Evaluation of light emitting diode suction traps for the collection of livestock-associated *Culicoides* species in South Africa

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ABSTRACT

Risk analysis of pathogens transmitted by *Culicoides* (Diptera; Ceratopogonidae) depends on the ability to detect all potential vectors attacking livestock in an area. Onderstepoort 220-V ultraviolet (UV) down-draught light traps are considered the gold standard for this purpose. To improve the flexibility of this trap in the field, in the absence of 220-V power, the possibility of using low-energy light emitting diodes (LEDs) was assessed. The efficiency of a standard 220-V Onderstepoort trap (30 cm 8 W fluorescent UV light tube) was compared to that of 220-V Onderstepoort traps fitted with either two, four or eight individual white LEDs. The Onderstepoort 220-V trap was also compared to a 12-V Onderstepoort trap fitted with an 8 W fluorescent UV light tube, a 12-V Onderstepoort trap with 12 individual white LEDs and 12-V and 220-V Onderstepoort traps fitted with 12 individual UV LEDs. Higher numbers of *Culicoides* as well as species diversity were collected with a brighter light source. The use of UV LEDs in both the 12-V and 220-V combinations was comparable to the Onderstepoort 220-V light trap with ration to species diversity collected. The Onderstepoort 220-V light trap is recommended if large numbers of *Culicoides* need to be collected.

Keywords. Culicoides imicola, onderstepoort light trap, ultraviolet.

INTRODUCTION

In addition to several species of protozoa, nematodes and filarial worms, viruses of global veterinary importance transmitted by *Culicoides* species (Diptera; Ceratopogonidae) include those that cause bluetongue, epizootic haemorrhagic disease, African horse sickness and Schmallenberg (Meiswinkel *et al*., 2004; Hoffmann *et al*., 2012; Veronesi *et al*., 2013; Purse *et al*., 2015). The extensive geographical expansion of these viral diseases in Europe, North America over the last two decades, and recently in Asia, indicated the involvement of several novel species of *Culicoides* in the transmission of these viruses (Meiswinkel *et al*., 2007; Rodríguez-Sánchez *et al*., 2008; Conraths *et al*., 2009; Pascall *et al*., 2019; King *et al*., 2020). An apparent wide-spread susceptibility to orbivirus infection in the genus *Culicoides*, as indicated by laboratory susceptibility studies (Carpenter *et al*., 2008; Venter *et al*., 2011; Del Rio López *et al*., 2012; Ruder *et al*., 2012), emphasized the need to collect and

identify all species that may potentially feed on livestock to pinpoint the vectors of these diseases.

The geographical distribution, seasonal incidence, and over-wintering of these viruses, in addition to susceptible vertebrate hosts, depends on the presence and abundance of competent arthropod vectors, i.e. specific *Culicoides* species. The success of disease risk analysis depends on an ability to detect all potential vectors in an area (Courtejoie *et al*., 2018). Successful integrated control, among other control methods, must include vector control. The reliable comparison of *Culicoides* abundance and species composition between sites will be pivotal in comparing the risk between areas. Presence and absence data of potential vectors form the basis of reliable risk models for disease occurrence and potential expansion (Courtejoie *et al*., 2018; Leta *et al*., 2019).

Due to the relative ease of use various light trap models with different, and yet unknown levels of efficiency, are extensively used for the monitoring of *Culicoides* diversity and abundance (Venter *et al*., 2009; Lühken & Kiel, 2012; Del Río López *et al*., 2013; Probst *et al*., 2015; McDermott *et al*., 2016). The superior ability of the Onderstepoort 220-V UV down-draught light trap to capture large numbers of *Culicoides*, with no major differences between traps regarding species composition, has been demonstrated on several occasions (Venter *et al*., 2009; Del Río López *et al*., 2013; Probst *et al*., 2015). As such both the European Centre for Disease Prevention and Control and the European Food Safety Authority recommend the 220-V Onderstepoort UV light trap as the surveillance tool of choice (Medlock *et al*., 2018). Compared to other light traps the Onderstepoort trap is relatively heavy (4 kg) and robust. This metal trap can, however, be left in situ for a couple of months. Taking into consideration the more powerful light source and fan of the Onderstepoort, it is not surprising that this trap is more efficient than the others (Venter *et al*., 2009).

The attractiveness of UV light, compared to other wavelengths of light, to nocturnal insects is well-known (Pohe *et al*., 2017). The greater attractiveness of UV light was also confirmed for proven biting midge vectors of viruses harmful to livestock such as *Culicoides imicola* Kieffer (Rowley & Jorgensen, 1967; Venter & Hermanides, 2006; Sloyer *et al*., 2019; Bray *et al*., 2020). Similarly green light-emitting diodes (LEDs) were shown to be equal or more attractive than UV light for some species of *Culicoides* (Bishop *et al*., 2006; Harrup *et al*., 2016). A drawback of a more attractive light source may be that it may result in a bigger by-catch of non-target insect and as such increase sorting time in the laboratory.

The dependence of the Onderstepoort trap on 220-V power supply limits the applicability thereof in rural areas and field situations. Certain species of wildlife act as reservoir hosts of orbiviruses transmitted by *Culicoides* species (Ruiz-Fons *et al*., 2014), e.g. breeding populations of zebras are considered a reservoir host of African horse sickness virus (Barnard, 1998). Wildlife-associated *Culicoides* species as such may play a decisive role in the transmission and overwintering of these viruses. Evaluations of a 12-V version of the Onderstepoort trap indicated it to be less efficient than the 220-V version (Venter *et al*., 2018). Comparisons of the Onderstepoort trap fitted with energy-efficient 12-V LEDs indicated that, although the standard 220-V trap was the most efficient, relative large

numbers of South African livestock-associated *Culicoides* species can be collected in Onderstepoort traps fitted with either blue or white LEDs (Venter *et al*., 2018).

It was shown that the brightness of the light source may influence the efficiency of the trap for some species of mosquitoes (Barr *et al*., 1960). Although several studies have dealt with the evaluation of the effectiveness of different wavelength of light (Bishop *et al*., 2004, 2006; Hope *et al*., 2015; Silva *et al*., 2015; González *et al*., 2016; Harrup *et al*., 2016; Venter *et al*., 2018; Mazumdar & Mazumdar, 2020) for the attraction of *Culicoides*, studies regarding the brightness of the light are limited. Light intensity was shown to play a significant role in increasing the numbers of especially *Cuicoides brevitarsis* Kieffer collected with green LEDs in Australia (Bishop *et al*., 2004).

In the present study, the potential improvement of the efficiency of LEDs traps was investigated by increasing the numbers of individual LEDs incorporated in a 220-V Onderstepoort trap. Subsequently the efficiency of 12-V and 220-V UV LED was compared to that of a standard 220-V Onderstepoort trap. The efficiency of these traps to collect South African livestock-associated *Culicoides* species and especially *C. imicola*, a proven vector of orbiviruses, was assessed.

MATERIALS AND METHODS

To assess the potential influence of the brightness of the light source on trapping efficiency, Onderstepoort down-draught traps fitted with either two, four or eight white LEDs (∼425–750 nm) (CE ROHS 5050 3LED; Sencart, Shenzhen, China) was compared to a standard 220-V Onderstepoort trap with a 30 cm 8 W fluorescent UV light tube (∼365 nm). In a second comparison, the efficiency of 12-V and 220-V Onderstepoort traps with 8 W fluorescent UV light tube, a 12-V Onderstepoort trap with 12 white individual LEDs and a 12-V and 220-V Onderstepoort trap with 12 UV individual LEDs (∼390 nm) were compared. While the 220-V traps were operated on the main electrical supply, the 12-V traps were operated on 12-V car batteries which were charged daily.

The evaluations were conducted at the Agricultural Research Council–Onderstepoort Veterinary Research (25°39′ S, 28°11′ E; 1219 m above sea level) in South Africa. To ensure that treatment means were independent of any effects due to site or occasion, the traps were compared in a randomized Latin square design (Perry *et al*., 1980). In the evaluation of the light brightness, the four traps were compared in three replicates of a 4 × 4 randomized Latin square design. The comparisons were conducted at four sites over 12 nights during spring between 10 and 22 September 2018. The UV LED and fluorescent UV light comparisons were done 2 months later in early summer 12–21 November 2018. The five trap designs were compared in two replicates of a 5 × 5 randomized Latin square design conducted over 10 nights.

The traps were operated from dusk to dawn at stables housing between 20 and 40 cattle each. During the day, the cattle were in open pens (900 m^2) with concrete flooring in front of the stables. To minimise interference between traps, trap sites were located at least 15 m apart, out of direct sight to each other. The traps were 1.4 m above ground level and as close to the cattle as practical possible. Insects were collected into distilled water to which

0.5% Savlon® (Johnson & Johnson, Johannesburg, South Africa) (Clorhexidine gluconate 0.3 g/100 mL and Cetrimide 3.0 g/100 mL) antiseptic was added to break the surface tension of the water and allow insects to sink to the bottom of the collection beaker (Goffredo & Meiswinkel, 2004; Venter *et al*., 2009). Moths and other insects bigger than *Culicoides* were excluded by polyester netting (mesh size 2 mm) placed around the entrance portals of the traps.

After retrieval in the morning, the collected insects were transferred to 80% ethanol (Goffredo & Meiswinkel, 2004) and stored in the dark at room temperature until analysed. Large collections (>1000) were sub-sampled (Van Ark & Meiswinkel, 1992). The *Culicoides* were sorted from the rest of the insects, sexed, and identified to species level using identification keys (Labuschagne, 2016). All females were based on abdominal pigmentation (Dyce, 1969), age-graded into nulliparous, parous, gravid or freshly blood-fed females. To complete the analyses, males and other insects captured were also counted.

Analysis of variance (anova) was used to differentiate between the trap treatments. The data were normally distributed, and treatment means were separated using Tukey's test with 95% confidence intervals. Data were analysed using the statistical program GenStat® (VSN International, 2012). Shannon diversity index were calculated for *Culicoides* species (Al Young Studios, 2020).

RESULTS

Comparison of white LEDs of various brightness to the 220-V Onderstpoort trap

In the comparison of the four traps fitted with either two, four, eight white LEDs or a standard 30 cm 8 W fluorescent UV light tube, 78 383 *Culicoides* specimens were collected in 48 collections made over 12 nights between 10 and 22 September 2018 (Table 1). More than half of the specimens, 62.1%, were collected in the standard 220-V Onderstepoort trap with the fluorescent UV light. The mean number, 4058.9 ± 2486.42, of *Culicoides* collected in this trap was significantly different ($P < 0.001$) from that collected in the traps fitted with white LEDs (Fig. 1A). The lowest mean number, 488.3 ± 473.22, collected in the trap fitted with two white LEDs was significantly ($P < 0.001$) different from that of any of the other traps. The mean number of *Culicoides* collected in the trap fitted with four, 943.1 ± 479.75, and eight white LEDs, 1041.6 ± 643.65 did not differ significantly.

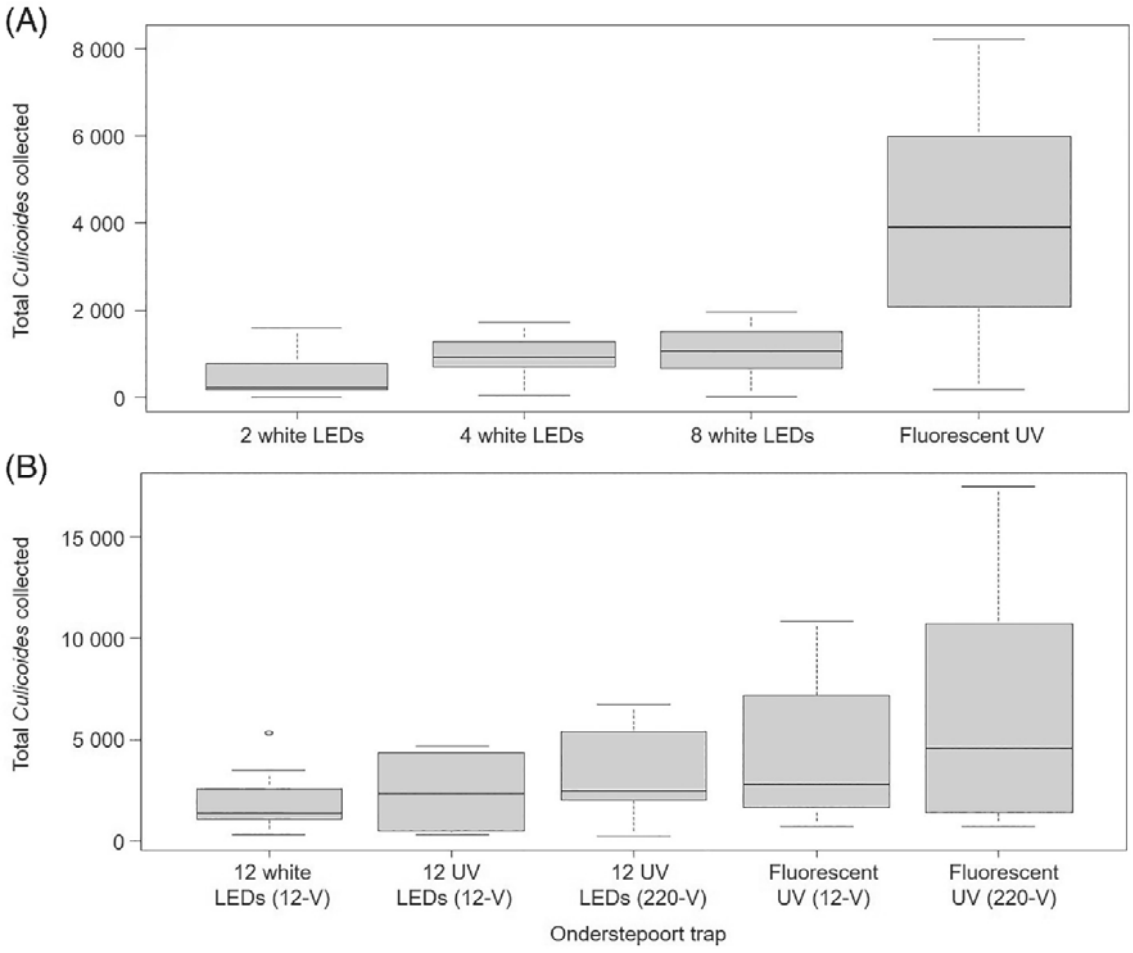

Fig. 1. *Culicoides* collected with down-draught Onderstepoort traps fitted with various numbers of white LEDs (A), light type and voltage configuration (B) during spring (10–22 September 2018) and early summer (12–21 November 2018) at the ARC-OVR, South Africa. Each box shows the group median separating the 25th and 75th quartiles, capped bars indicate maximum and minimum values, circles indicating the outliers

Table 1. Comparison of *Culicoides* midges collected with four down-draught 220-V Onderstepoort traps fitted with two, four or eight white LEDs or a standard 30 cm 8 W fluorescent UV light tube during spring (10–22 September 2018) at the ARC-OVR, South Africa.

	TWO WHITE LEDS	**FOUR WHITE LEDS**	**EIGHT WHITE LEDS**	**FLUORESCENT UV***
SPECIES RICHNESS	15	19	17	20
SHANNON DIVERSITY INDEX (H)	0.29c	0.36b	0.35b	0.41a
SHANNON DIVERSITY INDEX (H) EXCLUDING *CULICOIDES IMICOLA*	1.84b	2.13a	2.01a	2.02a
TOTAL *CULICOIDES* COLLECTED (%)	5860 (7.5)	11 317 (14.4)	12 499 (15.9)	48 707 (62.1)
MEAN COLLECTION SIZE (STD)	488.3 (473.22)c	943.1 (479.75)b	1041.6 (643.65)b	4058.9 (2486.42)a
RANGE IN COLLECTION SIZE	10–1585	50–1723	19–1966	180–8208
CULICOIDES*: NON *CULICOIDES	1: 0.36	1: 0.15	1: 0.47	1: 0.09
COMPARISON WITH FLUORESCENT TRAP*	0.12	0.23	0.26	1
MOST ABUNDANT SPECIES: *C. IMICOLA*				
TOTAL COLLECTED (%)	5563 (94.9)	10 633 (94.0)	11 732 (93.9)	45 085 (92.6)

MEAN NUMBERS COLLECTED (STD)	463.6 (463.29)c	886.1 (448.16)b	977.7 (616.99)b	3757.1 (2382.99)a
NULLIPAROUS (%)	3264 (58.7)	6226 (58.6)	6827 (58.2)	25 988 (57.6)
PAROUS (%)	2156 (38.7)	3764 (35.4)	4310 (36.7)	16 614 (36.9)
FRESHLY BLOOD-FED (%)	37 (0.7)	83 (0.8)	98 (0.8)	331 (0.7)
GRAVID (%)	34 (0.6)	111 (1.0)	79 (0.7)	673 (1.5)
MALES (%)	72 (1.3)	449 (4.2)	418 (3.6)	1479 (3.3)
CULICOIDES MAGNUS				
TOTAL COLLECTED (%)	140 (2.4)	175 (1.6)	221 (1.8)	654 (1.3)
MEAN NUMBERS COLLECTED (STD)	11.7 (6.89)	14.6 (9.37)	18.4 (0.0)	54.5 (0.0)
NULLIPAROUS (%)	93 (66.4)	137 (78.3)	158 (71.5)	484 (74.5)
PAROUS (%)	47 (33.6)	43 (24.6)	59 (26.7)	139 (21.3)
FRESHLY BLOOD-FED (%)	0 (0.0)	0 (0.0)	2 (0.9)	1 (0.2)
GRAVID (%)	0 (0.0)	0 (0.0)	9 (1.4)	0 (0.0)
MALES (%)	0 (0.0)	2 (1.1)	2 (0.9)	18 (2.8)
CULICOIDES LEUCOSTICTUS				
TOTAL COLLECTED (%)	28 (0.5)	117 (1.1)	145 (1.2)	1065 (2.2)
MEAN NUMBERS COLLECTED (STD)	3.5 (3.8)	11.7 (10.58)	14.5 (16.37)	88.8 (74.03)
NULLIPAROUS (%)	5 (17.9)	28 (24.1)	41 (28.3)	282 (26.5)
PAROUS (%)	1 (3.6)	4 (3.5)	12 (8.3)	68 (6.4)
FRESHLY BLOOD-FED (%)	0 (0.0)	1 (0.9)	1 (0.7)	6 (0.6)
GRAVID (%)	2 (7.1)	7 (6.0)	14 (9.7)	91 (8.5)
MALES (%)	20 (71.4)	76 (65.5)	77 (53.1)	618 (58.0)

* The standard 220-V Onderstepoort trap equipped with a 30 cm 8 W ultraviolet UV light tube. Twelve collections were made with each trap type. Numbers in the same row followed by different letters are significantly different at the 5% level.

The second most abundant species in each treatment is indicated in bold.

STD, standard deviation.

In agreement to the mean numbers collected, the highest (20 species) and lowest (15 species) species richness were recorded in 220-V fluorescent UV light trap and the trap fitted with two white LEDs, respectively. All the traps indicated *C. imicola* as being the dominant species. With a proportional representation ranging from 92.6% in the trap with the fluorescent UV light to 94.9% in the trap with two white LEDs. In agreement to the total mean numbers of *Culicoides*, the mean number of *C. imicola* collected in the trap with the fluorescent UV light, 3757.1 ± 2382.99, was significantly higher ($P < 0.001$) than that in the other traps. While the trap with two white LEDs collected significantly lower mean numbers (463.6 ± 463.29), the mean numbers collected in the trap with four (886.1 ± 448.16) and eight white LEDs (977.7 ± 616.99) did not differ significantly.

The Shannon diversity index differed significantly between the trap with the fluorescent UV light (0.41) and all the traps fitted with white LEDs ($P < 0.001$) (Table 1). There was no significant difference in the Shannon diversity indexes between the traps with four (0.36) and eight (0.35) white LEDs, however, both were significantly higher than that for the trap fitted with two white LEDs (0.29). When the Shannon diversity index was determined excluding the overriding dominance of *C. imicola*, the index for all the traps were higher ranging from 2.13 for the traps with the four white LEDs to 1.84 for the trap with two white LEDs (Table 1). The diversity index of the trap fitted with two white LEDs was, however, still significantly ($P < 0.001$) lower than that of any of the other traps (Table 1). In evaluating the

Culicoides species diversity, it can be mentioned that low numbers of *Culicoides schultzei* (Enderlein) and *Culicoides cornutus* de Meillon were only present in the trap with the two white LEDs. *Culicoides gulbenkiani* Caeiro were only present in the trap with four white LEDs.

The second most abundant species, with a proportional representation ranging from 1.6% to 2.4% in all the white LED traps was *Culicoides magnus* Colaco (Table 1). With a representation of 1.3%, it was the third most abundant species in the fluorescent UV light trap. *Culicoides magnus* was replaced by *Culicoides leucostictus* Kieffer, at 2.2%, as the second most abundant species in the fluorescent UV light trap (Table 1). All four traps indicated nulliparous females of these two species to be the dominant grouping. The trap fitted with the fluorescent UV light collected higher numbers of both these species then any of the traps fitted with white LEDs (Table 1).

With a proportional representation ranging from 57.6% in the trap with fluorescent UV light to 58.7% in the trap with two white LEDs, all four traps indicated nulliparous *C. imicola* females to be the dominant grouping. The proportional representation of parous *C. imicola* females, ranging from 35.4% in traps fitted with four white LEDs to 38.7% in traps fitted with two white LEDs, was similar (Fig. 1A). All four traps collected low numbers of freshly blood engorged, gravid females and males. The highest proportion of gravid *C. imicola* females (1.5%) was collected in the trap with the fluorescent UV light (Table 1). Males represent less than 5% of the collected *C. imicola* for all traps (Table 1).

The proportion *Culicoides* to non-*Culicoides* was the lowest 1: 0.09 in the trap with the fluorescent UV light, and the highest 1: 0.47 in the trap with eight white LEDs (Table 1).

Comparison of white and UV LEDs to the Onderstepoort trap

In the comparison of the five Onderstepoort traps, fitted with either 12-V white LEDs, 12-V or 220-V UV LEDs, 12-V or 220-V fluorescent UV light tubes, 186 927 *Culicoides* were collected in 50 collections made over 10 nights between 12 and 21 November, 2018. The highest mean numbers of midges collected in the 220-V fluorescent UV light trap (6561.0 ± 6186.56), accounting for 35.1% of the total number collected, was significantly different ($P = 0.019$) from that in the 12-V UV (2438.8 ± 1831.44) LED and 12-V white (1930.8 ± 1490.81) traps (Table 2). The lower mean numbers collected in the 12-V fluorescent UV light trap (42 96.2 ± 3697.59) and the 220-V UV LED trap (3465.9 ± 1831.44) were not significantly different from each other nor from that collected in the 220-V fluorescent UV light trap (Fig. 1B; Table 2).

Table 2. Comparison of *Culicoides* midges collected with five down-draught Onderstepoort traps fitted with 12 white or UV LEDs and a standard 30 cm 8 W fluorescent UV light tube during early summer (12–21 November 2018) at the ARC-OVR, South Africa.

	12 LEDS			**FLUORESCENT UV**	
	12-V white	**12-V UV**	**220-V UV**	**12-V**	**220-V***
SPECIES RICHNESS	21	19	17	20	21
SHANNON DIVERSITY INDEX (H)	0.34	0.36	0.37	0.42	0.33
SHANNON DIVERSITY INDEX (H) EXCLUDING *C. IMICOLA*	2.20	2.24	2.11	2.16	2.13
TOTAL *CULICOIDES* COLLECTED (%)	19 308 (10.3)	24 388 (13.1)	34 659 (18.5)	42 962 (23.0)	65 610 (35.1)
MEAN COLLECTION SIZE (STD)	1930.8 (1490.81)b	2438.8 (1831.44)b	3465.9 (2278.03)ab	4296.2 (3697.59)ab	6561.0 (6186.56)a
RANGE IN COLLECTION SIZE	357–5361	304–4698	239–6720	727–10 854	725–17 512
CULICOIDES*: NON *CULICOIDES	1: 0.42	1: 0.16	1: 0.19	1: 0.30	1: 0.21
COMPARISON WITH FLUORESCENT 220-V TRAP*	0.29	0.37	0.53	0.65	1
MOST ABUNDANT SPECIES: *C. IMICOLA*					
TOTAL COLLECTED (%)	18 214 (94.3)	22 939 (94.1)	32 493 (93.8)	39 787 (92.6)	62 032 (94.6)
MEAN NUMBERS COLLECTED (STD)	1821.4 (1468.87)b	2293.9 (1729.47)ab	3249.3 (2206.26)ab	3978.7 (3542.31)ab	6203.2 (6031.67)a
NULLIPAROUS (%)	11 060 (60.7)	16 247 (70.8)	21 420 (65.9)	25 793 (64.8)	39 337 (63.4)
PAROUS (%)	5904 (32.4)	5470 (23.9)	9545 (29.4)	12 289 (30.9)	19 539 (31.5)
FRESHLY BLOOD-FED (%)	264 (1.5)	216 (0.9)	247 (0.8)	458 (1.2)	748 (1.2)
GRAVID (%)	132 (0.7)	229 (1.0)	291 (0.9)	461 (1.2)	658 (1.1)
MALES (%)	854 (4.7)	777 (3.4)	990 (3.1)	786 (2.0)	1750 (2.8)
CULICOIDES MAGNUS					
TOTAL COLLECTED (%)	207 (1.1)	327 (1.3)	563 (1.6)	655 (1.5)	626 (1.0)
MEAN NUMBERS COLLECTED (STD)	20.7 (14.58)	32.7 (33.45)	56.3 (48.78)	65.5 (42.51)	62.6 (54.74)
NULLIPAROUS (%)	128 (61.8)	257 (78.59)	412 (73.18)	470 (71.76)	438 (69.97)
PAROUS (%)	70 (33.8)	69 (21.1)	128 (22.7)	138 (21.1)	164 (26.2)
FRESHLY BLOOD-FED (%)	2 (1.0)	0 (0.0)	0 (0.0)	5 (0.8)	3 (0.5)
GRAVID (%)	0 (0.0)	0 (0.0)	1 (0.2)	2 (0.3)	9 (1.4)
MALES (%)	7 (3.4)	1 (0.3)	22 (3.9)	40 (6.1)	12 (1.9)
CULICOIDES BEDFORDI					
TOTAL COLLECTED (%)	193 (4.1)	271 (5.7)	338 (7.1)	744 (1.7)	813 (1.3)
MEAN NUMBERS COLLECTED (STD)	19.3 (17.70)	27.1 (36.68)	37.56 (35.26)	74.4 (61.93)	81.3 (84.25)
NULLIPAROUS (%)	72 (37.3)	143 (53.0)	146 (43.2)	354 (47.6)	341 (41.9)
PAROUS (%)	15 (7.8)	22 (8.2)	34 (10.1)	76 (10.2)	72 (8.9)
FRESHLY BLOOD-FED (%)	1 (0.5)	3 (1.1)	0 (0.0)	10 (1.3)	3 (0.4)
GRAVID (%)	22 (11.4)	25 (9.3)	44 (13.0)	70 (9.4)	129 (15.9)
MALES (%)	83 (43.0)	77 (28.5)	114 (33.7)	234 (31.5)	268 (33.0)

* The standard 220-V Onderstepoort trap equipped with a 30 cm 8 W ultraviolet UV light tube. Ten collections were made with each trap type. Numbers in the same row followed by different letters are significantly different at the 5% level.

The second most abundant species in each treatment is indicated in bold.

STD, standard deviation.

The proportional representation of *C. imicola* was 92.6% (12-V fluorescent UV trap) or higher in all the traps (Table 2). The higher mean number collected in the 220-V fluorescent UV trap (6203.2 ± 6031.67) was significantly different from that collected in the 12-V white LED (1821.4 ± 1468.87) trap (Fig. 1B; Table 2). However, the lower mean numbers of *C. imicola* collected in the 12-V UV LED (2293.9 ± 1729.47), 220-V UV LED (3249.3 ± 2206.26)

and 12-V fluorescent UV light (3978.7 ± 3542.31) trap did not differ significantly from that collected in the 220-V fluorescent UV trap (Fig. 1B; Table 2).

Species richness was similar in the 220-V fluorescent UV and LED 12-V trap (Table 2). The 220-V UV LED trap collected the lowest number of species, 17 (Table 2). There were, however, no significant differences in the Shannon diversity indexes of any of the traps (Table 2). There was, like in the previous evaluation, an increase in Shannon diversity indexes if the overriding *C. imicola* numbers were excluded (Table 2).

A single specimen of *Culicoides bolitinos* Meiswinkel was only present in the 12-V white LED and single specimen of *Culicoides tropicalis* Kieffer only in the 220-V fluorescent UV light trap. *Culicoides glabripennis* Goetghebuer were only collected in low numbers in the 12-V white LED (four specimens) and 220-V fluorescent UV (three specimens). Similarly, *Culicoides neavei* Austen were only present in low numbers in the 12-V (two specimens) and 220-V fluorescent UV (three specimens) traps.

The second most abundant species collected in the white LED traps, representing between 1.1% and 1.6% of the total number of *Culicoides* collected, was *C. magnus* (Table 2). The second most abundant species in both the fluorescent UV traps were *Culicoides bedfordi* Ingram and Macfie (Table 2). The fluorescent UV light traps collected higher numbers of both species than any of the LED traps (Table 2).

As in the comparisons conducted in September, nulliparous *C. imicola* females were the dominant age grouping recorded in all the traps. The proportional representation of nulliparous females ranged from 60.7% in the 12-V white LED to 70.8% in the 12-V LED UV light trap (Table 2). The proportion of parous *C. imicola* females collected in the different traps was similar (Table 1). Characteristically of light traps, freshly blood engorged, and gravid females represented less than 1.5% of the *C. imicola* females collected (Table 2). The highest proportion (4.7%) *C. imicola* males was collected in the 12-V white LED trap (Table 2).

The ratio of *Culicoides* to non-*Culicoides* was similar as found previously. The highest ratios, 1: 0.42 were found in the 12-V white LED trap and the lowest in the 12-V UV LED, 1: 0.16 (Table 2).

DISCUSSION

Although the standard Onderstepoort 220-V collected significantly greater numbers than the 220-V traps baited with two, four or eight white LEDs, the present results indicated that increasing the number of individual LEDs may indeed improve the efficiency of white LED-baited traps for the collection of South African *Culicoides* livestock-associated species. Increasing the number of individual white LEDs from two to four or eight units increased the mean numbers collected by a factor of 1.9 and 2.1, respectively. There were, however, no significant difference in the mean numbers collected with four or eight white LEDs. The increase in efficiency was also reflected in the species richness and diversity. Bishop *et al*. (2004) recorded that a 42% rise in intensity of green LEDs will result in an almost three-fold increase in the number of *C. brevitarsis* collected, the results of the present study

evaluating light brightness was less profound. Increasing the brightness and/or light intensity of the light source may expand the range of attraction of the trap and as such result in a larger proportion of the field population being sampled.

In the comparison of five Onderstepoort traps fitted with either 12-V white LEDs, 12-V or 220-V UV LEDs, 12-V or 220-V fluorescent UV light tubes indicated that traps baited with UV light, in general, collected bigger numbers of *Culicoides* than white light. The lower mean numbers collected in the 12-V white LED were, however, not significantly different from that collected in white LED 12-V UV, LED 220-V UV and 12-V fluorescent UV trap. The standard Onderstepoort 220-V fluorescent UV light collected significantly higher numbers than the 12-V white and 12-V UV LED traps. These observations confirmed the well-established greater attractiveness of UV for *Culicoides* midges compared to either white fluorescent (Venter & Hermanides, 2006) or white incandescent light sources (Rowley & Jorgensen, 1967; Sloyer *et al*., 2019; Bray *et al*., 2020).

In contradiction to previous results (Venter *et al*., 2018) the lower mean numbers collected with 12-V fluorescent UV trap in the present study did not differ significantly from that collected with the 220-V fluorescent UV trap. Venter *et al*. (2018) found that the 220-V trap collected 2.4 more midges than the 12-V trap. In the present comparison, the 220-V trap collected only 1.5 more midges than the 220-V trap. Similar inconsistencies were recorded in previous trap comparisons. During a comparison done in winter in South Africa, the 220-V Onderstepoort trap collected on average 1.2 times more midges than a commercially available Triple Trap with no statistical difference in the efficiency of the two traps (Venter *et al*., 2013). The Triple Trap (The Kendal Group, Hubers cc, South Africa), marketed to reduce mosquito numbers in a particular area, is a 220-V down-draught light trap utilizing two 15 cm 4 W parallel UV light tubes to attract insects, which are then drawn into a container underneath the fan. Unique to this trap are the selected surfaces coated with TiO_2 (titanium dioxide), which with UV light acts as a photocatalyser and produces heat, CO_2 and H_2O (Fujishima *et al*., 2000). In contradiction to the comparison done winter, a comparison done in summer indicated that the mean number collected with the Onderstepoort trap was double that of the Triple Trap and of statistical significance (Venter *et al*., 2013). These apparent inconsistencies indicate that, although of high importance, light source may not be the only factor to influence trap efficiency. The strength of the fan in relation to the opening portals of the trap may also play a role. These observations accentuated the potential problems involved in the reliable comparison of light trap results.

Despite lower numbers collected, the 12-V version of the Onderstepoort as well as white / UV LED-baited traps will give a reliable representation of the abundances of *Culicoides*, species representation, and composition in an area as well as the physiological status of the population and as such the associated risk for transmission in an area. Other than for single specimens of some species with exceptionally low abundance, the findings on species composition and diversity as determined with the various 12-V traps were comparable. An increase in the number of individual collections made with a less efficient trap may overcome this problem. The use of UV LEDs in both the 12-V and 220-V combination was comparable to the Onderstepoort 220-V trap with ration to species diversity. These traps facilitate the prospect to collect in rural and wildlife areas in the absence of 220-V power supply. The Onderstepoort 220-V light trap is still recommended as the trap of choice if

large numbers of *Culicoides* need to be collected for virus detection in field collections or other biological studies.

Independent of light source or power supply all the traps, however, indicate *C. imicola* to be the dominant species and that the proportional representation did not differ significantly between treatments. The observation that the second most dominant species collected with fluorescent UV and white LEDs differ may indicate a preferential species attraction. Preferential species attraction to different light sources, especially wavelength, was shown for some Australian *Culicoides* species (Bishop *et al*., 2006). More detailed studies will be needed to confirm this observation for South African *Culicoides* species.

In the present study, age grading results for *C. imicola*, and the other species, as established by the various traps and light sources, were comparable and apparently not significantly influenced by the light source or trap type. As indicated by Braverman & Mumcuoglu (2009) pigmentation, as used in the present study, may not always reflect the true parity status of a field population. In the absence of transovarial transmission of orbiviruses in *Culicoides* (Osborne *et al*., 2015), the proportional representation of especially parous females may indicate the relative risk for potential virus transmission. In evaluating light trap parity results, it must be considered that virus infection, and especially orbivirus infections, may render infected females adverse to light (McDermott & Mullens, 2017; Mills *et al*., 2017). Light traps may as such underestimate the parous rate and infection prevalence in field populations in endemic areas. Typical to light traps operated near potential hosts all the traps in the present study collected low numbers of freshly blood engorged and gravid females as well as males. This accentuates the fact that light traps, placed near livestock, will mainly collect females actively flying around in search of a bloodmeal.

In the evaluation of light trap results, it must be considered that *Culicoides* are not homogeneously distributed in an area (González *et al*., 2017) and that light traps operated near cattle have an attraction range of less than 4 m (Venter *et al*., 2012; Elbers & Meiswinkel, 2015). In the absence of cattle or other livestock, the attraction range was reported to be between 30 and 50 m (Rigot & Gilbert, 2012; Kirkeby *et al*., 2013). It can therefore be envisaged that the random movement of animals in combination with yet unidentified factors may have a significant influence on the numbers of *Culicoides* collected on a nightly basis and trapping efficiency. This is reflected in the relative great variation found in the nightly numbers collected in the current study (Fig. 1).

The lower ratio of *Culicoides* to non-*Culicoides* collected with white light (1: 0.15–0.42), in comparison to the ratios collected with UV light (1: 0.09–0.30), may be of value considering the numbers of insects that need to be sorted after collection, as this will have an effect on the time needed to process the sample (Bishop *et al*., 2006). It may furthermore indicate that white light may attract a greater range of night flying insect species compared to UV light. The lower by-catch may indicate a reduced environmental impact of the UV light trap.

Due to their artificial stimuli, light traps unavoidably attract large numbers of non-target insects, e.g. beetles and moths and the results does not inevitably reflect the biting rate on the livestock involved (Gerry *et al*., 2009; Viennet *et al*., 2011; Scheffer *et al*., 2012). Despite the proven efficiency of the 220-V Onderstepoort trap, there is no information available on

the effectiveness of this, or any other trap, for the collection of *Culicoides* species. Although more than a million *C. imicola* can be captured in a single light trap in one night (Meiswinkel *et al*., 2004) we do not know what proportion of the midges in a given area is attracted by the light source and what proportion of the midges attracted is eventually captured by the trap. Although it is generally accepted that high proportions of nulliparous females and or males in light traps may be indicative of nearby larval habitats, no attempt has been made to estimate midge numbers from the availability of larval habitat. These factors will need to be taken into consideration in the development of more effective traps for the collection of *Culicoides* species.

CONCLUSIONS

The objectives of field collection are to identify potential vector *Culicoides* species, to study the geographic distribution of *Culicoides* vectors and to understand factors (e.g. climate, land cover, altitude, and host availability) that may influence the distribution and abundance of *Culicoides*. The field collection of potential vectors may also be used for the determination of seasonal vector free periods and routine monitoring in an area. In the absence of laboratory colonies, the live-collection field populations for biological and vector competence studies will be unavoidable.

Although less effective traps may necessitate an increase in the number of individual collections, all of the traps evaluated gave comparable results of the abundances of *Culicoides* species, as well as the physiological status of the population, and associated risk for transmission in an area. Although less efficient, these traps can be used to collect midges in rural and wildlife areas in the absence of 220-V power supply. The Onderstepoort 220-V trap is still recommended as the trap of choice if large numbers of *Culicoides* need to be collected for virus detection in field collections or other biological studies. The trap of choice will, however, depend on the aim of the study.

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Research). Acquisition of data: C.J.d.B. and S.N.B.B. (Agricultural Research Council–Onderstepoort Research). Analysis, interpretation of data and manuscript writing: C.J.d.B., G.J.V. All authors read and approved the final version of the manuscript.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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