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TECHNICAL NOTE

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Conditioned *Microplitis croceipes* Cresson (Hymenoptera: Braconidae) Detect and Respond to 2,4-DNT: Development of a Biological Sensor

ABSTRACT: We examined the ability of *M. croceipes* to learn, detect, and respond to 2,4-DNT, which is a volatile discriminator of trinitrotoluene (TNT). The percentage of conditioned wasps to detect and respond to the various concentrations of 2,4-DNT for ≥15 sec was measured. Significantly more of the conditioned wasps responded to the concentration of 2,4-DNT used for conditioning than other concentrations examined. Accordingly, percent conditioned wasps to respond ≥15 sec could be used as a suitable measure to screen air samples and distinguish between samples with or without the target odorant. The data recorded in this study indicate the measured behavior could be used to estimate the concentration of target odorants. Data in this study indicate *M. croceipes* can detect and respond to this compound, which provide further support for its development as a biological sensor.

KEYWORDS: forensic science, forensic entomology, *Microplitis croceipes*, parasitic wasp, biological sensor, 2,4-DNT

The use of animals to detect volatiles from compounds of human importance has long been recognized and utilized by law enforcement for tracking individuals or detecting narcotics (1). A more recent study determined that canines also could possibly be used by the medical profession to detect volatiles emitted by people inflicted with a particular disease, such as melanoma (2). However, the ability to learn and detect odors of human importance is not limited to vertebrates.

Forensic entomology is the study of insects and their use in forensic investigations including medicolegal, stored products and structural damage. In regards to the medicolegal, insects have primarily been used to estimate a minimal time of death of a corpse based on the development and succession of insects on it. However, because of the sensitivity of their olfactory system, it appears that insects also might be used to develop a novel method for detecting and locating chemicals of human importance (1).

Because there is a need for novel chemical detectors, there has been increased interest in insect learning and the potential for using them as conditioned biological sensors. Research on insect learning has received great attention and has been documented for a number of species (3–7). However, while some areas of insect learning have received considerable attention, others have been neglected. For example, very little data are available on the ability of conditioned insects to detect and respond to various concentrations of target odorants (7).

Microplitis croceipes, which is an important larval parasitoid of *Helicoverpa zea* (F.) (Lepidoptera: Noctuidae) and *Heliothis virescens* (Boddie) (Lepidoptera: Noctuidae) (8), has served as a model for a number of studies examining the learning and foraging behavior of insects (9–11). Like other wasps, *M. croceipes* uses olfactory and visual cues to locate and lay their eggs in *H. zea* and *H. virescens*, as well as to locate food resources (9,10), and it has been determined that the use of these mediating cues is improved through associative learning (12,13).

In order to ascertain the ability of insects to be conditioned and respond to target odorants, investigators must determine the appropriate behavioral responses of the trained insect to target odorants (5). These behaviors can be used to determine the ability of insects to discriminate between background and target odorants in context to natural learning situations (5). Takasu and Lewis (11) used the flight behavioral response of *M. croceipes* to determine the roles of odorant-learning when this insect is foraging for food. Olson et al. (14) used the same behavior, in addition to the reflexive behavior coiling when attacking and laying eggs in a host after learning host-associated odors, to demonstrate the robustness and practicality of using expressed behaviors of the conditioned insect to detect and monitor indicator odors of important human activity. Their study also demonstrated that the sensory system of *M. croceipes* holds great potential for its development as a biological sensor due to its ability to be conditioned, respond and discriminate target odors from background odors. However, many questions surrounding its ability to detect and discriminate between various concentrations of target odors remain unanswered.

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The objective in our study was to determine the threshold responses using percent of *M. croceipes* conditioned to associate food with the odors from 2,4-dinitrotoluene, which is a volatile discriminator of trinitrotoluene (TNT).

Material and Methods

Responses of *M. croceipes* to four concentrations of 2,4-dinitrotoluene (2,4-DNT) (Aldrich Chemical Company Inc., Milwaukee, WI) were recorded. Concentrations for 2,4-DNT were 0.05, 0.25, 0.5, 5.0 μg per 100-μL dichloromethane. The control for each odor being examined was dichloromethane alone.

Microplitis croceipes was reared on *H. zea* larvae, which is its natural caterpillar host, using methods previously described (15). Wasp colonies were maintained at 28°C and 60–70% RH, with 16:8 (L:D) hr. *Helicoverpa zea* larvae reared on a pinto bean artificial diet as described by Burton (16) were provided as hosts for *M. croceipes*. Adult wasps were held in Plexiglas® cages and provided with water and honey. Emergent females were not provided honey for 2 days and then were subsequently used in our experiments.

The method developed by Olson et al. (14) was adapted for conditioning and testing wasps to 2,4-DNT. This system was selected because it did not rely on active diffusion to expose the wasps to the odorant but generated airflow to transport the odorant to the wasps.

Twenty-five wasps (5 per day for 5 days) were conditioned and tested to each concentration of 2,4-DNT and the control (dichloromethane). The following methods were used to condition and test the ability of *M. croceipes* to learn and detect 2,4-DNT. For conditioning, the wasps were individually exposed to a filter paper treated with 100-μL dichloromethane containing 0.5-μg 2,4-DNT. However, before exposing the wasps to the target odorant, the treated filter paper was placed on a glass petri dish under a ventilation hood for 15 min to allow the solvent to evaporate. This step allowed the wasps to be conditioned solely to the 2,4-DNT and not the 2,4-DNT/dichloromethane solution. After allowing the solvent to evaporate, the treated filter paper was placed in a 25 × 8-cm 45/50 RodaViss® volatile collection chamber (Analytical Research Services, Inc., Gainesville, FL) with 1-cm openings at both ends (14). One opening was attached to a Cole-Parmer® correlated flow meter (Cole-Parmer, Vernon Hills, IL), which was attached to an Air Cadet® dual head vacuum/pressure pump (VWR, Willard, OH) pushing air at 40 mL/min during the wasp-conditioning period. The opposite end of the collection chamber was attached to the arm of a 5-cm glass tube (2 mm inside diameter) bent near its center 90°. The open end of the glass tube was inserted through a hole in a Teflon® plug (2 cm hole through center) attached to a 4-cm long plastic tube (approximate 2.5 cm diameter) with an aluminum foil cover attached to it. Once the foil was attached, seven holes (each approximately one mm diameter) separated by approximately two mm were placed in a circle near the center of the foil. A droplet (<0.5 mL) of 33% sucrose solution was placed in the center of the ringlet of holes as a food resource for the wasps. Five wasps for the treatment and four for the control were placed individually and sequentially on the aluminum foil and allowed to feed for 10 sec. During this time the wasps were exposed to the odor being pushed through the holes by the air pump. Each wasp had three consecutive 10 sec conditioning periods with approximately 3 min between each session. Each session was separated by a 15 sec quiescent period whereby the wasps were held individually in a 5-mL glass vial. After the final training session, the wasps were held individually in 5-mL glass vials for 15 min before testing.

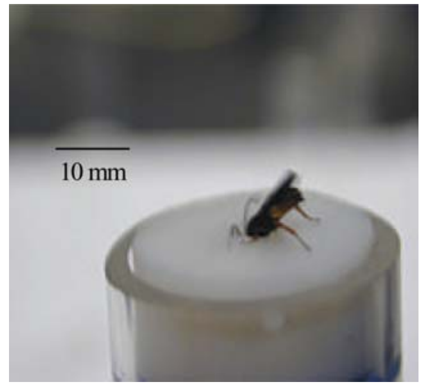

FIG. 1—*Conditioned wasp entering exhaust port emitting target odor.*

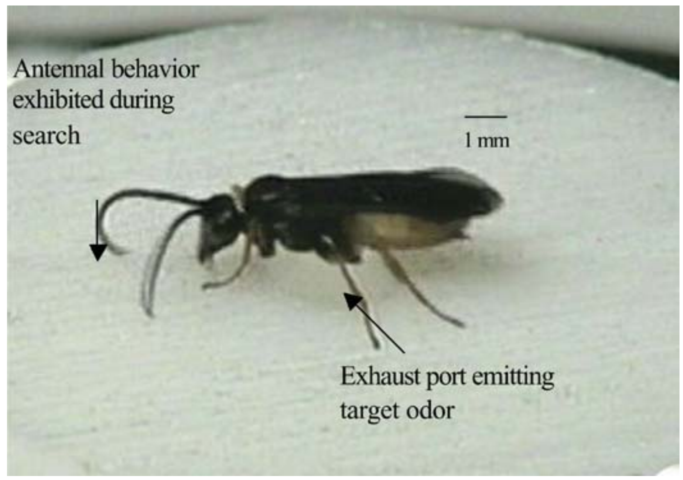

FIG. 2—*Conditioned wasp searching for target odorant.*

For testing, the procedures previously described for conditioning the wasps were used with some modifications. Instead of being attached to a plastic tube with an aluminum foil cap, the 5-cm glass tubing connected to the volatile collection chamber was attached to a Teflon® cap with an approximately 2-mm diameter hole (exhaust port) bored through its center and no sugar water was placed on the cap. The conditioned wasps were exposed solely to the odor being pushed through this hole. For testing, each conditioned wasp was released in close proximity (<1 cm) to the hole in the Teflon® cap. Wasps that entered the exhaust hole (Fig. 1) were recorded as positive responses. All wasps that exhibited positive responses were discarded. Those that exhibited a non-response for <15 sec were removed and re-examined two additional times in succession. Those responding for <15 sec during all three examinations were recorded as a no response, while those to respond for ≥15 sec were considered a positive response (Fig. 2). A chi-square test ($P < 0.05$) was used to analyze percent responses (17). Studies being presented were not blind. Individuals conducting the study knew the 2,4-DNT concentration being presented to the conditioned wasps.

To ensure that wasps being used in the control were not exposed to remnants of 2,4-DNT, which would result in a false-positive, two apparatuses were available each day for these experiments. One apparatus handled filter papers treated with 2,4-DNT/dichloromethane (treatment) and the other receiving filter papers treated only with dichloromethane (control). Additionally, these apparatuses were interchanged daily and, at the conclusion of each day's tests, were disassembled and cleaned with dichloromethane,

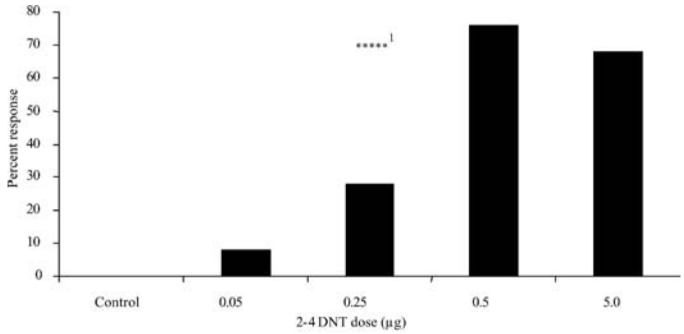

FIG. 3—*Percentage of M. croceipes conditioned to 5 µg 2,4-DNT to respond for ≥15 sec to one of four concentrations of the same odor (χ^2 = 47.97, df = 4, P = 0.0001, Zar 1984).*[1] *Significant difference determined between treatments (P < 0.05).*

air dried, and placed in a convection oven at 55°C for 24 h in order to remove any remnants of 2,4-DNT volatiles that might be present.

Results and Discussion

According to the monitored behaviors, *Microplitis croceipes* can be conditioned to associate food with 2,4-DNT (Fig. 1). However, the percentage of wasps to respond to the target odorant at the concentration used for conditioning was significantly different from responses to other concentrations of the same odorant ($\chi^2 = 47.97$, df = 4, $P = 0.0001$). Approximately 80% of the wasps conditioned to 0.5 µg 2,4-DNT responded to the same concentration. The percentage of conditioned wasps to exhibit the measured behavioral response for ≥15 sec was moderately reduced (70% response) when exposed to a concentration greater than that used for their training. Percent wasps to provide a positive response decreased by 30% when examining concentrations lower than that used for conditioning.

Microplitis croceipes has innate behavioral responses that only occur during host- (18), as well as food- (11), associated learning. The behavioral responses that were measured were only exhibited when conditioned wasps came in contact with the target odorant associated with food (Fig. 3). These results demonstrate that the exhibition of these behaviors will only occur in the presence of the target odorant. Therefore, the probability of recording a false-positive to a sample when monitoring these behaviors is remote and consequently provides greater confidence in the sensitivity and reliability of *M. croceipes* as a biological sensor.

Olson et al. (14) demonstrated the behavioral responses and the level of learning plasticity of *M. croceipes* are suitable for serving as part of a detection system for a variety of odors. Data from our study support their claim. However, Tomberlin et al. (unpublished data) determined that the behavior(s) being measured influences the degree to which the individual recording the observations could distinguish between odors and their concentrations. They determined that behaviors exhibited by wasps conditioned to a target odor in association with hosts, rather than food, were more dependable when attempting to distinguish between the target odorant and other odorants with similar molecular structure. That is, when using one set of behaviors (host association), the data led Tomberlin et al. (unpublished data) to conclude the wasps were extremely sensitive and able to distinguish between the target odor and the non-target odor with a similar molecular structure. In contrast, when measuring another behavior set (food association) to the same odorants, they were led to conclude that the conditioned wasps were not sensitive enough to make the distinction.

The suite of behaviors being recorded might also influence the ability of an observer to truly predict the level at which conditioned wasps detect low levels of a target odorant. In relation to our study, the data recorded using described behaviors indicate that conditioned wasps for the most part were unable to detect 2,4-DNT at 0.05 µg, which indicates conditioned *M. croceipes* are not very sensitive to this concentration. However, it might be that the conditioned wasps can detect low levels of a target odorant, and the suite of behaviors being monitored is not suitable.

Conclusion

Microplitis croceipes might be a prospect for the development of a chemical detection system (14). However, additional research is still needed. Future research efforts need to further examine the use of behaviors in association with host, which appear more sensitive, or possibly a combination of behaviors in association with food. Examining a combination of behaviors in association with food might increase the ability of an observer to distinguish between target odors and those with similar molecular structure, as well as detect low concentrations of the target odors. Furthermore, research efforts are needed that will focus on possibly digitizing behavioral responses of conditioned wasps to target odorants (19). Such studies would eliminate observer bias resulting in greater precision and accuracy when identifying target odors.

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