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ADULT BITING MIDGE RESPONSE TO TRAP TYPE, CARBON DIOXIDE, AND AN OCTENOL–PHENOL MIXTURE IN NORTHWESTERN FLORIDA

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ABSTRACT. The efficacy of Centers for Disease Control, ABC PRO, and counterflow geometry (CFG) mosquito suction traps to sample populations of adult *Culicoides* was investigated in northwestern Florida. These traps were baited either with a 4:1:8 mixture of octenol, 3-*n*-propylphenol, and 4-methylphenol alone or in combination with carbon dioxide (CO_2). Control traps were operated without the octenol–phenol mixture or CO_2. Four species, in order of descending abundance, were collected in all traps regardless of treatment: *Culicoides mississippiensis, C. barbosai, C. melleus,* and *C. furens.* Midge abundance from traps baited with octenol–phenol alone was not significantly different, regardless of species, when compared with traps without the mixture. However, when CO_2 or CO_2 plus the mixture was used, trap collections of *C. mississippiensis* and *C. barbosai* significantly increased, with the latter mixture exhibiting a synergistic effect on trap catch for both species. When this combination was used, the ABC PRO trap collected significantly more *C. mississippiensis,* whereas the CFG trap caught significantly more *C. barbosai* compared with all traps powered with 6-V batteries ($P <$ 0.05). The effects of CO_2 plus the octenol–phenol mixture on *C. melleus* collections appeared to be additive only for ABC PRO and CFG traps. Populations of *C. furens* were sporadic and too low (<0.5% of total collection) to determine any statistically meaningful differences. On the average, CFG traps powered with 12-V batteries only increased midge collection 1.2 times compared with similar traps powered by 6-V batteries. This increase was not significantly different ($P > 0.05$).

KEY WORDS *Culicoides mississippiensis, Culicoides barbosai, Culicoides melleus, Culicoides furens,* attractants, traps

INTRODUCTION

Adult biting midges (Ceratopogonidae), as a group, have been shown to impact human health and comfort (Kettle 1965, Linley et al. 1983). Annoyance from these biting pests can often become serious enough to curtail outdoor recreational and work-related activities. Moreover, pestiferous bloodfeeding midge populations can negatively impact economic growth (primarily tourism) along coastal environments (Linley and Davies 1971). The biting midge problem along Florida's coast often can be quite severe during certain times of the year. Linley (1990) stated that 4 species of *Culicoides* (*C. barbosai* Wirth and Blanton, *C. furens* (Poey), *C. melleus* (Coquillett), and *C. mississippiensis* Hoffman) could be considered serious pests in the state depending on time of year.

Control of biting midges has met with limited success. Because most larval developmental sites are in coastal wetlands (Blanton and Wirth 1979, Linley 1990), physical alteration or insecticide application to these habitats is not an option because of federal or state regulatory issues. Area-wide reduction of host-seeking adults via adulticides also seems to provide little long-term relief (Linley 1976). Therefore, novel control methods must be developed.

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An alternative method to reduce pestiferous populations of biting flies is by removal trapping where the target species is lured into traps away from potential animal or human hosts. Day and Sjogren (1994) reviewed 4 successful removal trapping programs for *Hippelates* eye gnats, tsetse flies (*Glossina pallidipes* Austen), stable flies (*Stomoxys calcitrans* (L.)), and tabanids that employed visual traps with and without olfactory attractants. These projects demonstrated that pestiferous populations could be reduced by attracting and killing the target species in relatively confined areas without the need for area-wide application of insecticides. Kline and Lemire (1998) demonstrated that a single line barrier of either attractant-baited traps or cloth targets, impregnated with the pyrethroid insecticide lambda-cyhalothrin, could reduce mosquito populations on a barrier island resort in southwestern Florida. Recently, Day et al. (2001) reported reduction of populations of *C. furens* by removal trapping by using similarly treated targets baited with attractants in mangrove (e.g., *Lagunularia* and *Rhizophora* spp.) swamps.

Another removal trap method against biting midges might employ suction traps, such as those used to monitor field populations of mosquitoes and *Culicoides.* These traps, in combination with semiochemicals, could be used to physically remove host-seeking midges from a targeted area, such as a backyard. Indeed, Kline et al. (1994) reported that Centers for Disease Control (CDC) suction trap collections of *C. furens* in Georgia salt marshes were increased when supplemented with carbon dioxide (CO_2) either alone or in combination with 1-octen-

3-ol. Moreover, field studies on other biting flies, such as tsetse flies (Brady and Griffiths 1993) and stable flies (Cilek 1999), showed that the attractiveness of octenol was further enhanced when mixed with methylphenol and propylphenols. The purpose of this study was to determine the attractancy of adult *Culicoides* to various types of suction traps augmented with and without CO_2 gas in combination with an octenol–phenol mixture. This was a prerequisite study to evaluate trap and semiochemical combinations for future use in removal trapping studies against these biting pests.

MATERIALS AND METHODS

Studies were conducted in a needle grass (*Juncus roemerianus* Scheele.) marsh in Bay County, Florida, from April 19 through June 4, 1999. Suction traps used to collect adult biting midges were model 512 CDC (John W. Hock Co., Gainesville, FL) as described by Sudia and Chamberlain (1965), and ABC PRO (American Biophysics Corp., East Greenwich, RI) and counterflow geometry (CFG; American Biophysics) as described by Kline (1999). The CDC and PRO traps were both run without light sources; the CFG trap does not contain a lighting system. All traps were powered by 6-V, 10-A-h rechargeable gel-cell batteries.

Treatments were CO_2 delivered from 9-kg (20-lb) compressed gas cylinders (release rate was 500 ml/min), a 4:1:8 mixture of octenol: 3-*n*-propylphenol: 4-methylphenol, and combination of CO_2 plus the octenol–phenol mixture. The CO_2 flow rate was controlled with a FLOWSET1 pressure regulator (American Biophysics) with output fixed at 15 psi, fitted with a 10-μm line filter, a 500-ml/min flow control orifice, and quick-connect luer fitting. The octenol–phenol mixture was released from 16-ml screw-capped glass vials via a single wick (Dills® pipe cleaner, United States Tobacco Sales and Marketing, Greenwich, CT) that protruded approximately 1 cm from a 0.3-cm-diameter hole in the cap (referred to as wick out by Kline et al. [1991]). Vials were located on traps adjacent to the CO_2 release point. Release rates (mg/h) were determined by weighing individual vials just before and after deployment in the field divided by the amount of time in the field (24 h). Controls consisted of each trap type without CO_2 or the octenol–phenol mixture. All test treatments were replicated 3 times.

All traps were suspended from metal poles about 1.8 m above ground level. Midges were collected in 30 × 30-mesh screened dark olive green heat-resistant polyester fabric bags (John W. Hock Co.), with the exception of the CFG trap where collections were removed from the outer clear plastic collection reservoir. A trap line was established along the margin of the marsh–upland interface. Traps were located 33 m apart. A 12 × 12 Latin square experimental design was followed that consecutively rotated all treatments between 12 location sites resulting in 12 consecutive 24-h collection periods (Cockran and Cox 1957). Each 12-day test was repeated 3 times. Number and species of *Culicoides* were recorded from each collection. Collections with counts less than 1,000 individuals per trap were completely counted and identified. When trap collections exceeded this number, each collection was placed, as evenly as possible, on a white 7 × 5-cm grid ruled in increments of 2.5 cm². At least 2 aliquots were then taken and all midges within each aliquot were counted and identified to species. The average number of each species from those aliquots was then multiplied by the number of squares covered. The taxonomic guide of Blanton and Wirth (1979) was used for identification.

In a recent publication, Kline (1999) stated that CFG traps should be operated at 12 V. Therefore, a separate test was conducted to compare biting midge collection abundance from 6-V– and 12-V–operated CFG traps. The study utilized a daily switchback design to minimize location bias between treatments (Kline 1999). Treatments consisting of one 6-V CFG trap and one 12-V CFG trap were alternated each night between locations. Traps were baited with CO_2 and evaluated in the same habitat, at the same time of year (March through May 2001, $n = 22$ days) with the same equipment and flow rate described above. Traps were located 33 m apart. All treatments were replicated 3 times each day. Collections were processed as above with the exception that all midges in these collections were counted. Each trap's suction capacity with 6-V and 12-V batteries was also indirectly recorded by measuring output wind velocity in meters per sec (m/sec) with a handheld digital anemometer (Fisher Scientific, Pittsburgh, PA) placed horizontally and directly beneath the fan exhaust.

Statistical analyses: The mean number of biting midges per trap, and within species treatment, were subjected to analysis of variance (PROC GLM of SAS Institute [1990]) after $\sqrt{x+1}$ transformation. A Student–Neuman–Keuls test was performed to determine significant ($P < 0.05$) differences among trap types and treatments within species (Sokal and Rohlf 1981). The 6-V vs. 12-V mean CFG trap comparison data were similarly analyzed.

RESULTS

Four species of ceratopogonids were captured in all traps and treatments; these were in descending order of abundance: *C. mississippiensis, C. barbosai, C. melleus,* and *C. furens.* Regardless of trap type, significantly more *C. mississippiensis, C. barbosai,* and *C. melleus,* were collected when traps were supplemented with either CO_2 or CO_2 plus the octenol–phenol mixture compared with the mixture alone or no treatment (Table 1). Octenol–phenol release rates averaged 8.40 ± 0.40 mg/h and did not differ significantly among traps or treatments ($F = 1.13$; df 7,208; $P = 0.34$).

Table 1. Mean (±SE) abundance of 3 Florida coastal *Culicoides* species attracted to 3 types of insect suction traps with and without carbon dioxide (CO_2) and with or without a 4:1:8 mixture of 1-octen-3-ol:3-*n*-propylphenol:4-methylphenol.[1]

Treatment	*n*	*C. mississippiensis* CDC	*C. mississippiensis* ABC PRO	*C. mississippiensis* Counterflow
None	36	4.9 ± 2.5Aa	2.3 ± 0.7Aa	3.4 ± 0.6Aa
Octenol–phenol mix	36	6.2 ± 1.7Aa	6.2 ± 2.6Aa	3.3 ± 0.8Aa
CO_2	36	89.1 ± 20.3Bb	83.9 ± 16.7Bb	78.3 ± 16.9Bb
CO_2 + octenol–phenol mix	36	110.2 ± 23.5Bb	192.0 ± 32.2Bc	95.4 ± 22.6Bb

[1] Means with the same capital letter in the same column (for trap comparison), or with a lowercase letter in the same row (for treatment comparison), within each species, are not significantly different ($P < 0.05$; Student–Neuman–Keuls mean separation test).

The combination of CO_2 plus the octenol–phenol mixture increased some *C. mississippiensis* and *C. barbosai* collections by more than 100 times compared with the octenol–phenol mixture alone. This combination also appeared to be synergistic for both species and increased collections as much as 3-fold compared with CO_2 alone. *Culicoides mississippiensis* were collected in significantly ($P <$ 0.05) greater numbers in ABC PRO traps baited with CO_2 plus the octenol–phenol mixture compared with similarly baited CDC and CFG traps, all operated with a 6-V battery (Table 1). Also, significantly more *C. barbosai* were collected from the CFG trap when this same semiochemical mixture was used and collections compared with similarly baited CDC and ABC PRO traps. Depending upon trap type, collections of *C. melleus* could be increased nearly 25- to 46-fold with the addition of CO_2 (compared with the octenol–phenol mixture alone or no odor, respectively). However, compared with CO_2 alone, collections increased only approximately 1.1 times with CO_2 plus the octenol–phenol mixture, while collections in the CDC trap decreased. Populations of *C. furens* were sporadic and too low (<0.5% of total collection) to determine any statistically meaningful differences. Therefore, this species was omitted from the data set.

A 7-fold difference was found in output wind velocity of CFG traps powered with 12-V batteries (average 0.7 ± 0.1 m/sec) compared with traps run with 6-V batteries (average < 0.1 m/sec). However, midge collections from traps powered with 12-V batteries were not significantly greater than those from traps powered with 6-V batteries ($F = 1.38$; df 43,131; $P = 0.24$; 12-V mean = 517.7 ± 59.1; 6-V mean = 447.9 ± 48.4). No clearly marked differences in species composition or order of abundance was observed between 12-V and 6-V collections.

DISCUSSION

Traps powered by 6-V batteries and baited with CO_2, or a combination of CO_2 plus octenol–phenol, collected significantly more biting midges compared with octenol-phenol alone or no attractant. ABC PRO traps collected significantly more *C. mississippiensis,* whereas CFG traps collected more *C. barbosai* when baited with a combination of CO_2 plus octenol-phenol compared with other similarly baited 6-V–powered traps. The reason for trap preference by either species is, at present, unknown. However, CO_2 has long been recognized to increase collections of bloodfeeding Diptera. Nelson (1965) reported the effect of CO_2 as a potent attractant for ceratopogonids and our study corroborated his evidence. Moreover, Kline et al. (1994) and Ritchie et al. (1994) reported that the combination of CO_2 and octenol often resulted in a synergistic effect on trap collections for some species of *Culicoides.* We found this to be true for *C. mississippiensis* and *C. barbosai* even though our semiochemicals combined octenol with methyl- and propylphenols. But ABC PRO and CFG traps baited with this same mixture only increased *C. melleus* collections additively.

Interestingly, a significant increase in midge collection did not occur when CFG traps were powered with 12-V compared with 6-V batteries. Collection abundance between traps run with 12-V batteries did not always increase over those in traps run with 6-V batteries. In fact, 50% of the traps powered by 12-V batteries collected contained fewer midges than corresponding collections from traps powered by 6-V batteries. The reason for this is unknown. Increased fan output velocity, spatial abundance (i.e., evenness) of midge populations, habitat, species composition, trap location, or a combination of these may have separately or collectively influenced the number of midges taken in various collections.

In conclusion, suction trap collections of *C. mississippiensis, C. barbosai,* and *C. melleus* can be increased (in some cases synergistically) by the addition of a mixture of octenol:3-*n*-propylphenol:4-methylphenol to CO_2. Additional investigations are warranted to determine the field effectiveness of this combination as a possible tool for monitoring biting midge populations or its deployment in a removal trapping program.

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Table 1. Extended.

C. barbosai			C. melleus		
CDC	ABC PRO	Counterflow	CDC	ABC PRO	Counterflow
2.5 ± 1.4Aa	0.6 ± 0.2Aa	0.7 ± 0.3Aa	0.3 ± 0.2Aa	0.2 ± 0.1Aa	0.4 ± 0.2Aa
2.4 ± 1.3Aa	1.1 ± 0.4Aa	3.0 ± 1.1Aa	0.3 ± 0.1Aa	0.4 ± 0.2Aa	0.4 ± 0.2Aa
20.5 ± 8.3Bb	23.0 ± 5.7Bb	25.4 ± 9.4Bb	8.5 ± 4.2Bb	9.2 ± 2.8Bb	7.5 ± 2.8Bb
42.9 ± 12.5Bb	79.0 ± 38.5Bb	81.0 ± 33.1Bc	7.0 ± 2.5Bb	11.6 ± 3.0Bb	8.1 ± 3.6Bb

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