

## Olfactory Responses of *Microctonus aethioides* Loan, an Adult Parasitoid of the Alfalfa Weevil, *Hypera postica* (Gyllenhal): A Matter of Host Location

الإستجابة لحاسة الشم عند حشرة، *Microctonus aethioides* طفيل  
الطور البالغ لسوسة البرسيم، *Hypera postica* في تحديد موقع العائل

إدوارد س إستيلي، عبدالعزيز م ع محمد، ديفيد ب هوج

Edward C. Steele<sup>1</sup>, AbdulAziz M.A. Mohamed<sup>1,2</sup>, and David B. Hogg<sup>1</sup>

<sup>1</sup>Department of Entomology, University of Wisconsin-Madison, 237 Russell Labs, 1630 Linden Drive, Madison, WI 53706

<sup>2</sup>Directorate of Plant Wealth, Agriculture Affairs, Ministry of Municipality Affairs and Agriculture  
P.O. Box 251, Kingdom of Bahrain. E-mail: ama\_mohamed@hotmail.com

**Abstract:** *Microctonus aethioides* is an important parasitoid in the biological control program of the alfalfa weevil, *Hypera postica*, a pest of alfalfa. Basic biological information about female *M. aethioides* orientation toward its host habitat was assessed in Y-tube olfactometer choice arena. The response of the parasitoid to volatiles from the microhabitat complex, adult weevil, host frass, healthy plants, naturally damaged plants, artificially damaged plants and control odors were tested. The parasitoid was attracted to the host-plant complex of cues representing the microhabitat in most trials, while the response to other odor sources as a means of locating the host were discussed.

**Keywords:** *Microctonus aethioides*, adult parasitoid, alfalfa weevil, *Hypera postica*, host location, olfactometer, olfactory responses.

**المستخلص:** أجريت هذه التجربة لتقييم حاسة الشم لدى أنثى حشرة *Microctonus aethioides* طفيل الطور البالغ لسوسة البرسيم، *Hypera postica* في تحديد موطن تواجد العائل عبر اختبار مصادر متنوعة للروائح المنبثقة عن الأوساط المختلفة وذلك استخدام الأنبوبة ذات الفرعين. ومن مصادر الروائح التي تم اختبارها الروائح المنبثقة عن: موطن العائل الدقيق والذي يشمل على السوسة والنبات المصاب بالسوسة، الطور البالغ للسوسة، مخلفات السوسة، نبات برسيم غير مصاب، نبات برسيم مصاب بالسوسة، نبات برسيم مصاب على نحو متعمد، وهواء نقي كضوابط. دلت النتائج المتحصل عليها أن أنثى الطفيل تنجذب نحو الروائح المنبثقة عن وسط تواجد السوسة على العائل النباتي المتمثل بالموطن الدقيق في أغلب محاولات التجربة مقارنة بالروائح المنبثقة من مصدرا واحد. كلمات مدخلية: طفيل، *Microctonus aethioides* الطور البالغ لسوسة البرسيم، *Hypera postica*، إستجابة حاسة الشم، جهاز تقييم حاسة الشم

### Introduction

The cues and stimuli used by parasitoids have long been studied by parasitoid biologists, who in recent years have made enormous advances in understanding these methods of host location (Godfray, 1994, for current review). Parasitoids use a variety of subtle cues to locate their host, which can be divided into three categories: cues from the microhabitat or host plant; indirect cues from the host such as frass; and direct cues from the host itself (Godfray, 1994). A parasitoid may use one or more of these olfactory chemical cues to locate their host's habitat and then the host, and many of these

processes are understood well for many species. However, some parasitoid's methods of host location have not been studied and are as of yet not understood. One such parasitoid is *Microctonus aethioides* Loan (Hymenoptera: Braconidea) an adult parasitoid of the alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) a pest of alfalfa, *Medicago sativa* L.

*M. aethioides*, a species of European origin, was first introduced into the United States in 1948 as a biological control agent of the sweetclover weevil, *Sitona cylindricollis* (Fahraeus), and later discovered to parasitize the alfalfa weevil (Coles and Puttler, 1963). Since its recovery in 1961, the

parasitoid has established throughout the alfalfa growing area of the northeastern and upper Midwestern United States (Bryan *et al.*, 1993 for detailed establishment sites), and become an important parasitoid in the biological control program of the alfalfa weevil responsible for maintaining the weevil population at or below economic levels in the area of establishments (van Driesche and Gyrisco, 1979; Kingsley *et al.*, 1993).

*M. aethioides* is a bivoltine, solitary endoparasitoid that lays a single egg into the haemocoel of the adult alfalfa weevil, where development of the parasitoid immature stages take place. In Wisconsin, the parasitoid overwinters as a first instar larva inside the overwintering adult weevil and emerges in the early spring (i.e., late April to early May) to pupate in the soil. Upon emergence by Mid-May, the first generation females attack the surviving nonparasitized overwintered weevil adults (spring population) and the developing parasite larvae sterilizes the weevil (Dysart and Day, 1976). The parasitoid larvae eventually kill these adults, emerge and complete the pupal period in the soil. The second generation emerges from these hosts in the summer (i.e., mid to late-June), attacks and sterilizes the newly emerged adult weevils (summer population) which are actively feeding on the alfalfa, where the parasitoid will then overwinter as first instar larvae inside the hibernating weevils until next spring (Brunson and Coles, 1968; Abu and Ellis, 1976; van Driesche and Gyrisco, 1979).

Female *M. aethioides* has adapted a unique ovipositional behavior of utilizing the weevil mobility as stimulant that guides the females to final stage of host location. Although a few biological studies concerning *M. aethioides* biology, and its relationship with its host, have been carried out (Loan and Holdaway, 1961; Fusco and Hower, 1974), some basic biological information such as host habitat location is unknown and absent from the literature. It is unknown whether the parasitoid is utilizing different environmental cues to locate the host. The goal of this study was to explore the different cues associated with both the plant and the host, and to determine which odors or combination of odors the parasitoid uses to locate the host's habitat and the host itself.

## Materials and Methods

### 1. Host and parasitoid culture

A colony of *H. postica* adults, which were reared from field-collected larvae during the spring, were

sustained in the laboratory to provide a supply of unparasitized adult weevils for the experiment. After eclosion adult weevils were kept in 1.0 liter containers with organdy cloth lids, each containing twenty-five weevils. A fresh supply of alfalfa was kept in the container for the weevils and old alfalfa removed regularly. Containers were frequently exchanged for clean ones, and dead weevils were removed. The containers were kept at a relatively constant temperature of 21° C and 16 h photoperiod on an open-air bench.

The *M. aethioides* used in this study were reared from field-collected overwintered alfalfa weevil adults. The weevils were kept in the same manner as described above, in groups of twenty-five in 1.0 liter containers. These containers had cheesecloth at the bottom in order to facilitate parasitoid pupation. The weevils were supplied with fresh alfalfa and the containers were kept in rearing chambers which maintained a light/temperature regime fluctuating between sixteen hours of light at 23.3°C and eight hours of dark at 15°C. The containers were checked daily for emergence of any *M. aethioides* larvae and weevils were frequently moved to new containers to limit disturbance of parasitoid pupation. The pupae were kept in the containers until the adult parasitoid eclosed. Only newly emerged and mated female *M. aethioides* were used in this study.

### 2. The Olfactometer

To determine the response of the parasitoid to odors relating to the host, experiments were conducted in a two-armed airflow olfactometer. The olfactometer consisted of a Y-shaped glass tube 15 mm in diameter. The base and the two arms of the Y-tube were 75 mm long. Each arm was connected to an odor source made of a plastic cylinder container, 26 mm in diameter and 67 mm long that was connected to a flowmeter. The flow rate of the air was regulated at 1.5 l/min through each arm. Air was allowed to run through a mesh hole at one end of the plastic tube, through the arm of the Y-tube to the trunk of the tube containing the parasitoid. The parasitoid was initially placed at the opening of the central trunk of the Y-tube and could walk freely into either arm of the tube. Between each trial the olfactometer was dismantled and cleaned with water and 90% EtOH, then allowed to dry completely.

### 3. Assay Test

Six different odors as well as a control were

offered in combination and passed through the olfactometer. The odor source was placed in one arm of the olfactometer and another odor source was placed in the other arm, in order to determine the preference of the parasitoid for an associated cue(s).

**Adult Alfalfa Weevil.** Adult weevils from a maintained colony of nonparasitized weevils were used. The weevils used in the trials were isolated and starved for 48 hours prior to the test in order to allow time for any odors from the plant, as well as associated feeding damage odors that may have been lingering on the weevil, to dissipate.

**Control Odor.** The control odor was an empty tube that had been thoroughly cleaned before the experiment. Air that passed through this tube offered a choice to the parasitoid of no odor.

**Adult Weevil Frass.** Alfalfa weevil frass was gathered from containers used to hold the weevils during rearing. The frass was moistened with drops of water prior to the trial to increase freshness by allowing the frass to release its odor.

**Host-Plant Complex.** A complex cue representing the microhabitat of the weevil was used, and consisted of alfalfa weevil adults, frass and fresh alfalfa as well as alfalfa with fresh feeding damage.

**Healthy Plant.** Health alfalfa plant not fed upon by an adult weevil was used.

**Naturally Damaged Plant.** Host plant that had been fed on and damaged by feeding weevil adults was used. Alfalfa weevil adults were confined with the plants for 48 h. After time expiration the weevils were removed and the plants were maintained for a 48 h prior to the trials

**Artificially Damaged Plant.** Alfalfa was intentionally damaged fifteen minutes prior to the trial by cutting out sections of leaves to artificially simulate feeding damage.

#### 4. Experimental Procedure

Each female parasitoid was cooled by placing the petri dish containing them on an ice-pak to slow down their activity prior to its transfer to the olfactometer. The test started by placing the parasitoids into the central trunk section of the olfactometer after the odor source had been connected to the tube and allowed to pass through. The moment the parasitoid became active and started movement toward the fork of the tube the trial commenced. Each parasitoid was observed until it showed a response to one of the odors or until 30 minutes elapsed. A positive response was measured when the parasitoid was within a distance of 15 mm from the base of the arm.

Six trials were conducted to determine the parasitoids response to different chemical cues associated with the host and its habitat. Trial 1 compared cues from the host-plant complex versus a naturally damaged plant (N=7); trial 2 compared cues from the host-plant complex versus a healthy plant (N=12); trial 3 compared cues from the host-plant complex versus an artificially damaged plant (N=7); trial 4 compared cues from a naturally damaged plant versus an artificially damaged plant (N=10); trial 5 compared cues from the host-plant complex versus a starved adult weevil (N=7); and trial 6 compared air versus cues from weevil frass (N=7).

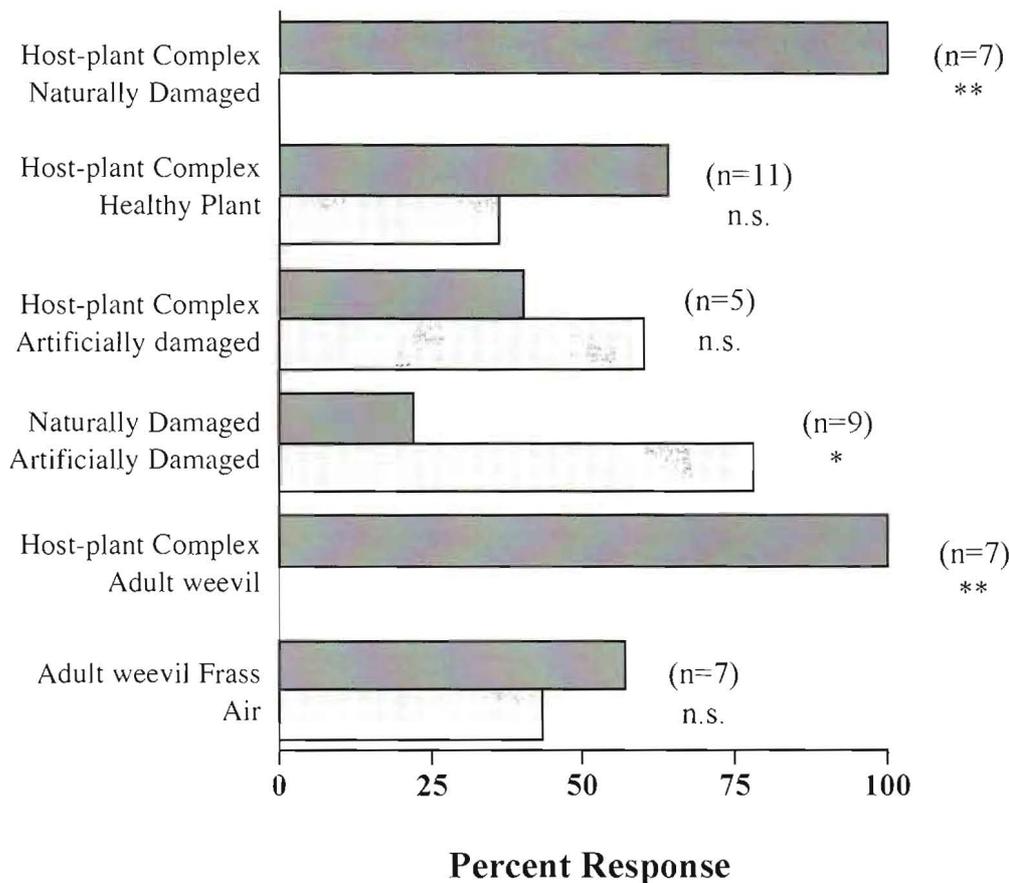
#### Results

*M. aethiopoulos* females significantly oriented toward cues of alfalfa damaged by feeding activity of adult *H. postica* over naturally damaged plant from which adult weevils were removed 48 h prior to the test ( $\chi^2 = 14.00$ ; d.f. = 1;  $P < 0.001$ ) (Fig. 1). When the naturally damaged plant was compared with the healthy plant and the artificially damaged plant, the parasitoid females showed no differences in their attraction to either cue sources when tested independently (Fig. 1). However, parasitoids showed a statistically significant orientation toward the artificially damaged plant to naturally damaged plant from which adult weevils were removed ( $\chi^2 = 5.6$ ; d.f. = 1;  $P < 0.025$ ) (Fig. 1).

When given a choice between the host-plant complex compared to a starved adult weevil alone, again *M. aethiopoulos* females responded significantly toward the host-plant complex cues over the starved weevil ( $\chi^2 = 14.00$ ; d.f. = 1;  $P < 0.001$ ). A non-significant response was found when the parasitoid was given a choice between the indirect cue of weevils' frass and the control odor of air.

#### Discussion

The data collected in the study were quite interesting, as they allowed some insight into the way in which female *M. aethiopoulos* may locate its host. When analyzing the frequency of response by the parasitoid to a particular cue source we saw certain trends in the data that made sense. The results showed that *M. aethiopoulos* females were strongly orientated toward host-plant complex cues. Such response was not surprising, as several products related to the host and feeding damages have been shown to contain chemical cues that facilitate host location by parasitoids.



**Fig. 1.** Response of female *M. aethiopoies* to various cue sources in a Y-tube olfactometer. Asterisks indicate that the responses to the stimuli in the two arms of the olfactometer were significantly different from those,  $p$ -value = 0.05, Chi-square test. n.s. = non significant. Number of the individuals tested given in parenthesis.

Parasitoids response to the host-plant complex versus the healthy plant showed a higher response preference to the host-plant complex (Fig.1). Again, this response makes sense, as it would be important for the parasitoid to be able to locate not only the plant but the host on the plant as well, so it would be more attracted to the combination of host and plant than just the plant alone, as also revealed in the parasitoid adults *Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) (Eller *et al.*, 1988), *Cotesia glomerata* (L.) (Hymenoptera: Braconidae) (Mattiacci *et al.*, 1994) *Cotesia rubecula* (Marshall) (Hymenoptera: Braconidae) (Agelopoulos and Keller, 1994; Geervliet *et al.*, 1994) response to infested plants over uninfested prey habitat.

It is possible that the parasitoid responses to the healthy plant was due to strong odors emanating from the freshly cut alfalfa, which could easily cause such a bias in the responses (Fig. 1). The parasitoid responses to the healthy plant could have been valid responses if the parasitoid was attracted to the healthy plant itself, as other studies on parasitoids have shown that parasitoid females locate the plant first and then find and attack the host

(Sato and Ohsaki, 1987). Indeed, Vinson (1975) observed that the parasitoid *Cardiochiles nigriceps* (Viereck) (Hymenoptera: Braconidae) searches host free tobacco plants, showing that the parasitoid is first attracted to the plant regardless of the presence or absence of the hosts.

Many parasitoids orientate towards cues originating not from the host directly, but from indirect cues that are a product of the host's activity, such as odors released by feeding damage. In a tritrophic study, Mattiacci *et al.* (1994) found that when Brussels sprouts are fed upon by *Pieris brassicae* L. (Lepidoptera: Pieridae), the plants emit volatile compounds which attract the insect's parasitoid *C. glomerata*. Storeck *et al.* (2000) found similar results indicating that herbivore-induced plant-derived cues play an important role in host preference by the generalist aphid parasitoid *Aphidius colemani* (Hymenoptera: Braconidae), a parasitoid of green peach aphid, *Myzus persicae* (Sulzer). However, in the present study when a comparison of the naturally damaged plant versus the host-plant complex was conducted the parasitoid showed preference response of 100% for the host-

plant complex, which we found a bit surprising (Fig. 1). The lack of the parasitoid response to the naturally damaged plant free of adult hosts could have resulted from the declined rate of the volatile cues due to the plant's 48 h isolation period prior to the test making it less obvious to the parasitoid.

The non-significant differences in the parasitoid response to either artificially damaged or the host-plant complex cues could have resulted from the strong release of plant volatiles due to the fresh cut to induce artificial damage that biased the parasitoid responses (Fig. 1). Similarly, attraction to artificially damaged plants by parasitoids has also been reported as in *Campoletis sonorensis* (Cameron) (Hymenoptera: Ichneumonidae), an endoparasitoid of *Heliothis* = (*Helicoverpa*) *zea* (Boddie) (Lepidoptera: Noctuidae) a pest of cotton (Elzen *et al.*, 1983), as well as *C. glomerata* L. a specialized larval endoparasitoid of *Pieris rapae* L. (Lepidoptera: Pieridae) which was attracted to volatiles emanating from artificial damaged *Brassica oleracea* (Blaakmeer *et al.*, 1994). However, the significant influence of the artificially damaged plant in eliciting a strong response in the parasitoid behavior over the naturally damaged plant was a matter of experimental artifact.

The significant response by the parasitoid to the host-plant complex over the starved adult host was not unexpected (Fig. 1). It is not known if the weevil produces active volatile chemicals used by *M. aethioides* as host location cues. However, in a close-range observation on *M. aethioides* ovipositional behavior, host odor(s) was found to play a role only in host recognition by the parasitoid (Mohamed, 2003). Thus, for *M. aethioides* it seems that the complex of cues are only important to host location whereas host odor(s) is important in host recognition preceding the parasitization.

Indirect chemical cues such as the weevil's frass may contribute to the factors involved in host location by the parasitoid, although this cannot be confirmed at all in the study (Fig. 1). While there are no other studies that we can compare with involving parasitoids of adult hosts, different host-parasitoid relationships found that host frass had some role in influencing the behavioral response of the parasitoid toward the host. Lewis and Jones (1971) and Jones *et al.* (1971) found that the parasitoid *M. croceipes* is attracted to chemicals in the frass of its host, the corn earworm, *H. zea*. Additionally, Eller *et al.* (1988) indicated that the corn earworm's frass was the most important factor in the host-locating flight behavior of *M. croceipes*. Ramachandran *et al.* (1991) also found that female *Microplitis demolitor* (Cresson) (Hymenoptera:

Braconidae) a parasitoid of the soybean looper, *Pseudoplusia includens* (Walker) (Lepidoptera: Noctuidae) utilizes the hosts' frass to locate the host. Insect by products along with chemical cues emitted from the plant as a result of feeding damage can add to the complex cues that are utilized by the parasitoids to locate their hosts in the fields.

In conclusion, the results of this preliminary study show that *M. aethioides* females use chemical cues to locate its host's habitat. The cues originating from the complex of microhabitat and host were strong enough in our preference trials to be the overwhelming choice by the parasitoid in three out of four such trials. Some of the unexpected results in our data have been easily explained, as hindsight often allows. From a management perspective, natural enemy conservation is important and can be considered at the field level in terms of time of cutting and clear cutting. Time of cutting alfalfa should not interfere with peak activity of the parasitoid in the field, and degree-days can be used to determine the best time to harvest alfalfa while enhancing the beneficial activities of *M. aethioides*. The practice of clear cutting should also be considered, as it may remove both the host and its parasitoid. A better practice of the parasitoid conservation is to leave a refuge area, thus allowing the parasitoid survivorship to continue. More work needs to be done to determine the type of chemicals that play a major role in the methods of host location by *M. aethioides*, and the results of this study will be helpful to those doing further work in the area, as well as better management practices in the field.

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