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Breeding sites of *Culicoides* midges in KwaZulu-Natal

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Abstract

Locating breeding sites of disease vectors is an essential part of their control and the subsequent control of the diseases that they transmit. African Horse Sickness (AHS) is vectored by *Culicoides* midges and, while information is available on their breeding sites, not much data is available in KwaZulu-Natal (KZN). Tent traps were made and placed at 90 locations in the KZN midlands. Catch numbers were correlated to site properties using the generalised linear modelling procedure on untransformed data with a negative binomial distribution and a log link function. Sites with increasing ground moisture, increasing incident radiation and increasing wetness duration were found to positively increase the number of midges collected from them. Possible applications of the results are discussed.

Keywords: *Culicoides*, African Horse sickness, control, breeding sites
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Introduction

South Africa is the enzootic range for African horse sickness (AHS) virus (Mellor & Hamblin, 2004). The virus is transmitted by ceratopogonid flies of the genus *Culicoides* and the two species proven to be the vectors of AHS are *Culicoides imicola* and *C. bolitinos* (Du Toit, 1944; Venter *et al.*, 2000; Paweska *et al.*, 2003). *Culicoides* larvae often occupy an ecotone where there is a wet substrate rather than free standing water (Mellor *et al.*, 2000). They breed in both salt and fresh water marshes, animal dung, rotting vegetation such as fallen banana trees and forest floor cover, in tree holes, in waterlogged pastures, on beaches, around leaking irrigation pipes and water troughs, in muddy farm yards, and in areas of both high and low organic and faecal matter content. They have been found in sewage installations and drainage channels. Most commonly they are found along the edges of pools, streams and any permanent water body (Service, 1971; Braverman *et al.*, 1974; Lubega & Khamala, 1976; Mellor & Pitzoltis, 1979; Edwards, 1982; Mellor *et al.*, 2000; Mullen, 2002). These diverse habitats make larvicidal control of midges very difficult.

Braverman *et al.* (1974) used a simple flotation method whereby samples of possible habitat were added to salty water and the larvae simply floated to the surface. This worked well for many of the species except for *Culicoides imicola* which was shown by Nevill (1967) to sink when sampling by this method. Lubega & Khamala (1976), Mellor & Pitzoltis (1979) and Randall (1982) incubated samples from the soil in controlled environment facilities, using effective temperatures of between 25 °C and 28 °C (Bishop *et al.*, 1996), and constant light (Bishop *et al.*, 1998). Emergence traps in the field are the most effective (Pajor, 1987).

If horse owners are aware of some habitat parameters in which *Culicoides imicola* breed, they will be able to take appropriate steps to suppress the population, which could have a positive impact on the incidence of African Horse Sickness disease vectors.

Materials and Methods

Tent traps (Pajor, 1987) were constructed for the collection of emerging midges. The central stake of the gauze tent supported a collecting bottle containing a 15% Savlon™ solution for preservation of the midges. Modifications to the Pajor design included a smaller tent collection area of 75 cm x 75 cm and a tin can as the gauze and collecting bottle support. Ninety tent traps were sampled at twelve farms in the Howick area of KwaZulu-Natal during the height of the midge season in February, 2007. Farms that had previously had outbreaks of the disease were selected. Tent traps were placed at five strategic locations across the twelve farms to produce replicated midge count data for five possible breeding locations. The possible breeding locations varied in ground cover (kikuyu or another type of vegetation), vegetation length (long (over 30 cm), short (under 30 cm) or none), incident radiation (sun, shade or deep shade), moisture (dry, wet, or did it squelch under foot) and duration of wetness (permanently wet or temporarily wet by rains or irrigation). Traps were left on site for seven days. Catches were removed to 85% alcohol, sorted and *Ceratopogonidae* were identified to species and reproductive status in females (G. Venter, Pers. Comm. Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110).

The number of midges caught (total catch, males and females) was analysed using generalized linear modeling procedure in Genstat v9 (2006). The data followed a negative binomial distribution, as the data was non-normal count data where the relationship between the variance and the mean was not equal. A log link function was used (McConway *et al*., 2006). The significance of the parameter estimates in the accumulated analysis of deviance was used to analyse ceratopoginid emergence in breeding locations differing in moisture, duration of wetness and incident radiation.

Results and Discussion

Catch numbers were very low (Table 1). Approximately 32% of midges caught were males and 68% were females. An increase in the level of the factor was assumed to increase the probability of raising the catch of midges. More females ($P < 0.001$) were caught in moister soil with more incident radiation and increases in male midge numbers were found to be highly correlated to increases in both ground moisture and wetness permanence (Table 2). Ground cover and vegetation length did not significantly affect the numbers of midges caught.

Table 1 Species and total catch data of the female developmental stages and sexes of *Culicoides* midges caught by *in situ* larval emergence traps

Culicoides species	Nulliparous	Parous	Gravid	Female	Male
C. bolitinos		3		3	1
C. glabripennis	1			1	
C. gulbenkiani		1	3	4	
C. imicola	4	1		5	1
C. leucostictus	30	5		35	18
C. magnus	5			5	2
C. neavei	1			1	
C. nevilli	1			1	
C. nivosus					1
C. pycnostictus	7	7	1	15	8
C. schultzei					1
C. trifasciellus					1
C. zuluensis	37	10	1	48	23
Total	86	27	5	118	56

Low yields from emergence traps are common (G. Venter, Pers. Comm.). The data show however, that *Culicoides bolitinos* that have only been found thus far in dung (Meiswinkel & Paweska, 2003) were found in dung and under low trees in very deep shade. The percentage emergence of juvenile male midges (32%) is interesting in comparison to adult midge data (Jenkins, 2008) of 8%. Midges are far more likely to emerge from very wet (squelchy) sites as opposed to drier ones. Dripping irrigation pipes that are moved frequently may possibly not be a concern. Such sites as dripping taps, gutter down-pipes, septic tank overflows, leaking reservoirs, edges of drainage ditches and small depressions that are always wet, are of greater concern. Both male and female catches were significantly increased by an increase in ground moisture, and total midges increased where wetness was permanent, although female midges did not emerge from permanently wet sites. Barnard (1998) and Whitman & Baylis (2000) also found more females where temperatures were higher, as in sunny sites.

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Table 2 Parameter estimates of untransformed male and female midge numbers analysed by negative binomial generalized linear model with dispersion parameter k = 1 with a log link function in Genstat v9, indicating the emergence of midges from breeding locations different in moisture, incident radiation and duration of wetness

Parameter	Sex	Estimate	Std error	T prob
Constant	Male	-4.725	0.966	<0.001**
	Female	-3.956	0.689	<0.001**
Moisture ◊	Male	1.726	0.497	<0.001**
	Female	1.226	0.260	<0.001**
Permanence of wetness ○	Male	1.930	0.787	0.014*
Incident Radiation ♦	Female	1.414	0.349	<0.001**

Levels of the treatment factors : ◊ Moisture: dry (0) → wet (1) → squelchy (2).
○ Permanence of water wetness: temporarily wet (0)→ permanently wet (1).
♦ Incident Radiation: deep Shade (0) → dappled shade (1) → full sun (2).

Conclusion

Breeding sites for *Culicoides* midges are small, widely spread and not uniformly chosen by any one species. Increases in moisture, permanence of wetness, and incident radiation all positively increase midge catches. Environmental modifications are highly effective at reducing midge populations at suitable breeding sites. Larvicidal activity should be focused on very wet sites that do not dry out but receive a lot of sun if they are to be most effective.

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