SHORT COMMUNICATION

Effect of Octenol on Engorgement by Tabanus nigrovittatus (Diptera: Tabanidae)

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ABSTRACT

Adult female Tabanus nigrovittatus Macquart (Diptera: Tabanidae) were field collected from a salt marsh in Essex County, Massachusetts. The horse flies were transported back to and tested in the laboratory to determine the effects of octenol (1-octen-3-ol) on engorgement. Flies exposed to octenol strips had a significantly higher engorgement response compared with control flies. To our knowledge, this is the first study to demonstrate an important link between an odor stimulus and the feeding response in Tabanidae. Research examining the link between odor attractants and repellents on the engorgement response is lacking or limited in most hematophagous Diptera. Understanding the role odors have on ingestion is essential to knowing how to interrupt feeding behavior of blood-feeding arthropods, especially for important vectors.

KEY WORDS blood feeding, odor attractants, octenol

Tabanus nigrovittatus Macquart (Diptera: Tabanidae) is found in the salt marshes of the Atlantic coast. This fly is a notorious nuisance to tourists, locals, and livestock alike, especially because its 3- to 4-wk presence on the marsh coincides with the peak tourist season of the summer. T. nigrovittatus is an excellent blood-feeding insect to study because the flies can be collected in extremely high numbers on the marsh during the season, they have been shown to readily feed through a parafilm membrane (Stoffolano 1979), and considerable information already exists on phagostimulants (Friend and Stoffolano 1983; Friend 1984, 1991), food diversion (Stoffolano 1983), and oogenesis and oviposition (Magnarelli and Stoffolano 1980, Graham and Stoffolano 1983).

To our knowledge, research examining the effects of odor on probing and ingestion is lacking for most hematophagous Diptera. Most, if not all, research involving host-seeking stimuli and odor attractants is concerned with trap effectiveness and control measures (Takken and Kline 1989, Jaenson et al. 1991). Those studies that do examine the role of odors only investigate probing and not ingestion (Hopkins 1964, Gatehouse 1970). There is a general absence in the literature of studies that look at the function of odors in the next behavioral step of obtaining a bloodmeal (i.e., engorgement).

Our laboratory used octenol to study how it affects engorgement. Octenol (1-octen-3-ol), identified from ox breath and originally used in tsetse fly research (Vale and Hall 1985), is an odorous compound that has been identified as an olfactory stimulant for several hematophagous insects, including tabanids (Hayes et al. 1993, Foil and Hribar 1995). Octenol is used in box traps and has proven to be an effective attractant for increasing trap collection of T. nigrovittatus (Hayes et al. 1993, Foil and Hribar 1995).

Materials and Methods

Collecting and Maintaining Flies. Female host-seeking T. nigrovittatus were collected from box traps on the salt marsh in Essex County, Massachusetts, during July 2005. Flies were moved from black box traps on the marsh to metal screened cages (24 by 24 by 45 cm) and given access to granulated sugar and water during transportation back to and while housed in the laboratory. Females were maintained at 25–27°C and 50–60% RH. Before experimentation, all flies were deprived of granulated sugar for 16 h and were tested 1 d after being collected in the field. The exact chronological ages of all flies used are unknown. However, the first collection date for the 2005 experiments occurred on 4 July 2005, and field collections were made every other day consecutively throughout the season. Therefore, all flies used in experimentation were assumed to have only been in the field traps for at most 2 d.

Feeding Assay. After the starvation period, flies were cold immobilized in the freezer. Each experimental group (control and octenol) consisted of 20 flies, and eight replicates in total were performed. Citrated beef blood was warmed on a hot plate to 37°C and continuously stirred. The bottoms of 500-ml plas-
tic deli cups were cut off; parafilm was fitted over the opening and secured to the cup with a rubber band. A single cup was placed on top of each cage with the parafilm positioned on the bottom to act as a membrane for the flies to probe. The warmed blood was poured into each cup, and a lamp with a 60-W bulb was positioned over it to provide adequate light and to keep the blood warmed. The flies were placed in the cages and allowed to feed ad libitum for 1 h. Octenol strips (BioSensory Inc., Willimantic, CT) were placed on top of the cage next to the cups of blood for the octenol-exposed treatments. The octenol release rate at room temperature in an open area is 0.0075 g/h (BioSensory Inc.). Octenol feeding assays were performed in a different laboratory from that containing the control cages to ensure no odor stimulus affected any other feeding assays, and only octenol-exposed cages were used for octenol-exposed flies for the same reason. The two laboratories were not compared beforehand using a nontreatment to ensure equivalency; however, optimal lighting and temperature were ensured before each experimental replication.

Dissection Technique and Analysis. After the feeding assays, flies were held at −20°C for approximately an hour. Once the flies were dead they were submerged in 70% ethanol, and each fly was then held up to a light bulb to check for the presence of a bloodmeal. Flies looked deep red if there was a bloodmeal in the midgut and looked yellow if lacking a bloodmeal. Any questionable individuals were dissected, and the midgut was checked for the presence of blood. The data were analyzed using a t-test to compare the means of the control and the treatment group.

Results and Discussion

In total, 320 flies were used in this study. A t-test was used to compare the means of the controls (2.375 ± 1.4497) and the octenol-exposed flies (7.250 ± 1.4497) (JMP, SAS Institute 2005). We found that 36.3% of the 160 control flies exposed to octenol took a bloodmeal, whereas only 10.4% of the 160 control flies took a meal. Thus, octenol significantly stimulates blood feeding in _T. nigrovittatus_ (F = 5.65, df = 14, P < 0.05).

It is not intuitively surprising that an odor attractant stimulates engorgement in a hematophagous fly. However, it is an important missing link in the literature when investigating the sequence of events a fly must respond to provide a bloodmeal. Dethier (1954) and Gatehouse (1970) both failed to show any effect of odors on the probing response of the tsetse fly and stable fly, respectively. However, Rudolfs (1922) demonstrated that the probing response in mosquitoes (_Aedes_ spp.) was enhanced by odors (CO₂ and ammonia). Although Dethier (1954) was unable to show any effect of odors on probing by tsetse, he states, “... it is not unlikely that olfactory stimuli may play a part.”

Because we did not make any observations of individual flies, it is impossible to make a direct correlation in this study between an individual fly probing and its engorgement. It is possible that the observed increased engorgement rate might be due to the octenol, perceived by the antennae, positively modulating the central excitatory state (as described by Dethier et al. 1965) and thereby affecting sensitivity to thermal stimuli, probing, and ultimately engorgement. This remains to be tested.

There is abundant literature describing how hematophagous insects locate their host and all the factors involved. There is also a wealth of knowledge available on how hematophagous insects ingest their bloodmeals, the destination of meals, and the factors affecting feeding physiology. This study demonstrates an important link between an odor stimulus and the feeding response (i.e., ingestion) in _T. nigrovittatus_. Understanding how to interrupt feeding, especially when trying to control obnoxious pests such as _T. nigrovittatus_, requires knowing not only what attracts the flies to their hosts but also recognizing each physiological and behavioral step that stimulates the fly to successfully feed. Thus, understanding the role of attractants on ingestion is essential, especially for those arthropods that serve as vectors of various parasites or pathogens.

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