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EVALUATION OF ATTRACTANTS IN TRAPS FOR GREENHEAD FLY (DIPTERA: TABANIDAE) COLLECTIONS ON A CAPE COD, MASSACHUSETTS, SALT MARSH

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ABSTRACT. Field studies evaluated 1-octen-3-ol (octenol), carbon dioxide (dry ice), and a combination of both attractants in comparison with unbaited traps for collecting greenhead flies on a Massachusetts salt marsh. The carbon dioxide (CO_2)-baited trap collections, and the CO_2 with octenol-baited trap collections were more than 300% greater than the unbaited collections. The octenol-baited traps collected 83% more greenhead flies than unbaited traps. Afternoon trap collections were 2.5 times greater than morning collections. Dissections and measurements of a sample of the flies collected indicated that the "cryptic species" composition of greenhead flies was 77% *Tabanus nigrovittatus* and 23% *Tabanus conterminus*. Ovarian tracheole examinations of a sample of the flies showed that 99% of the greenhead flies had laid eggs before being trapped. The use of CO_2 as an attractant in the traps could be cost effective in small problem areas, and the use of octenol in the box traps should be evaluated in large areas.

INTRODUCTION

Greenhead flies (*Tabanus nigrovittatus* Marquart and *Tabanus conterminus* Walker) and other species of Tabanidae are important pests in many marsh and beach areas on Cape Cod in Massachusetts. The Cape Cod Greenhead Fly Control District (the District) has evaluated and used box traps since the 1960s to control greenhead flies in the salt marsh breeding areas (Wall and Doane 1980). Because the control program is based upon the use of box traps to reduce the number of flies, this study was undertaken to evaluate attractants that may increase the number of flies collected in the traps.

Previous studies of CO_2 and 1-octen-3-ol (octenol) as attractants for tsetse flies and mosquitoes have shown promising results for increasing trap collections (Hall et al. 1984; Vale and Hall 1985a, 1985b; Takken and Kline 1989; Kline et al. 1991). Octenol in combination with dry ice, and without dry ice, increased the effectiveness of canopy traps for collecting *Tabanus molestus* Say and other species in a Georgia study (French and Kline 1989). In Florida, it was shown that octenol combined with CO_2 increased collections of the tabanid *Diachlorus ferrugatus* (Fabricius) (Kline et al. 1990).

MATERIALS AND METHODS

The study was conducted on a salt marsh south of Gray's Beach near Alm's House Road, Yarmouthport, MA. The marsh vegetation included marsh grasses (*Spartina alterniflora, Spartina patens,* and *Distichlis spicata*), woody glasswort (*Salicornia virginica*), and sea lavender (*Limonium carolinianum*).

The box traps used for collecting the flies were the type used by the District, but their tops were modified to facilitate removal of trapped flies (Fig. 1). The plywood traps were rectangular (82 cm long × 80 cm wide × 62 cm high), and each trap had corner legs to anchor the trap into the marsh at a height of about 70 cm. The bottom of the trap was of anodized screen (18 × 16 mesh) attached to a wood frame (45 × 60 cm) that extended upward 34 cm on a 45° angle to form an inverted "V". At the top of the "V" there was an opening (1 cm wide × 25 cm long) through which the flies entered the trap.

The removable trap top was a pyramid-shaped wood frame that was covered with anodized screen. The top height was 45 cm, and it was constructed on a base with 4 sloping triangle-shaped sides. The base of the top was made so that it fit securely on the rectangular box trap. The piece of wood forming the apex of the removable top was 10 cm wide × 15 cm long × 3 cm thick, with a 6-cm hole in its center. The metal lid of a 1-gallon (3.78 liter) glass jar was attached to the trap top. The center of the lid was cut out to permit flies in the pyramid top of the trap to move into the glass jar through a cone-shaped screen. It was open 10 cm at the base and 2 cm at the top. It prevented trapped flies from leaving the jar and reentering the box trap.

To remove flies from a trap, a flannel-lined plastic table cloth was placed over the pyramid top. However, the glass jar was not covered. The flies were attracted by the light and entered the glass jar. After several minutes, the jar was carefully removed, and a lid was placed on the jar. The new lid had cotton taped in its center, to which chloroform was added to anesthetize the captured flies. They were then transferred to a labeled container, and were taken to the laboratory for identification and processing.

Twelve traps were used and they were posi-

Fig. 1. The pyramid-shaped top attached to a box trap on the salt marsh was useful for collecting, concentrating, and removing trapped tabanid flies.

tioned in a row about 31 m from the upland edge of the marsh. The distance between traps was about 19 m.

Twelve morning or afternoon collection periods of 3–4 h duration were used. The morning collections began by 0900 h and were completed by 1320 h; the afternoon collections occurred between 1100 h and 1520 h. Only one collection was 4 h long in both the morning and afternoon collections. At the beginning of each collection period, the collectors' names, time, temperature, percent cloud cover, wind speed and direction, and 24-h rainfall information were recorded.

The attractants (baits) were placed inside the traps according to a randomized schedule. Three unbaited traps, 3 octenol-baited traps, 3 CO_2-baited traps, and 3 traps baited with both octenol and CO_2 were used during each collection period. A modified Latin Square design was used to avoid bias due to trap location. Thus, during each of 3 replicates of 4 collection periods, each trap had been used unbaited and with each of the various types of attractants.

The octenol (Aldrich Chemical Company, Milwaukee, WI) bait was a clear liquid that was placed inside the trap, at the middle of the inverted "V" opening. Four milliliters of octenol was added to a 5-ml microreaction vial (Supelco Inc., Bellefonte, PA) with a screw-top lid. The octenol odor was released by capillary attraction and evaporation from a pipe cleaner wick. The pipe cleaner was bent to pass through the vial lid as an inverted "U", similar to the method described as "wickout" by Kline et al. (1991). The CO_2 bait was released from dry ice as it sublimated from the solid to the vapor state. Approximately 1–1.5 kg of dry ice was placed in an insulated plastic container (BioQuip Products, Inc., Gardena, CA) and suspended near the top of the trap. Some dry ice remained in each container at the end of every trial with the CO_2 attractants.

Two types of wicks also were evaluated in 3 trapping periods. The standard pipe cleaner wick was compared to a 0.5 cm diam lamp wick. The lamp wick released the octenol vapor by extending from octenol in a microreaction vial, through its neoprene septum in the screw-top lid, and out about 4 cm. Trap numbers 1, 5, and 9 were left open. The fly collections were obtained in a series of 3 consecutive traps (numbers 2–4, 6–8, and 10–12) with octenol from pipe cleaner wicks, from lamp wicks, or without attractant. The 3 trapping periods were conducted to obtain comparison samples of flies collected in each of the 3 series of consecutive traps.

One trial compared the number of days octenol remained in a microreaction vial with either a pipe cleaner wick or a lamp wick. Each type of wick releasing octenol from a vial was placed in an open wood box (32 × 32 × 32 cm) and observed daily until the octenol had evaporated.

Finally, to evaluate the effect of trap location upon the collection data, 2 morning and 2 afternoon collections were conducted. All 12 of the traps were used simultaneously (unbaited) for the location comparisons.

Processing of the tabanid collections included counting, identification, dissection for cryptic species determinations, and ovary preparation and examination for parity determinations. The collection from each trap was numbered, and a collection record sheet was used to document attractant, trap number, and total flies collected, and for measurement data on 3 specimens of the *Tabanus nigrovittatus–T. conterminus* complex. Records of ovarian examinations for tracheation in these 3 specimens also were documented.

Flies were identified by using the morphological keys of Stone (1938). To separate the 2 "cryptic species," the body length was measured with calipers, and the head of the fly was removed and pasted onto a slide with a mixture of flour and water. One antenna was removed and also placed on the slide under a cover slip. A digital Microcode II micrometer and a 10× digital Filar ocular (Boeckeler Instruments, Tucson, AZ) were used to measure the width of the head, frons, and scape. Those measurements were used in the formula developed by Sofield et al. (1984) for a computer program to differentiate *T. nigrovittatus* from *T. conterminus.*

At the time the aforementioned sample of flies was dissected for cryptic species determinations, the ovaries also were removed and placed on the slide (under a cover slip) near the antennal scape. The dried ovaries were later examined at 100× and 400× magnification to determine the condition of the ovarian tracheoles. The methods described by Detinova (1962) and by Kardos and Bellamy (1961) were used to distinguish females that had laid eggs from those that had not oviposited.

The collection data were evaluated using analyses of variance on the log-transformed numbers of flies. The least significant difference procedure was used for multiple comparisons of the overall means of attractants.

RESULTS

The female greenhead fly collection data from baited and unbaited traps are listed in Table 1. Seven species of Tabanidae were trapped. There were 38,834 (99%) *T. nigrovittatus–T. conterminus* complex, 37 *Tabanus lineola* Fabricius, one *Tabanus calens* Linnaeus, 10 *Chrysops sackeni* Hine, 3 *Chrysops atlanticus* Pechuman, and one *Chrysops frigidus* Osten Sacken. Based upon the dissections, measurements, and the identification formula used with a sample of 445 flies (up to 3 per collection), 77% of the cryptic species were *T. nigrovittatus* and 23% were *T. conterminus.*

Table 1. Collections in greenhead fly traps during 3–4-h periods at Gray's Beach Marsh, Yarmouthport, MA, July 16–August 21, 1992.[1]

Attractant	Maximum	Mean no. ± SE	Group[2]
CO_2 and octenol	1,757	400.6 ± 71.7	A
CO_2	2,475	415.1 ± 82.0	A
Octenol	776	171.0 ± 29.7	B
None	416	93.4 ± 16.8	C

[1] Twelve trials, 3 replicates per trial (n = 36).

[2] Attractant groups with same letter are not statistically different ($P > 0.05$)

Four trials in which each of the 12 traps were unbaited collected 240, 78, 280, and 259 tabanids. The first and the last of these trials were morning collections, the other 2 were afternoon collections. The average sample of flies collected in the 4 trials at the 12 locations ranged from 10 to 36. The 3 largest 4-trial average collections were 36 in trap 7, 26 in trap 1, and 22 in trap 11. The 3 smallest 4-trial average collections were 9 in trap 2, and 10 in traps 5 and 10.

The number of greenhead flies collected per trial ranged from 548 on August 4 to 13,162 on July 28, 1992. Both of those collections were made in the afternoon on warm days. However, the cloud cover during the small collection was widespread (beginning at 80% sky coverage and ending at 100%). Cloud cover was sparse during the large collection (beginning at 5% and ending at 15%).

Analyses of the trapping data showed that each of the attractants significantly increased the number of greenhead flies collected, in comparison to the number collected in unbaited traps ($P < 0.05$). The times of day during which the collections were obtained affected the number of flies collected, and the afternoon collections were significantly larger than the morning collections ($P < 0.05$). The average number of flies trapped during the afternoon collections was 2.5 times greater than during the morning. In the afternoon, the average number of greenheads trapped was 2,331; in the morning it was 918.

The total number of greenhead flies trapped in the CO_2-baited traps was 14,944 (range, 221–5,547); in CO_2 and octenol-baited traps it was 14,422 (range, 237–4,673); in the octenol-baited traps it was 6,157 (range, 59–1,889), and in the unbaited traps it was 3,363 (range, 31–1,053). Thus, 38% of the collected greenheads were from traps using CO_2 alone as the attractant, 37% were from traps using CO_2 with octenol as the attractant, 16% were from traps using octenol as the attractant, and 9% were from traps without out an attractant. In these collections, the CO_2-baited traps collected 344% more greenhead flies than the unbaited traps, the CO_2 with octenol 329% more, and the octenol-only-baited traps 83% more than the unbaited traps.

The sample of 445 flies dissected to determine cryptic species comprised 111 from octenol-baited traps, 114 from CO_2-baited traps, 110 from traps with octenol and CO_2 bait, and 110 from unbaited traps. The *T. nigrovittatus* : *T. conterminus* ratio using octenol-baited traps was 84: 27, using CO_2 it was 80:34, using octenol with CO_2 it was 89:21, and in unbaited traps it was 89:21. Thus, the percentage of *T. nigrovittatus* in the samples from the baited and unbaited traps ranged from 70 to 81%; 77% of the sample dissected were *T. nigrovittatus*.

The ovarian tracheole examinations showed that 99% of the 426 flies examined had laid eggs. Both the CO_2-trapped and the octenol-trapped samples of dissected flies contained 2 nulliparous (flies that had not oviposited) and 106 parous (flies that had laid eggs) females. The unbaited trap sample contained one nulliparous and 101 parous female flies. The CO_2 with octenol-baited trap sample contained no nulliparous and 108 parous females.

The trials to compare the 2 types of wicks in octenol-baited traps produced 739 greenhead flies in lamp wick vials and 737 in pipe cleaner wick vials. In the simultaneous unbaited trap comparisons, 265 greenhead flies were trapped. In the single evaluation of the duration of release, the octenol was released over a 16-day period from a vial with a pipe cleaner dispenser and over a 12-day period with a lamp wick dispenser.

DISCUSSION

The data obtained from this project provide good insight into the effectiveness of CO_2 and octenol as attractants in traps for greenhead fly collections. The results with CO_2 (with and without octenol) are similar to those obtained in studies by others for trapping mosquitoes and different tabanid species. The CO_2 and the CO_2 with octenol-baited traps collected 344 and 329% more greenhead flies than unbaited traps, respectively. The traps baited with octenol alone collected 83% more flies than unbaited traps. The variation in collection numbers obtained at the different trap locations was not surprising. The random assignment of bait and trap and the trial replications minimized the effects of location and time on the attractant results evaluations.

The dry ice alone, and in combination with octenol, was much more effective than octenol alone as a greenhead fly attractant. Unfortunately, its high cost and the short time that it lasts precludes the use of dry ice as a CO_2 source in large-scale control operations. The use of CO_2 from gas cylinders also is cost prohibitive and impractical. However, the 83% greater greenhead fly collections in traps baited with octenol alone than in unbaited traps merits follow-up for improving greenhead fly control on Cape Cod. Increasing the size of the vial containing the octenol attractant would extend the length of the period between replenishments. If traps were baited only twice per season with octenol (e.g., early June and late August), the increased trap efficiency and control results may be cost effective.

The use of dry ice or gas cylinders as sources of CO_2 could be cost effective in some instances. For example, resort hotels (or other property owners) that have relatively small beaches may find that the material and labor expenses for routine day-long baiting of traps with either of these CO_2 attractants provides a worthwhile level of greenhead fly control.

There was no evidence that any of the attractants, or that the unbaited traps, selectively collected either of the cryptic species. Thus, an octenol-baited trap program or the others could be expected to be equally effective for collecting both *T. nigrovittatus* and *T. conterminus*.

Ninety-nine percent of the flies dissected for ovarian tracheation had laid eggs before being trapped. These findings are similar to those of Wall and Doane (1980), and other investigators in the eastern United States. None of the baits tested increased collections of nulliparous female greenhead flies. Obviously, a method for collecting female greenhead flies before they oviposit would be highly desirable for improving control.

We believe that this study should be followed by a large-area project using octenol alone as an attractant in box traps on Cape Cod. If that reduced the greenhead fly problem in the study area, the use of octenol bait in all of the Cape Cod Greenhead Fly Control District traps could result in a further reduction of this biting fly problem.

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