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Research paper

Comparison of different light sources for trapping *Culicoides* biting midges, mosquitoes and other dipterans

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ABSTRACT

The response of *Culicoides* biting midges, mosquitoes and other dipterans to different wavelengths was evaluated in a farm meadow in northern Spain. A total of 9449 specimens of 23 species of *Culicoides*, 5495 other ceratopogonids (non-biting midges), 602 culicids and 12428 other mixed dipterans were captured. Centers for Disease Control and Prevention (CDC) suction light traps fitted with five light emitting diodes (LEDs) (white, green, red, blue, ultraviolet) were run for 15 consecutive nights. Significantly more *Culicoides* were collected in those traps fitted with green, blue or ultraviolet (UV) lights than in red and white-baited LED traps for the most abundant species captured: *C. punctatus* (37.5%), *C. cataneii* (26.5%) and *C. obsoletus*/*C. scoticus* (20.4%). Similar results were obtained for non-*Culicoides* ceratopogonids, mosquitoes and other mixed dipterans. Wavelengths in green (570 nm) resulted effective for targeting some *Culicoides* species, culicids and other midges. In a second trial, the effectiveness of 4-W white and UV tubes was compared to traps fitted with UV LED and a standard incandescent light bulb. More specimens of all taxa were collected with fluorescent black light (UV) traps than with the other light sources, except culicids, which were recovered in high numbers from fluorescent white light traps.

1. Introduction

Various trapping devices for insects are commercially available, which are used for nuisance reduction, monitoring or surveillance of Diptera (Lühken et al., 2014). Light traps are one of the most commonly used devices, particularly to attract insects with phototaxis. The use of light traps for sampling dipterans with relevance as disease vectors has been studied by many researchers since the mid-twenties (Odetoyinbo, 1969). Among the wide range of light traps developed, CDC-light traps (Centers for Disease Control and Prevention light traps) were introduced originally for arbovirus surveillance and other short-term mosquito investigations. They provide a reliable method for monitoring disease vectors with minimal exposure (Cohnstaedt et al., 2008), avoiding unsafe methods such as animal or human bite collection. Since their introduction, several modifications to these traps have been made to improve their effectiveness, being the modern models (CDC miniature trap models 512 and 1212), the most common tools for monitoring *Culicoides* species. These traps have routinely been used in surveillance programs in the USA (Smith and Mullens, 2003), but also in many European countries, including France (Venail et al., 2012), Spain (Pérez et al., 2012; González et al., 2013) or Portugal (Ramilo et al., 2012). Black lights (UV) are superior to white light in terms of specimens and species collected (Venter et al., 2009), depending on the type of trap (design, size and intensity of light source, etc.).

In an effort to develop a highly effective visual target for improved surveillance of different economically important vectors, Burkett et al. (1998) used for first time a new generation of lighting technology based on super-bright light-emitting diodes (LEDs). These are energy efficient, often producing a greater total photon flux (TPF) than incandescent globes in the visible spectrum (400–780 nm), making them optimal for battery operation (Bishop et al., 2004). LEDs have become widely available and popular substitutes for incandescent light over the past 18 years. Their advantages include greatly reduced power consumption, high efficiency, accuracy in specific wavelength achievement, cool

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operating temperatures, durability, less prone to shock damage, compact size, excellent colour saturation, and monochromatic light production in a wide variety of possible wavelengths (Hoel et al., 2007).

Several recent studies have tested LED colours and whether LEDs can serve as effective substitutes for incandescent lamps in standard CDC mosquito traps for mosquito surveillance as well as to determine the most appropriate colour for attracting these vectors (Tchouassi et al., 2012). Blood-feeding Diptera such as mosquitoes, sand flies and biting midges, often are attracted to specific wavelengths of light: UV, blue and green (Wilton and Fay, 1972; Mellor and Hamilton, 2003; Bishop et al., 2004; Burkett and Butler, 2005; Fernández et al., 2015). LED tests on *Culicoides* biting midges have been done in Australia (Bishop et al., 2004, 2006), Africa (Tchouassi et al., 2012), and most recently in South America (Silva et al., 2015) and Europe (Hope et al., 2015). Only a few publications describe the attractiveness of LEDs to different Diptera species.

Therefore, the objective of this study was to determine wavelength preference of adult *Culicoides*, culicids, and selected non-target dipterans, using LEDs technology and standard fluorescent and incandescent light sources.

2. Material and methods

2.1. Study area

Trapping studies were done at Neiker-Tecnalia, Basque Institute for Agricultural Research and Development, Vitoria-Gasteiz, Northern Spain, coordinates: 42° 51′ 43″ N; 2° 38′ 84″ W, elevation 517 masl. This area consists primarily of extensive sheep farming, with a flat landscape bearing a variety of trees and bushes. Sheep flocks were enclosed at night until the next morning to avoid interferences in the study. Traps were placed in the middle of a meadow (200 m × 150 m), which was occasionally irrigated, creating temporary pools of water that provided suitable conditions for the development of *Culicoides* species as well as other dipterans.

2.2. Collection methods

The first trial occurred from mid-July to early August 2013 over 15 consecutive nights. Traps were hung 15 m apart in a randomised block design at a height of 1.5 m and separated by 15 m from each other to prevent interference between traps. Five CDC-miniature portable light traps model 512 (John W. Hock Company, Florida, U.S.A.) featured five different LED platform arrays (Bioquip, Rancho Dominguez, U.S.A). Different LED bulbs emitted light which was: white between 425–750 nm, red 660 nm, green 570 nm, blue 430 nm and UV 390 nm. Adapters consisted of eight LED units oriented in all directions (360°). Each day, all traps were rotated to new positions to reduce sampling point specific differences.

A second trial was run over 12 nights in mid-August. Traps were hung, rotated and positioned same manner as the first trial. Two CDC standard miniature traps (John W. Hock Company, Florida, U.S.A., model 1212) were used, one equipped with a 4-W UV light (320–420 nm) and the other with a 4-W white light (peaks at 450 and 580 nm). The other two CDC-miniature portable traps model 512 were baited with a UV LED array light (390 nm) and with an incandescent bulb. The portable models were connected to the power supply by means of transformers (6 V to 220 V).

All traps fitted with the same model of fan and dimensions were operated overnight from dusk till dawn. Dipterans were collected into 500 ml plastic jars containing water and a drop of detergent and were emptied early in the morning. Trapping was repeated during extra nights in case of strong wind and/or trap failure. Insects collected were stored in 70% ethanol until processing. In total, four Diptera groups were studied: *Culicoides*, other ceratopogonids, culicids referred as mosquitoes and other mixed Diptera. *Culicoides* specimens were identified to species level based on the appropriate keys for northern Spain biting midges (González, 2014). Other common ceratopogonids and culicids were identified at genus level (González and Goldarazena, 2011; Schaffner et al., 2001). For the common members of the subgenus *Avaritia*, *Culicoides obsoletus* and *C. scoticus* (sibling species) were grouped, while *C. chiopterus* and *C. dewulfi* were identified by their characteristic morphological features (Nielsen and Kristensen, 2011). The number of *Culicoides* collected were counted and sexes pooled to simplify the data analysis, as for example *Culicoides* males are relatively rare representing only 0.9% of the total collections.

2.3. Statistical analysis

All data analyses were performed using the program R 3.3.0 (Fox, 2005; R Core Team, 2016) and graphs were prepared with SPSS statistics 23 (IBM corporation, Armonk, U.S.A). Data were analysed with generalized linear models (GLMz) using Poisson response as variables are discrete. Due to data overdispersion, a binomial negative response was applied to compare captures among light traps with the following criteria: if residual deviance was double the degrees of freedom, data were readjusted with negative binomial response (McCullagh and Nelder, 1989). Post-Hoc multiple comparisons of mean trap catches between the different traps were assessed using Tukey's test.

3. Results

A total of 27974 specimens of Diptera was collected over the 27 consecutive nights divided into two independent experiments (15 nights and 12 nights), giving a total of 123 collections. The majority (53.4%) were ceratopogonids. *Culicoides* midges comprised 33.7% of the total ($n = 9449$), while other ceratopogonids represented 19.6% ($n = 5495$). A total of 22 species/6485 specimens of *Culicoides* biting midges were collected in the first trial and 11 species/2964 specimens in the second trial.

The most abundant species were *Culicoides punctatus* (67.7%; $n = 6400$), *C. cataneii* (13.5%; $n = 1282$) and *C. obsoletus*/*C. scoticus* (9.6%; $n = 906$). Other species collected in declining order of abundance were: *Culicoides alazanicus* (3.1%; $n = 291$), *C. festivipennis* (1.6%; $n = 148$) and *C. kibunensis* (1.2%; $n = 117$). The remaining 16 species comprised less than 3.2% of the total collections. Non-biting midges within Ceratopogonidae were represented by *Forcipomyia* (92.6%; $n = 5090$), *Dasyhelea* (4.4%; $n = 242$), *Atrichopogon* (2.8%; $n = 154$) and *Stilobezzia* (0.2%; $n = 9$). Culicids comprised a total of 602 specimens (2.2% of the total collections) representing four genera: *Culex*, *Culiseta*, *Anopheles* and *Aedes*. Other mixed Diptera (Nematocera suborder), specially Chironomidae, Sciaridae and Cecidomiidae, were also recorded in a single group which accounted for 12428 individuals (44.4% of the total collections).

In the first trial (Table 1, Fig. 1A), there were significant differences in the total mean numbers of *Culicoides* collected between traps ($X^2 = 194.91$, d.f. = 4, $P < 0.001$). Comparison of the efficacy of different LEDs in *Culicoides* collections indicated that UV-LED traps ($X \pm SD = 159.8 \pm 121.9$) and green-baited LED traps (118.8 ± 74.3) collected significantly higher numbers of *Culicoides* than traps using white (34.7 ± 25.2) and red (21.8 ± 12.5) LEDs. Blue LED-baited traps (96.2 ± 61.3) were not significantly different from each other ($P > 0.05$). Similar patterns were observed for the most common *Culicoides* species: *C. punctatus*, *C. cataneii* and *C. obsoletus*/*C. scoticus,* but with subtle differences. Green LED light traps showed the highest numbers of captures for the 18 remaining species (10.7 ± 9.3) ahead of UV LED light (7.9 ± 9.3), and significantly