EFFECT OF CARBON DIOXIDE ON THE COLLECTION OF ADULT *CULICOIDES* SPP. (DIPTERA: CERATOPOGONIDAE) BY A NEW MODIFICATION OF BLACK LIGHT NEW JERSEY LIGHT TRAPS[1]

LACEY WIESER-SCHIMPF, LANE D. FOIL AND FREDERICK R. HOLBROOK[2]

Department of Entomology, Louisiana Agricultural Experiment Station, Louisiana State University Agricultural Center, Baton Rouge, LA 70803

ABSTRACT. Comparisons were made between black light-modified New Jersey traps with and without dry ice as a carbon dioxide bait. The black light trap with CO_2 collected significantly more nulliparous and parous empty *Culicoides variipennis* than the black light trap without CO_2. No significant difference was detected in the number of gravid, blood engorged or male *C. variipennis* collected by the 2 trap types. The baited trap collected a larger proportion of nulliparous females than the black light trap. No difference in the proportion of males or other female parity groups was detected between the 2 trap types. The total number or proportion of each parity group of *C. stellifer* was not significantly different between the 2 trap types.

INTRODUCTION

Culicoides variipennis (Coq.) is a known vector of bluetongue virus (Price and Hardy 1954, Jones et al. 1981) in the United States. Accordingly, numerous studies have been conducted to determine the most advantageous trapping technique for *C. variipennis* (Nelson 1965, Lillie et al. 1979, Holbrook 1985, Anderson and Linhares 1989). The use of a high intensity light source has been recommended by the World Health Organization/Food and Agriculture Organization (WHO/FAO) working team (Holbrook et al. 1985) to catch the largest percentage of parous flies for virus isolation studies. Wieser-Schimpf et al. (1990) reported that a black light-modified (ultraviolet) New Jersey light trap collected 10 times more parous *C. variipennis* than the standard New Jersey light trap with a 110-V, 40-W incandescent bulb.

Incandescent and black light-modified CDC miniature light traps baited with CO_2 collect more nulliparous and parous empty *C. variipennis* than the same traps without CO_2 (Holbrook 1985, Anderson and Linhares 1989). However, the CO_2 baited traps collect fewer gravid females than the unbaited traps (Anderson and Linhares 1989, Holbrook and Bobian 1989).

Our objective was to determine if a 15-W black light (ultraviolet fluorescent tube) plus CO_2 was more attractive to nulliparous and parous empty females but also less attractive to gravid *C. variipennis* than the unbaited trap. Details for trap modifications are also provided.

MATERIALS AND METHODS

Standard New Jersey light traps (Hausherr's Machine Works, Toms River, NJ) were modified to allow for switching between the incandescent and black light (U.V.) sources by connecting the 2 light sources to a 2-way toggle switch (Model 247-965 Master Electrician, Chicago, IL) in 2 parallel series (Fig. 1). The black wires from the ballast, the outlet plug and the fan were connected to one terminal of the incandescent fixture. An extension wire (12-10 gauge) connected the other terminal of the incandescent fixture to the bottom switch terminal. When the switch is turned to the up position, the incandescent light is on. The white ballast wire is connected to the top switch terminal and the black light is on when the switch is turned to the down position. An additional extension wire is connected from the middle switch terminal to the other fan wire and the white outlet plug wire. The fan runs without either light when the switch is turned to the off position. The ballast (Model 700, U.S. Fluorescent Dist. Co., N. Miami Beach, FL) is attached to the traps by securing one end to the roof support and the other end to a 5 × 1.25 cm mending plate (Model V118, National Manufacturing Co., Sterling, IL) with a 6.4 × 19 mm machine screw and nut (Fig. 2). A hole is drilled in the mending plate at the distance required to connect it to a side support. The outlet for the 15-W fluorescent bulb (Stinger B1515, deJay Corp., Greeneville, TN) was attached to the other side support. The switch was mounted on a piece of wood and attached to the roof support.

Trapping was conducted at 2 sites (ca. 3 km apart) on the Louisiana State University Agricultural Experiment Station, Baton Rouge, LA. Site 1 was at the Dairy Improvement Center, and site 2 was located at Ben Hur Research Farms.

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[2] USDA-ARS-ABADRL, P.O. Box 3965, University Station, Laramie, WY 82071.

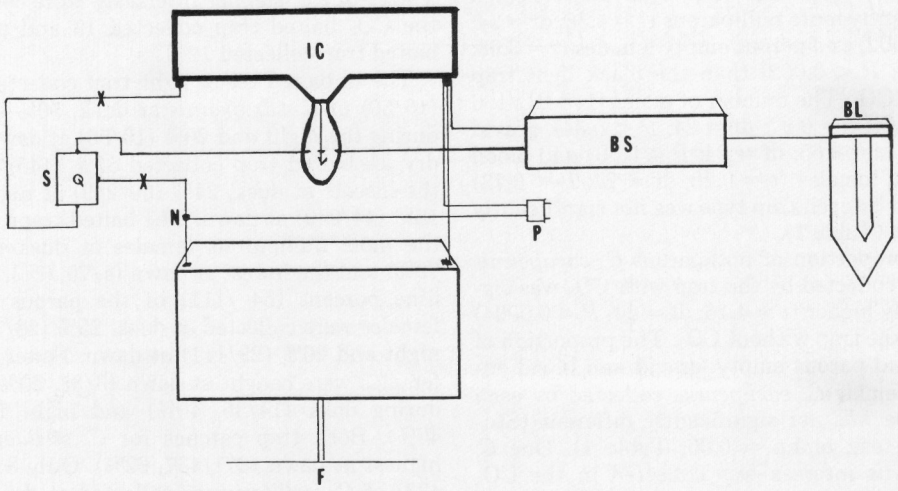

Fig. 1. Diagram of the wiring of the trap modification. IC = incandescent fixture; BS = ballast; BL = black light; P = plug; F = fan; S = switch; N = wire nut; X = extension wire.

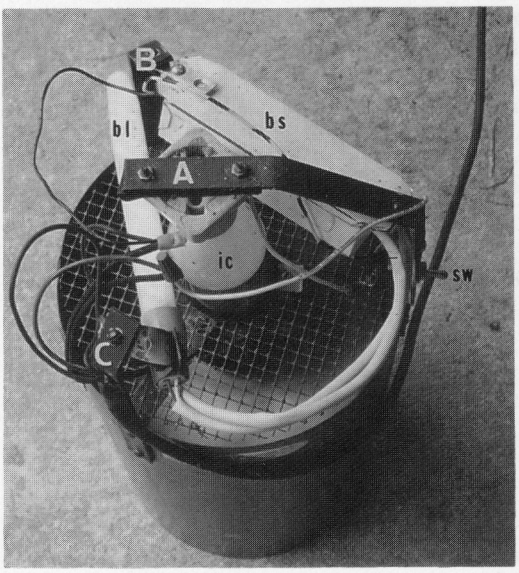

Fig. 2. New Jersey light trap with the roof removed to expose the supports (A, B, C) on which the incandescent light (ic), black light (bl), switch (sw) and ballast (bs) are attached.

Comparisons were made between the U.V. traps without dry ice and U.V. traps plus a black cloth bag containing ca. 3.5 kg of dry ice hung ca. 50 cm above the trap entrance. Following the experimental design of Holbrook (1985), one trap of each type was run at each site. The 2 traps were placed at diagonally opposite corners of a building in each site. Traps were run from 2 h prior to sunset to 2 h after sunrise on 2 consecutive nights per week unless climatic conditions were unfavorable. Trap position was alternated nightly to negate position effects. Thirteen weekly collections were made at each site from May 15 to August 15, 1990.

Three collections were made per night for 5 weeks from June 12, 1990 to July 16, 1990 at each site. During this period bags of dry ice and the collection jars were replaced 2 h after sunset and 2 h before sunrise. Traps were run for 4 h during both the dusk and dawn collection and 5.5–6 h during the night collection. Insects were collected and preserved in 70% ethanol. Adult *Culicoides* spp. were sorted by sex and females separated into 4 gonotrophic categories: nulliparous, parous empty, gravid and blood engorged. The parity status was determined on the basis of abdominal pigmentation (Dyce 1969, Potter and Akey 1978, Akey and Potter 1979).

Data were analyzed with a Student's *t*-test (SAS Institute 1985) and tested for significant differences in numbers of males and nulliparous, parous empty, gravid and blood engorged females in each trap. The dependent variable in each analysis was log (n + 1) transformed. Proportions of each sex and parity level between the 2 trap types were compared with a Student's *t*-test (alpha = 0.05) using arc sine transformations of the data (Snedecor and Cochran 1980).

RESULTS

The black light trap with CO_2 collected significantly (ca. 2 times 1,585/813) more *C. vari-*

ipennis than the trap without CO_2 ($t = 2.53$; df = 24; $P < 0.02$). The trap with CO_2 collected significantly more nulliparous ($t = 4.36$; df = 24; $P < 0.0002$) and parous empty females ($t = 3.58$; df = 24; $P < 0.002$) than the black light trap without CO_2. The number of males (148 BL/196 BL + CO_2, $t = 0.97$; df = 24; $P < 0.34$), gravid females ($t = 0.55$; df = 24; $P < 0.59$) and blood engorged females ($t = 0.28$; df = 24; $P < 0.78$) collected by each trap type was not significantly different (Table 1).

The proportion of nulliparous *C. variipennis* females collected by the trap with CO_2 was significantly higher ($t = 4.16$; df = 24; $P < 0.0004$) than in the trap without CO_2. The proportion of males and parous empty, gravid and blood engorged female *C. variipennis* collected by each trap type was not significantly different (Student's *t*-test; alpha = 0.05; Table 1). One *C. variipennis* intersex was collected in the CO_2 baited trap.

Although the baited trap collected approximately 50% more nulliparous and parous empty female *Culicoides stellifer* (Coq.) (Table 1), no significant difference was detected between the 2 trap types (nulliparous: $t = 1.66$; df = 24; $P < 0.11$; parous empty: $t = 0.80$; df = 24; $P < 0.43$). Furthermore, no significant differences were found in the number of gravid or blood engorged females or males collected by each trap type. The proportion of flies in each parity group collected by each trap was not significantly different (Student's *t*-test; alpha = 0.05; Table 1). Thirty-one *C. stellifer* intersexes were collected; the CO_2 baited trap collected 19 and the unbaited trap collected 12 flies.

The unbaited black light trap collected 30% (15/50) of *C. variipennis* at dusk, 50% (25/50) during the night and 20% (10/50) at dawn. The dry ice baited trap collected 58% (145/249) of the insects at dusk, 24% (60/249) at night and 18% (44/249) at dawn. The baited traps caught the most nulliparous females at dusk (55/70, 79%) and the fewest at dawn (4/70, 6%). Forty-nine percent (54 /111) of the parous empty females were collected at dusk, 25% (28/111) at night and 26% (29/111) at dawn. Fewer gravid females were caught at dawn (3/35, 20%) than during dusk (14/35, 40%) and night (13/35, 40%). Both trap catches for *C. stellifer* were highest at dawn (271/437, 62%). Only 3% (11/437) of *C. stellifer* were collected at dusk, and 35% (155/437) were collected during the rest of the night.

Five other *Culicoides* spp. were collected. The carbon dioxide-baited black light trap collected the following *Culicoides* species: *C. biguttatus* (Coq.) (1 ♀), *C. haematopotus* Malloch (9 ♀♀, 2 ♂♂), *C. venustus* Hoffman (20 ♀♀, 2 ♂♂), *C. lahillei* Iches (4 ♀♀) and the ornithophilic species, *C. crepuscularis* Malloch (360 ♀♀, 76 ♂♂, 72 intersexes). The unbaited black light trap collected: *C. haematopotus* (10 ♀♀, 1 ♂), *C. venustus* (12

Table 1. The number and gonotrophic status of female *Culicoides variipennis* and *Culicoides stellifer* collected by 2 New Jersey trap types: 15-W black light with CO_2 (CO) and 15-W black light (BL), 1990.

	Culicoides variipennis								*Culicoides stellifer*							
	Nulli-parous		Parous empty		Gravid		Engorged		Nulliparous		Parous empty		Gravid		Engorged	
Date	CO	BL	CO	BL	CO	BL	CO	BL	CO	BL	CO	BL	CO	BL	CO	BL
May 15–16	40	23	79	56	85	90	3	74	8	1	3	1	0	0	0	0
May 22–23	47	30	73	48	51	107	0	2	37	276	7	98	5	3	0	0
May 29–30	10	3	24	17	66	49	1	5	212	37	100	17	25	5	0	1
June 5–6	155	13	190	19	35	27	13	8	7	9	5	9	0	0	0	0
June 12–13	22	0	12	2	7	13	1	0	140	71	24	25	1	6	1	0
June 19–20	25	0	38	0	3	0	0	0	15	12	2	1	0	0	0	0
June 26–27	17	1	24	2	8	4	2	0	17	2	18	6	1	1	0	0
July 2–5	28	1	102	7	18	32	3	2	215	26	50	4	6	0	2	0
July 10–11	1	0	4	0	6	6	0	0	20	1	4	3	1	0	2	0
July 16–17	9	0	29	5	11	9	1	0	20	5	2	0	2	2	0	0
July 25–26	17	2	40	7	7	0	2	1	53	35	10	10	0	0	0	0
Aug. 1–2	25	0	9	2	2	0	0	0	2	0	2	3	0	0	0	0
Aug. 14–15	21	4	20	1	3	3	0	0	12	1	7	3	0	0	0	0
Total[1]	417[a]	77[b]	644[a]	166[b]	302[a]	330[a]	26[a]	92[a]	758[a]	476[a]	234[a]	180[a]	41[a]	17[a]	5[a]	1[a]
%[2]	26.3[a]	9.5[b]	40.6[a]	20.4[a]	19.1[a]	40.6[a]	1.6[a]	11.3[a]	71.0[a]	69.6[a]	22.2[a]	26.3[a]	3.9[a]	2.5[a]	0.5[a]	0.2[a]

[1] For each gonotrophic state, trap catches followed with the same letter are similar (Student's *t*-test alpha = 0.05).

[2] Percentage of flies in each gonotrophic state collected from each trap type. For each gonotrophic state, percentages followed with the same letter are similar (Student's *t*-test alpha = 0.05). Percentage of flies collected by each trap type equals 100% when percentage of males collected is included.

♀♀), *C. lahillei* (6 ♀♀) and *C. crepuscularis* (386 ♀♀, 32 ♂♂, 38 intersexes).

DISCUSSION

Nelson (1965) demonstrated that CO_2 was important in directing host-seeking (nulliparous and parous empty) biting midges to hosts by adding dry ice to traps. Numerous studies have supported this observation (Lillie et al. 1979, Holbrook 1985, Anderson and Linhares 1989). We collected significantly more nulliparous and parous empty flies in the traps baited with CO_2.

Anderson and Linhares (1989) and Holbrook and Bobian (1989) concluded that CO_2, to some extent, repelled gravid female *C. variipennis*. In our study, however, there was no significant difference in the number of gravid females collected between the CO_2 baited black light trap and the unbaited trap (302/330). Therefore, the black light modified New Jersey trap with strong suction can be used with CO_2 without the potential repellent effect on gravid females.

Culicoides variipennis activity occurred throughout the night; there were differences in the proportions of flies collected by the 2 trap types during the 3 collection periods. Trap catches for the unbaited black light were greatest during the night, and lowest at dawn. The dry ice baited trap collected most of the flies at dusk, and the fewest at dawn. This trend was observed for nulliparous, parous empty and gravid females and is consistent with vehicle-mounted trap collections (Akey and Barnard 1983). Therefore, addition of CO_2 to traps during the dusk peak appears to be the most expedient trapping strategy for *C. variipennis*.

Culicoides stellifer population activity was different than that of *C. variipennis*. Only 3% of *C. stellifer* were collected at dusk and activity increased throughout the night. Peak activity for both trap types was at dawn.

A large percentage (11.4%) of the *C. crepuscularis* intersexes were collected. These appeared to be genetically intended males which became altered morphologically to resemble anterior-posterior type gynandromorphs. The head, mouthparts and wings were modified to resemble females, while the abdomen and terminalia remained typically male. Beck (1958) and Smith and Perry (1967) collected intersexes of *C. crepuscularis* and reported mermithid parasitism. We did not examine specimens for mermithid parasitism.

Wieser-Schimpf et al. (1990) reported that the black light trap collected considerably more undesirable insect species, increasing sorting time 2.5-fold, than the incandescent trap. The addition of CO_2 did not attract significantly more undesirable insect species than the black light alone. Only the increased number of nulliparous *C. variipennis* had an effect on the collection sorting time. Addition of CO_2 resulted in a 2-fold increase (946/496) in specimens appropriate for virus isolation studies (parous empty and gravid). Therefore, the black light modified New Jersey light trap baited with CO_2 can be recommended for collecting *C. variipennis* for virus isolation studies from sites with available alternating current sources.

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