap analyses which are summarized in Table 2:

(1) *C. gulbenkiani* larval habitats were found on irrigated pastures grazed by cattle and horses. The substrate was rather dry to slightly moist and rich in decomposed plant material with low specific mass (Sites 3 & 4, Table 2). This substrate could also have been derived from old horse or cow dung, or could have been this dung itself, especially where it was kept moist by irrigation and shaded by the surrounding lanky pasture (Sites 2, 3 & 4). Such sites are frequently encountered as horses tend not to graze those portions of the pasture where they defaecate and urinate. These habitats are aerobic ones.

(2) *C. magnus* habitats were sandy (1,41 g/cm^3) to silty (2,02 g/cm^3), waterlogged or almost waterlogged soils with plant cover. The organic material in these habitats consisted either of wet, decomposed to rotting plant material (Site 1) or dissolved raw manure (Site 5). These habitats are fairly anaerobic.

[2] Gel-u-cap S.A. (Pty) Ltd.

TABLE 2 Description of the main *Culicoides* larval habitats located by means of emergence traps in the Stellenbosch area during November 1986

Site	Description	Soil analyses			No. of *Culicoides* collected	*Culicoides* species abundance (%)
		Specific mass g/cm³	% water in sample	% water at field capacity		
1. Welgevallen drainage depression	Well-drained area. Collects rainwater and drainage from stables. Some parts continuously wet. Traps set over these moist areas. Substrate consists of sandy soil covered by vegetation and a layer of organic matter. Grazed by sheep and sometimes cattle	1,41 (top 10–50 mm)	50,12	50,85	476	*C. distinctipennis* 33,6 *C. zuluensis* 32,4 *C. magnus* 31,9 *C. imicola* 1,0 *C. gulbenkiani* 0,4 *C. pycnostictus* 0,4 *C. nivosus* 0,2
2. Welgevallen horse camp	Irrigated kikuyu paddock. Emergence traps set in moister sections where grass is longer. Old moist horse manure common here, 1 trap set over ± 8 day-old manure. Soil clay-like and medium moist	1,48 (top 0–80 mm)	42,26	46,36	107	*C. gulbenkiani* 87,9 *C. imicola* 7,5 *C. magnus* 2,8 *C. distinctipennis* 0,9 *Culicoides* sp. 49 0,9
3. Welgevallen cattle paddock	Irrigated kikuyu pasture with long grass up to 300 mm. Soil dry and sandy. On the surface there was little organic matter and very old disintegrated cow dung. Plant particles 5–15 mm long and 1–3 mm wide. Top layer slightly moist with pleasant earthy smell	0,28 (top 0–10 mm) 0,98 (10–100 mm)	25,78 17,78	49,16 39,77	356 —	*C. gulbenkiani* 98,2 *C. distinctipennis* 0,6 *Culicoides* sp. 49 0,6 *Culicoides* sp. 72 0,6
4. Elsenburg near dairy	Irrigated kikuyu pasture used for cattle and sheep. Mixed with the kikuyu were other grasses and white clover. The compacted sandy soil was covered by 20–30 mm layer of dark organic matter (humus), decomposed plant debris and old cow dung	0,27 (surface layer)	26,69	51,28	252	*C. gulbenkiani* 100,0
5. Elsenburg near rugby field	Traps were set on 2 adjacent pastures kept wet by rain or irrigation, previously grazed short by cattle and sheep. Soil very moist to wet with fine particle size (silt). Very little surface organic matter except for some rotting plant particles and/or diluted manure	2,02 (top 0–20 mm)	76,70	67,80	377	*C. distinctipennis* 54,1 *C. magnus* 33,4 *C. pycnostictus* 10,1 *C. imicola* 1,3 *C. zuluensis* 1,1

TABLE 3 Identification of the bloodmeals of *Culicoides* species collected in light traps in the Stellenbosch area during November 1986

Culicoides species	Host				Total positive	Total tested
	Cattle	Horse	Sheep	Chicken		
C. imicola	10	—	17	—	27	41
Culicoides sp. 49	4	2	—	—	6	12
C. gulbenkiani	10	—	1	—	11	11
C. zuluensis	4	—	1	—	5	6
C. magnus	15	—	3	—	18	22
C. distinctipennis	—	—	—	1	1	1
C. pycnostictus	—	—	—	1	1	2
C. nivosus	—	—	—	—	—	1
Totals	43	2	22	2	69	96

(3) *C. zuluensis* also preferred a wet, almost waterlogged, organically rich habitat (Site 1) where the particle size of the soil was somewhat larger (1,41 g/cm^3). These habitats tend to be anaerobic.

(4) *C. imicola* could be found in dry as well as wet kikuyu paddocks, especially where horses were kept (Site 2).

(5) Although no effort was made to find the larval habitats of *C. distinctipennis*, it was twice collected in association with *C. magnus* (Sites 1 & 5).

Good *C. gulbenkiani*, *C. magnus*, *C. zuluensis* and also possible *C. imicola* larval habitats were found within 40–150 m of the original Welgevallen light trap site from which Venter *et al.* (1987) had made collections. This supports the present collection of large numbers of these species at this trap site.

Bloodmeal identification

Ninety-six engorged *Culicoides* belonging to the 8 commonest species were tested for bloodmeal identification. Of these, 69 were positive. Most of the specimens that were negative belonged to the very small species *C. imicola* and its sibling *Culicoides* sp. 49 which, if only partially engorged, probably contained too little blood for an identification to be made (Table 3).

C. imicola, *C. gulbenkiani*, *C. magnus* and *C. zuluensis* fed on both cattle and sheep. They are therefore potential vectors of virus diseases of both host species. However, because cattle are carriers of bluetongue virus these 4 *Culicoides* species would also be ideally suited for transmitting bluetongue virus from cattle to sheep. The ability of bluetongue virus to pass through the gut barrier of these 4 species and then multiply, particularly in the salivary glands, must now be investigated.

C. gulbenkiani and *Culicoides* sp. 49 both breed in cow dung. The bloodmeal identifications show that both species also feed on cattle. From the epidemiological point of view this intimate association between cattle and these species would make them ideal vectors of viruses of this host.

Culicoides sp. 49 was the only species found to have fed on horses. This very likely reflects our inability to sort out sufficient engorged specimens from amongst the *Culicoides* caught by traps set close to various types of potential hosts. In bloodmeal identification studies done on *Culicoides* species collected elsewhere in South Africa, *C. imicola*, *C. zuluensis* and *C. gulbenkiani* were also shown to have fed on horses (E. M. Nevill & J. H. van Gas, unpublished data, 1987). These species together with *Culicoides* sp. 49 are therefore potential vectors of certain viral diseases of horses in the Stellenbosch area.

The results of these bloodmeal identification tests can also help explain the comparative abundance or absence of certain *Culicoides* species in the light trap and emergence trap collections. For example, *C. distinctipennis* was common in emergence traps, yet few were collected in light traps set near cattle, horses or sheep. On the other hand this species and *C. pycnostictus* were common in light traps set in wooded areas away from stock. Since engorged specimens of these species tested positive against anti-chicken serum, their high numbers in wooded areas may be due to their being attracted to roosting wild birds.

Likewise *C. imicola* was comparatively rare in emergence traps but was caught in large numbers in light traps, especially near sheep. Bloodmeal tests showed 17 of the 27 positive specimens of this species to have fed on sheep, thus accounting for the high numbers collected near these animals. This finding lends support to the theory that the species composition of light trap catches, made in the proximity of host animals, can well be an indicator of host preference.

CONCLUSIONS

Venter *et al.* (1987) and G. J. Venter (unpublished data, 1987) showed that *C. gulbenkiani*, *C. magnus*, *C. zuluensis* and *C. imicola* alternated as the dominant species in light trap collections made over 2 years at Welgevallen Research Farm at Stellenbosch. The present 12-day study at various sites in the Stellenbosch area, during November 1986, confirmed the presence and abundance of the same species. However, *Culicoides* sp. 49, which was previously grouped under *C. imicola* by Venter *et al.* (1987), was also found to be a common species.

The placing of light traps near different species of livestock, the use of emergence traps and the identification of *Culicoides* bloodmeals showed that light trap catches were in fact influenced by the proximity of suitable larval habitats and by *Culicoides* host preferences. In addition the relatively uniform environment of irrigated pastures, which dominated the livestock sampling sites, appeared to favour the maintenance of 1 or more of the 8 species of *Culicoides* frequently encountered in these situations. Of these, 5 species were found to occur commonly near, and to feed on cattle, horses and sheep. The vectors of *Culicoides*-borne virus diseases of these animals should be amongst these 5 species. Two other *Culicoides* species fed on birds and must be considered if a vector for a poultry pathogen is being sought.

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