The efficiency of light-emitting diode suction traps for the collection of South African livestock-associated *Culicoides* species

Gert J. Venter[1,2,*], Solomon N.B. Boikanyo[1] and Chantel J. de Beer[1]

[1]Epidemiology, Vectors and Parasites, Agricultural Research Council-Onderstepoort Veterinary Research, Pretoria, South Africa

[2]Department of Veterinary Tropical Diseases, University of Pretoria, Pretoria, South Africa

*Correspondence: Gert J. Venter, Department of Epidemiology, Parasites and Vectors, Agricultural Research Council–Onderstepoort Veterinary Research, Private Bag X5, Onderstepoort, Pretoria 0110, South Africa. Tel.:+27 (0)12 529 9181; Fax:+27 (0)12 529 9180; E-mail: venterg@arc.agric.za

Abstract. *Culicoides* biting midges (Diptera: Ceratopogonidae) are the vectors of a range of arboviruses causing important veterinary diseases e.g., bluetongue and African horse sickness. The effective monitoring of *Culicoides* species diversity and abundance, not only at livestock but also near potential wildlife hosts will be essential for risk management. Currently the Onderstepoort 220 V black light trap is extensively used for this purpose. Reducing its power requirements, by fitting low energy light emitting diodes (LEDs), can lead to greater flexibility in monitoring efforts. Comparison of the efficiency of a standard 220 V Onderstepoort trap (8 W fluorescent black light) to 220 V Onderstepoort traps fitted with either red, white, blue or green LEDs also to 12 V LED traps and a 12 V Onderstepoort trap indicated the 220 V Onderstepoort trap to the most efficient. All the trap results showed nulliparous *Culicoides imicola* females to be the dominant grouping. The Onderstepoort 12 V trap, or those with blue or white LEDs, can be used in field situations to determine the most abundant species.

Key words: Attraction, *Culicoides, Culicoides imicola*, LEDs, Light colour

Introduction

Blood feeding midges in the genus *Culicoides* (Diptera: Ceratopogonidae) are involved as vectors of several viruses, protozoa and filarial nematodes of medical and veterinary importance (Meiswinkel *et al*., 2004; Purse *et al*., 2015). Amongst the orbiviruses (Reoviridae) transmitted by *Culicoides* species, those causing bluetongue (BT) in ruminants and African horse sickness (AHS) in equines are of global economic importance (Purse *et al*., 2015). The detection of these viruses in field-collected specimens as well as susceptibility results in the laboratory suggest that susceptibility to these orbiviruses may be widespread in the genus *Culicoides* and that it is not restricted to a few livestock associated species (Carpenter *et al*., 2015; Venter *et al*., 2015).

Asymptomatic circulation of bluetongue virus (BTV) has been observed in a wide range of wild ruminants and it has become accepted that all ruminants may be susceptible to infection (Barnard, 1997). Similarly, an indigenous African equid, the zebra (*Equus burchellii*) is considered to be the primary cycling host of African horse sickness virus (AHSV) (Barnard & Paweska, 1993). Additionally, Hartmann's mountain zebra (*Equus zebra hartmannae*) was also recently implicated as a potential cycling host of AHSV (Becker *et al.*, 2018). These wildlife species may act as cycling or amplifying hosts and can as such play an important role in the epidemiology and spread of these viruses (Niedbalski, 2015).

The effective monitoring of *Culicoides* species diversity and abundance, not only at livestock hosts but also near wildlife species, which act as cycling or amplification hosts, will be fundamental in clarifying the epidemiology of these diseases and the implementation of appropriate risk management strategies. Despite shortcomings, suction light traps have become the most popular and widely used tool for the monitoring of *Culicoides* midges (McDermott & Mullens, 2017). Light traps are easy to operate and can provide large numbers of *Culicoides* at reasonable costs and effort. The efficiency and superiority of the 220 V Onderstepoort black light trap has been demonstrated on several occasions (Meiswinkel *et al.,* 2004; Venter *et al*., 2009; Probst *et al*., 2009; Del Río López, 2012). Dependence on a 220 V power supply, linked to a limited range of attraction (Venter *et al*., 2012), however, restricts its application in field situations where collections need to be made near wildlife.

The development of light emitting diodes (LEDs) provided an opportunity for the incorporation of these energy efficient light sources in the design of improved visual targets for the monitoring of night active insects (Burkett *et al*., 1998; Cohnstaedt *et al*., 2008; Jenkins & Young, 2010; Snyder *et al.,* 2016). An advantage of LEDs is that they often produce a greater total photon flux in the visible spectrum than incandescent globes for the same power input, rendering them suitable for 12 V operation (Bishop *et al*., 2004). Reducing the power requirements of suction light traps, without decreasing efficiency, by fitting LEDs, can lead to a greater flexibility in monitoring efforts (Hope *et al*., 2015) and will enable trapping in the vicinity of wild life and near potential larval developmental sites in the field.

Studies evaluating various colours of light have been conducted with Australian (Bishop *et al*., 2004; 2006), South American (Silva *et al*., 2015) and European (Hope *et al*., 2015; González *et al.,* 2016) *Culicoides* species. While these studies suggest attraction specifically to ultraviolet (UV), blue and green light they also suggest that specific *Culicoides* species may react differently to various wavelengths of light.

In the present study the efficiency of different coloured LEDs were compared for the attraction of South African life stock associated *Culicoides* species. *Culicoides* (*Avaritia*) *imicola* a proven vector of both BTV and AHSV, is the most abundant life stock associated *Culicoides* in southern Africa (Meiswinkel *et al*., 2004).

Material and methods

To determine the applicability of LEDs for the collection and monitoring of South African livestock associated *Culicoides* the efficiency of 220 V Onderstepoort traps fitted with white, blue, green or red LEDs was compared to that of the standard 220 V black light Onderstepoort trap. In this study conducted at the end of summer in April/May 2017, the 30 cm 8W fluorescent black light tube was replaced by 12 individual LEDs, either white, blue, green or red, per trap. In a subsequent study in the winter of June/July 2017, blue, green, and white LEDs fitted to standard Onderstepoort traps, altered to operate at 12 V, by replacing the fan, were compared to a standard 220 V Onderstepoort trap. As in the first comparison, 12 LEDs per trap were used. A standard 220 V Onderstepoort trap, altered to operate at 12 V, was also included. To ensure that treatment means were independent of any effects due to site or occasion the five traps were compared in two replicates of a 5 X 5 randomized Latin square design (Perry *et al*., 1980) conducted at five sites over10 nights in each comparison.

All evaluations were conducted at the Agricultural Research Council–Onderstepoort Veterinary Research (25˚39′ S, 28˚11′ E; 1219 m above sea level). Traps were operated from dusk to dawn next to barns housing between 20 and 40 cattle each. Traps were hung 1.4 m above ground level and as close to the cattle as practically possible. To prevent interference between traps all traps were located at least 15 m apart. Insects were collected into distilled water to which 0.5% Savlon® (Johnson & Johnson, South Africa) (Clorhexidine gluconate 0.3 g/100 ml and Cetrimide 3.0 g/ 100 ml) antiseptic were added to break the surface tension of the water and allow midges to sink to the bottom of the collection beaker.

After retrieval in the morning, the insects were transferred to 80% ethanol and stored until analysed. Large collections were sub-sampled (Van Ark & Meiswinkel, 1992) and

Culicoides were identified down to species level (Labuschagne, 2016). Females were based on abdominal pigmentation (Dyce, 1969), age-graded into nulliparous, parous, gravid or freshly blood-fed. Males and other insects captured were also counted.

Analysis of variance (ANOVA) was used to differentiate between the treatments. The data were normally distributed and treatment means were separated using the Fisher's protected t-test least significant difference (LSD) at a 5% significance level (Snedecor & Cochran, 1980). The statistical program GenStat® (VSN International, 2012) was used.

Results

Comparison of 220 V LED traps

In the evaluation of the 220 V traps, 228 966 *Culicoides* midges were collected in 50 collections made over 10 nights in late summer from 24 April to 6 May 2017. More than half of these, 52.5%, were collected in the standard Onderstepoort trap (Table 1). The mean number (12 032) of midges, ranging from 4940 to 19 890, in the Onderstepoort trap was significant higher ($P<0.001$) than in any of the 220 V traps fitted with LEDs (Table 1). While the mean numbers collected in the traps with white (4469.5) and blue (4783.5) LEDs did not differ, those collected with green LEDs (1337.9) were significantly lower (Table 1). Although up to 1592 midges could be collected in a single night with red LEDs the mean numbers (273.7) were significantly lower ($P<0.001$) than those in the other traps (Table 1).

Culicoides midges belonging to at least 16 species were collected. Species richness ranged from 12 in the traps with white and blue LEDs to 10 in those with green and red LEDs (Table 1). Single specimens of *Culicoides coarctatus* Clastrier and Wirth and a species belonging to the Nigripennis group were only present in the trap

Table 1. Comparison of *Culicoides* midges collected with four 220 V downdraught light traps fitted with different coloured LED lights and the standard Onderstepoort trap during late summer (24 April to 6 May 2017) at the ARC-OVR, South Africa.

	Onderstepoort 220 V	White LED	Blue LED	Green LED	Red LED
No. of collections	10	10	10	10	10
Species richness	11	12	12	10	6
Total *Culicoides* collected (%)	120 320 (52.5)	44 695 (19.5)	47 835 (20.9)	13 379 (5.8)	2737 (1.2)
Mean collection size (STDV)	12 032.0 **a** (4840.94)	4469.5 **b** (5030.90)	4783.5 **b** (5174.11)	1337.9 **c** (870.86)	273.7 **d** (472.66)
Range in collection size	4940-19 890	1404-18 384	276-15770	346-2405	26-1592
Culicoides:non *Culicoides*	1:0.03	1:0.03	1:0.03	1:0.05	1:0.1
Comparison with Onderstepoort trap	1	0.37	0.40	0.11	0.02
C. imicola:					
Total collected (%)	119 705 (99.5)	44 460 (99.5)	47 561 (99.4)	13 261 (99.1)	2696 (98.5)
Mean numbers collected (STDV)	11970.5 **a** (4 828.05)	4446.0 **b** (5022.20)	4756.1 **b** (5162.65)	1326.1 **c** (864.55)	269.6 **d** (466.92)
Nulliparous (%)	92 087 (76.9)	34 136 (76.8)	35 792 (75.3)	9768 (73.7)	2116 (78.5)
Parous (%)	26 783 (22.4)	9888 (22.2)	11 294 (23.7)	3326 (25.1)	501 (18.6)
Freshly blood-fed (%)	242 (0.2)	63 (0.1)	81 (0.2)	56 (0.4)	74 (2.7)
Gravid (%)	192 (0.2)	82 (0.2)	87 (0.2)	31 (0.2)	0
Males (%)	401 (0.3)	291 (0.7)	316 (0.7)	80 (0.6)	5 (0.2)

Numbers per row followed by a different letter were significantly different at the 5% level.

with blue LEDs. Low numbers of *Culicoides bedfordi* Ingram and Macfie were only present in the Onderstepoort trap.

All the traps, however, showed *C. imicola* to be dominant (Table 1). Its proportional representation ranged from 98.5% in the red LED trap to 99.5% in the Onderstepoort and white LED traps (Table 1). As with the total mean numbers of *Culicoides* collected those in the Onderstepoort trap (11970.5) were significantly higher (P<0.001) than in the other traps (Table 1). Similar mean numbers were collected in the white (4446.0) and blue (4756.1) LED traps (Table 1). The red (269.6) and green (1326.1) LED traps collected the least (Table 1). The second most abundant species in all of the traps, representing 0.4% of the total number collected, was *Culicoides enderleini* Cornet and Brunhes.

With the proportional representation ranging from 73.7% in the green to 78.5% in the red, all five traps showed nulliparous *C. imicola* females to be the dominant grouping (Table 1). The highest proportion of freshly blood engorged *C. imicola* females (2.7%) was collected with red LEDs. Gravid *C. imicola* females and males represented less than 1% of the total *C. imicola* (Table 1).

The proportion *Culicoides* to non-*Culicoides* was 1:0.3 in the Onderstepoort, white and blue LED traps. It was somewhat higher in the green (1:0.05) and red (1:0.1) ones (Table 1).

Comparisons of 12 V LED traps

In the comparison of the 12 V blue, green, white LED traps and a 12 V black light Onderstepoort trap with the 220 V black light Onderstepoort trap, 21 754 *Culicoides* midges were collected in 50 collections made in winter from 23 June to 6 July 2017. The mean numbers collected in the 220 V (1077.3), accounting for 49.5% of the total, was significantly higher (P<0.001) than that in the 12 V traps (Table 2). The higher mean numbers collected in the 12 V Onderstepoort trap (442.1) was not significantly different from collections in the

Table 2. Comparison of *Culicoides* midges collected with four 12 V down-draught light traps fitted with different coloured LED lights and the standard Onderstepoort trap during winter (23 June to 6 July 2017) at the ARC-OVR, South Africa.

	220 V	12 V			
	Onderstepoort	Onderstepoort	Blue LED	Green LED	White LED
No. of collections	10	10	10	10	10
Species richness	14	6	8	7	9
Total *Culicoides* collected (%)	10 773 (49.5)	4421 (20.3)	1904 (8.8)	1550 (7.1)	3106 (14.2)
Mean collection size (STDV)	1077.3 **a** (1192.92)	442.1 **bc** (867.56)	190.4 **bc** (132.76)	155.0 **c** (223.44)	310.6 **b** (379.20)
Range in collection size	66-4041	20-2864	40-429	13-768	70-1353
Culicoides: non *Culicoides*	1:0.05	1:0.07	1:0.1	1:0.2	1:0.1
Comparison with 220 V Onderstepoort trap	1	0.41	0.18	0.14	0.29
C. imicola					
Total collected (%)	10 683 (99.2)	4374 (99.4)	1886 (99.1)	1534 (99.0)	3079 (99.1)
Mean numbers collected (STDV)	1068.3 **a** (1188.87)	437.4 **bc** (859.47)	188.6 **bc** (132.31)	153.4 **c** (223.00)	307.9 **b** (377.04)
Nulliparous (%)	7431 (69.6)	2991 (68.4)	1297 (68.8)	998 (65.1)	2137 (69.4)
Parous (%)	3063 (28.7)	1351 (30.9)	579 (30.7)	520 (33.9)	904 (29.4)
Freshly blood-fed (%)	24 (0.2)	7 (0.2)	1 (0.1)	2 (0.1)	9 (0.3)
Gravid (%)	50 (0.5)	9 (0.2)	4 (0.2)	6 (0.1)	10 (0.3)
Males (%)	115 (1.1)	16 (0.4)	5 (0.3)	8 (0.5)	19 (0.6)

Numbers per row followed by a different letter were significantly different at the 5% level white (310.6), blue (190.4) or green (155.0) LED traps (Table 2). The mean numbers in the green LED however, were significantly lower than in the white LED trap.

Fourteen of the 15 species collected were present in the 220 V trap. Single specimens of *C. coarctatus, Culicoides huambensis* Caeiro and *Culicoides schlutzei* (Enderlein) were only present in the 220 V trap and a single *C. bedfordi* was only collected in the white LED trap. Species richness, 6 and 7 species respectively, was lower in the 12 V Onderstepoort and green LED traps (Table 2).

The proportional representation of *C. imicola* was 99% or higher in all traps (Table 2). The mean numbers collected in the 220 V (1068.3) was significantly higher than in any of the 12 V traps (Table 2). While the lower mean numbers of *C. imicola* collected in the green LED trap (153.4) was significantly lower than what was collected in the white (307.9) it did not differ from that of the Onderstepoort 12 V (437.4) or blue LED trap (188.6) (Table 2). Similar numbers were collected in the 12 V Onderstepoort, blue and white LED traps (Table 2).

As in April/May nulliparous *C. imicola* females were the dominant age grouping in all of the traps. The proportional representation of nulliparous females ranged from 65.1% in the green LED to 69.6% in the 220 V trap (Table 2). As is characteristic of light traps placed near livestock, freshly blood engorged and gravid females represented less than 0.5% of the *C. imicola* collected (Table 2). The highest proportion (1.1%) of *C. imicola* males were collected in the 220 V trap (Table 2). As also seen in April/May, the second most abundant species, representing 0.5% of the total number, were *C. enderleini*.

The ratio of *Culicoides* to non-*Culicoides* was similar to that found previously. The highest ratios, 1:0.05 and 1:0.07, were found in the 220 V and 12 V Onderstepoort traps (Table 2).

Discussion

The differential attractiveness of various coloured LEDs fitted in a 220 V Onderstepoort trap indicates that colour plays a role in defining the effectiveness of the trap for the collection of *C. imicola*. *Culicoides imicola* is based on its abundance at livestock, host preference, geographical distribution, oral susceptibility and field isolation of the virus considered the most important vector of BTV and AHSV in South Africa (Meiswinkel *et al*., 2004; Purse *et al*., 2015). The importance of *C. imicola* as an orbivirus vector is emphasized by the fact that this species are found widely distributed in Africa, the Mediterranean, Laos, Vietnam and southern China (Meiswinkel *et al.,* 2014). The 220 V Onderstepoort black light trap collected significantly more midges and *C. imicola* than either 220 V or 12 V traps fitted with various colours of LEDs. The greater attractiveness of black light is a well-known phenomenon for a number of adult aquatic and terrestrial insect species (Pohe *et al.,* 2017) as well as *Culicoides* (Rowley & Jorgensen, 1967; Venter & Hermanides 2006).

Bishop *et al.* (2006) indicated that *Culicoides brevitarsis* Kieffer, considered as a primary vector of BTV in Australia, might be under-represented in UV traps and that suction traps baited with green LEDs collect significantly greater numbers and also a greater diversity of *Culicoides* species than either a standard incandescent or blue- or UV-LED baited traps. In collections in the Brazilian savannah, with *Culicoides lutzi* Costa Lima being the most frequent species collected, green LED traps accounted for 53.9% of all individuals sampled, followed by incandescent (35.0%) and blue lights (11.2%) (Silva *et al*., 2015). These differences however were not statistically significant. Hope *et al*. (2015) found greater numbers of *Culicoides dewulfi* Goetghebuer and *Culicoides pulicaris* (Linnaeus) in traps fitted with green LEDs compared to UV LEDs. *Culicoides obsoletus* Meigen and *Culicoides* *scoticus* Downes and Kettle, on the other hand, responded randomly to all wavelengths of LEDs used with the exception of red, which was less attractive to all species (Hope *et al*., 2015). Overall, LED traps collected fewer *Culicoides* than the CDC UV light suction trap (Hope *et al*., 2015; González *et al*., 2016). Contrary to these results, green LEDs, as found with both 220 V and 12 V traps in the present survey, were less effective for the collection of *C. imicola* and South African livestock associated *Culicoides* species. Despite lower mean numbers of all species, 10 of 18 collected in these two surveys were present in the green LED traps.

There were no significant differences in the mean numbers of *C. imicola* collected with the 220 V traps fitted with white and blue LEDs. In agreement with published results (Bishop *et al.,* 2004; Hope *et al.,* 2015; González *et al.,* 2016) the lowest species diversity, number of *Culicoides* and of *C. imicola* were collected in the traps with red LEDs. It should, however, be noted that more than a 1500 *Culicoides* can be collected during periods of high abundance. All 220 V and 12 V traps, irrespective of light source or colour, found *C. imicola*, representing more than 98% as being dominant and *C. enderleini* as the second most abundant species.

Since transovarial transmission of orbiviruses is apparently absent in the genus *Culicoides*, the proportion of parous females can indicate the relative risk of potential virus transmission (Osborne *et al.,* 2015). In the present study, all of the traps showed nulliparous females, ranging from 73.7% to 78.5% in April and from 65.1% to 69.4% in June/July, to be the dominant age grouping. Age grading results for *C. imicola,* as established by the various traps, were comparable and were apparently not influenced by the colour of the light. This is in agreement with previous results for *C. imicola* (Venter & Hemanides, 2006) but contrary to what was found for *Culicoides sonorensis*

Wirth and Jones, where black light may be biased towards the collection of parous females (Anderson & Linhares, 1989).

Despite significantly lower numbers collected 12 V Onderstepoort traps as well as white and blue LED traps will give a reliable indication of species diversity and representation of the abundant species in South Africa as well as the physiological status of the population and associated risk of transmission in an area. Except for single specimens of some species, collected on one or two occasions, the composition and diversity as determined with the different traps were comparable. Due to the low abundance of these species in all of the traps, direct comparisons may be unreliable.

The comparison of the 12 V blue, green, white LEDs traps and 12 V Onderstepoort trap with the 220 V Onderstepoort trap indicated the 220 V trap to be more efficient than any of the 12 V traps evaluated. The higher mean numbers collected in the 12 V Onderstepoort trap were not significantly different from those collected in the blue and white LED traps. Although the same light source, a 30 cm 8 W black fluorescent light tube, was used in the Onderstepoort 220 V and 12 V traps significantly more *Culicoides* were collected in the 220 V trap. This may indicate that light source is not the only variable that will determine trap efficiency and that the strength of the down draft of the fan and the intensity of the light (Snyder *et al.,* 2016) may play a role. It should be remembered that a trap, due to the strength of the fan in relation to the entrance portals, may not capture all insects attracted to the light.

As proficiently reviewed by McDermott & Mullens (2017) it needs to be emphasized that researchers must constantly be aware of the shortcomings of light traps and that the collection method must be relevant to the research question. Energy efficient 12 V traps will be suitable to identify the most abundant species in a given area or at a specific host. 12 V traps were in fact, recently used to determine *Culicoides* diversity in the rural Eastern Cape, South Africa (Riddin, 2017) and also in Namibia (Liebenberg *et al.,* 2016). In these situations, the 12 V traps allowed effective sampling over a wide and diverse geographic area independent of the availability of a 220 V electricity infrastructure. The most efficient traps in these situations will be the 12 V Onderstepoort or 12 V traps fitted with either blue or white LEDs. If large numbers of *Culicoides* are needed for virus detection, or studies involving live specimens, traps that are more powerful may be appropriate.

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References

Anderson, J.R. & Linhares, A.X. (1989) Comparison of several different trapping methods for *Culicoides variipennis* (Diptera: Ceratopogonidae). *Journal of the American Mosquito Control Association*, **5**, 325–334.

Barnard, B.J.H. (1997) Antibodies against some viruses of domestic animals in southern African wild animals. *Onderstepoort Journal of Veterinary Research,* **64**, 95–110.

Barnard, B.J.H. & Paweska, J.T. (1993) Prevalence of antibodies against equine viruses in zebra (*Equus burchelli*) in the Kruger National Park 1991-1992. *Onderstepoort Journal of Veterinary Research,* **60**, 175–179.

Becker, E., Venter, G.J., Greyling, T., Molini, U. & van Hamburg, H. (2018) Evidence of African horse sickness virus infection of *Equus zebra hartmannae* in the south-western Khomas Region, Namibia. *Transboundary and Emerging Diseases*, 65, 278–280.

Bishop, A.L., Bellis, G.A., McKenzie, H.J., Spohr, L.J., Worrall, R.J., Harris, A.M. & Melville, L. (2006) Light trapping of biting midges *Culicoides* spp. (Diptera: Ceratopogonidae) with green light-emitting diodes. *Australian Journal of Entomology,* **45**, 202–205.

Bishop, A.L., Worral, R., Spohr, L.J., Mckenzie, H.J. & Barchia, I.M. (2004) Response of *Culicoides* spp. (Diptera: Ceratopogonidae) to light emitting diodes. *Australian Journal of Entomology*, **43**, 184–188.

Burkett, D.A., Butler, J.F. & Kline, D.L. (1998) Field evaluation of colored light-emitting diodes as attractants for woodland mosquitoes and other Diptera in north central Florida. *Journal of the American Mosquito Control Association*, **14**, 186–195.

Carpenter, S., Veronesi, E. Mullens, B. & Venter, G. (2015) Vector competence of *Culicoides* for arboviruses: three major periods of research, their influence on current studies and future directions. *Revue scientifique et technique, Office International des Epizooties*, **34**, 97–112.

Cohnstaedt, L.W., Gillen, J.I. & Munstermann, L.E. (2008) Light-emitting diode technology improves insect trapping. *Journal of the American Mosquito Control Association,* **24,** 331–334.

Del Río López, R. (2012) *Control strategies and vector competence of* Culicoides *species vectors of bluetongue virus in Iberian Peninsula and Balearic Islands*, PhD thesis, Universitat de les Illes Balears.

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

González, M., Alarcón-Elbal, P.M., Valle-Mora, J. & Goldarazena, A. (2016) Comparison of different light sources for trapping *Culicoides* biting midges, mosquitoes and other dipterans. *Veterinary Parasitology*, **226**, 44-49.

Hope, A., Gubbins, S., Sanders, C., Denison, E., Barber, J., Stubbins, F., Baylis, M. & Carpenter, S. (2015) A comparison of commercial light-emitting diode baited suction traps for surveillance of *Culicoides* in northern Europe. *Parasites and Vectors*, **8**, 239.

Jenkins, A.B. & Young, M.B. (2010) Investigation of *Culicoides* spp. preference for light colour and source using light emitting diodes and fluorescent light. *South African Journal of Animal Science*, **40**, 514–518.

Labuschagne, K. (2016) *The* Culicoides *Latreille (Diptera: Ceratopogonidae) species of South Africa*. PhD thesis, University of Pretoria.

Liebenberg, D., Piketh, S., Labuschagne, K., Venter, G., Greyling, T., Mienie, C., de Waal. T. & van Hamburg, H. (2016) *Culicoides* species composition and environmental factors influencing African horse sickness distribution at three sites in Namibia. *Acta Tropica,* **163**, 70–79.

McDermott, E.G. & Mullens, B.A. (2017) The dark side of light traps. *Journal of Medical Entomology,* XX(X), 1–11 doi: 10.1093/jme/tjx207

Meiswinkel, R., Venter, G.J. & Nevill, E.M. (2004) Vectors: *Culicoides* spp. In: Infectious Diseases of Livestock (ed. by J.A.W. Coetzer & R.C. Tustin), Oxford University Press, Cape Town, p. 93–136.

Niedbalski, W. (2015) Bluetongue in Europe and the role of wildlife in the epidemiology of disease. *Polish Journal of Veterinary Sciences*, **18**, 455–461.

Osborne, C.J., Mayo, C.E., Mullens, B.A., McDermott, E.G., Gerry, A.C., Reisen, W.K., Reisen, W.K. & MacLachlan, N.J. (2015) Lack of evidence for laboratory and natural vertical transmission of bluetongue virus in *Culicoides sonorensis* (Diptera: Ceratopogonidae). *Journal of Medical Entomology*, **52**, 274–277.

Perry, J.N., Wall, C. & Greenway, A.R. (1980) Latin square designs infield experiments involving sex attractants. *Ecological Entomology*, **5**, 385–396.

Pohe, S.R., Winterbourn, M.J. & Harding, J.S. (2017) Comparison of fluorescent lights with differing spectral properties. *New Zealand Entomologist*, Vol. 0, 1–11.

Probst, C., Kampen, H., Werner, D., Gethman, J., Conraths, F.J. & Beer, M. (2009) Comparison of the efficacy of four light trap models for the collection of *Culicoides* biting midges in Germany, *Revue d'élevage et de Médecine Vétérinaire des pays Tropicaux,* **62**, 139.

Purse, B.V., Carpenter, S., Venter, G.J., Bellis, G. & Mullens, B.A. (2015) Bionomics of temperate and tropical *Culicoides* midges: Knowledge gaps and consequences for transmission of *Culicoides*-borne viruses. *Annual Review of Entomology*, **60,** 373–392.

Riddin, M.A. (2017) *The epidemiology of African horse sickness in the Eastern Cape, South Africa*. PhD thesis, Rhodes University.

Rowley, W.A. & Jorgensen, N.M. (1967) Relative effectiveness of three types of light traps in collecting adult *Culicoides*. *Journal of Economic Entomology*, **60**, 1478–1479.

Silva, F.S., Brito, J.M. & Costa-Neta, B.M. (2015) Field evaluation of light-emitting diode as attractant for blood-sucking midges of the Genus *Culicoides* Latreille (Diptera: Culicomorpha, Ceratopogonidae) in the Brazilian Savanna. *Entomological News,* **125,** 1–6.

Snedecor, G.W. & Cochran, W.G. (1980) Statistical Methods, 7th ed. Iowa State University Press, Ames, IA.

Snyder, D., Cernicchiaro, N. & Cohnstaedt, L.W. (2016) Sugar-feeding status alters biting midge photoattraction. *Medical and Veterinary Entomology*, **30**, 31–38.

Van Ark, H. & Meiswinkel, R. (1992) Subsampling of large light trap catches of *Culicoides* (Diptera: Ceratopogonidae). *Onderstepoort Journal of Veterinary Research*, **59**, 183–189.

Venter, G.J. (2015) *Culicoides* spp. (Diptera: Ceratopogonidae) as vectors of bluetongue virus in South Africa – a review. *Veterinaria Italiana*, **51**, 325–333.

Venter, G.J. & Hermanides, K.G. (2006) Comparison of black and white light for collecting *Culicoides imicola* and other livestock-associated *Culicoides* species in South Africa. *Veterinary Parasitology*, **142,** 383–385.

Venter, G.J., Labuschagne, K., Hermanides, K.G., Boikanyo, S.N.B., Majatladi, D.M. & Morey, L. (2009) Comparison of the efficiency of five suction light traps under field conditions in South Africa for the collection of *Culicoides* species. *Veterinary Parasitology*, **166,** 299–307.

Venter, G.J., Majatladi, D.M., Labuschagne, K., Boikanyo, S.N.B. & Morey, L. (2012) The attraction range of the Onderstepoort 220 V light trap for *Culicoides* biting midges as determined under South African field conditions. *Veterinary Parasitology*, **190,** 222–229.

VSN International (2012) GenStat for Windows, 15th ed. VSN International, Hemel Hempstead.