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KAIROMONES AND THEIR USE FOR MANAGEMENT OF ENTOMOPHAGOUS INSECTS: III. STIMULATION OF *Trichogramma achaeae*,[1] *T. pretiosum*,[1] AND *Microplitis croceipes*[2] WITH HOST-SEEKING STIMULI AT TIME OF RELEASE TO IMPROVE THEIR EFFICIENCY[3,4]

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Abstract—Frass from larvae of the corn earworm, *Heliothis zea* (Boddie) and scales from *H. zea* moths (that are known to contain the host-seeking stimulus, tricosane) stimulate and orient host-seeking activity in female *Microplitis croceipes* (Cresson), a larval parasite of *H. zea*, and *Trichogramma* spp., egg parasites of *H. zea*. When larval frass, moth scales, and tricosane were used as sign stimuli (releasers) for *M. croceipes*, *T. pretiosum* (Riley), and *T. achaeae* Nagaraji and Nagarkatti, respectively, at time of their release from laboratory containers, parasite performance improved, resulting in significantly increased rates of parasitization over that of unstimulated parasites. Stimulation of *M. croceipes* with larval frass had an overriding effect on this parasite's innate tendency to disperse upon release, thereby increasing the numbers remaining and prolonging their retention in the target area. Supplying the appropriate host-seeking stimuli to these 3 hymenopterous parasites of *H. zea* at time of their release to improve their efficiency greatly increases the probability of their effective utilization in pest management systems.

Key Words—kairomones, *Trichogramma achaeae*, *Trichogramma pretiosum*, *Microplitis croceipes*, *Heliothis* spp., host-seeking stimuli, insect behavior, releasers, pest management, biological control.

[1] Hymenoptera: Trichogrammatidae.
[2] Hymenoptera: Braconidae.
[3] In cooperation with the University of Georgia College of Agricultural Experiment Stations, Coastal Plain Station, Tifton, Georgia 31794.
[4] Mention of a proprietary product does not constitute endorsement by the USDA.

INTRODUCTION

The success of an insect-management program dependent on the field release of laboratory-reared entomophagous insects is governed by a multitude of biotic and abiotic factors, any one of which may be determinant. Of necessity, the primary initial concern is the successful establishment and retention of the parasite in the target area. In nature, chemical guidance systems—kairomones (Brown et al., 1970, Whittaker and Feeny, 1971)—emanating from the host and/or its byproducts aid the parasite in effectively orienting to its host once within the host habitat. Recent studies by Lewis et al. (1975*a*,*b*) have shown that the efficiency of released *Trichogramma* spp. was significantly improved by the application of the host-seeking stimulus, tricosane, in target areas. However, the probability of field-released parasites encountering these host-seeking stimuli, either natural or introduced, is left to chance. These parasites may just as readily follow their primary innate response, upon release, to escape and disperse before settling into a normal behavioral pattern. Oriented movement may then again predominate at indeterminant distances from the release site.

During 1973 and 1974, kairomones were evaluated as sign stimuli (releasers)[5] at time of parasite release to determine whether the egg parasites, *T. achaeae* and *T. pretiosum* (Riley), and the larval parasite, *Microplitis croceipes* (Cresson), could be stabilized and oriented in a host-seeking pattern, thus increasing their frequency of retention in the target area and thereby improving their overall efficiency.

METHODS AND MATERIALS

Microplitis croceipes

Greenhouse Tests. In tests conducted on 0.75-m-high cement tables within a 7.6 × 7.6-m greenhouse section, the bottom halves of 10-cm petri dishes were placed atop 3 overturned 15.2-cm-high plastic planter pots arranged in a triangular design with ca. 0.5 m separation between pots. Each petri dish contained one 3rd- or 4th-instar larvae of the corn earworm, *Heliothis zea* (Boddie) obtained from the Tifton, Georgia, laboratory colony. The larva was mounted on a single crowder pea leaf with a minuten, nadeln pin sticking from a 3 × 6 × 10-mm cork beneath the leaf. The pin passed through the larva in one of the last 3 abdominal segments entering from the ventral surface, thus retaining the larva on the leaf. Also, newly deposited frass from *H. zea* larvae was spotted on the leaf to aid the searching parasite in locating the

[5] Sign stimuli (releasers) are stimuli that evoke a particular instinctive behavior or so-called fixed-action pattern, a sequence of coordinated motor actions that appear without the animal having to learn it by the usual learning process (Hess, 1965).

host, since frass elicits a host-seeking response by *M. croceipes* (Lewis and Jones, 1971). Laboratory-reared *M. croceipes* were collected in 2-dr vials from emergence cages (Lewis and Burton, 1970) when 3–6 days old. Prior to their release, fresh frass from larvae that has fed the previous 24 hr on crowder pea foliage was smeared over the entire lip of the release vial to ensure contact by the parasite upon emerging. One such vial containing a single parasite was then placed upright, at ground level, equidistant between the 3 pots. The parasites were allowed 30 min to locate larvae, after which larvae were collected and dissected to determine percentage parasitization (Jones and Lewis, 1971). The test was replicated 5 times on each of 7 days. Temperatures within the greenhouse ranged from 25.0°C to 28.9°C during the studies.

In other tests, *M. croceipes* were exposed as above to 13-methylhentriacontane (1 mg/ml hexane), an identified host-seeking stimulus of this parasite (Jones et al., 1971).

Field Tests. The stability of stimulated and unstimulated *M. croceipes* was observed under field conditions by releasing them onto a potted crowder pea plant ca. 25 cm high. Again frass from *H. zea* larvae that had developed on crowder pea foliage was smeared on the lip of the 2-dr release vial. Unstimulated parasites were released from untreated vials. Each parasite was permitted to crawl from the vial to the potted crowder pea plant. Stability of the parasites was then measured by their retention and antennal examination of the pea plant vs. their dispersion without initiating search.

Trichogramma achaeae

Laboratory Tests. Effects of stimulating the egg parasite, *T. achaeae*, with the host-seeking stimulus, tricosane, before their release was measured in 14-cm petri dishes. Laboratory and field bioassays have shown tricosane (a biochemical isolated from moth scales of *H. zea*) to elicit orientation and stimulate parasitism by *T. evanescens* (Jones et al., 1973). Eggs of *H. zea*, 1–2 days old, collected from the Tifton laboratory culture were arranged in 2 intersecting rows of 4 eggs/dish. Then 2 female *Trichogramma* spp., cultured as described by Lewis and Redlinger (1969), with the substitution of *H. zea* eggs for those of *Cadra cautella*, were collected from emergence tubes into a 2-dr shell vial and transferred to a release vial. The inner surface of the release vial had been treated with ca. 1/8 ml tricosane (1 mg/100 ml hexane) and allowed to dry. After the parasites had been confined for 5 min on the treated surface, they were released into petri dishes containing the eggs. Unstimulated parasites were released from untreated vials. Parasites were permitted 40 min to 1.25 hr to parasitize eggs. Eggs were then removed and dissected (Lewis and Redlinger, 1969) to determine percentage parasitization. Paired comparison (30 replications) were evaluated on 6 days.

Field Tests. The effect of stimulation with tricosane (1 mg/100 ml hexane) at time of release was evaluated on *T. achaeae* in field plots of early to late green tassel stage White Cross Bantam sweet corn 18 rows wide by 30.4 m long. Nine sites, 1.8 m long (3 lines equidistant from one another at 3 locations) were marked with plastic nursery tags in each plot. Then at each site, 20 *H. zea* eggs were attached to the upper leaf surfaces at the top one third of the corn plants (ca. 1.5 m high) with a saliva-moistened camel's hair brush. Circular sections of ca. 100 eggs of *H. zea* containing developing *T. achaeae* (av. 2/egg) were individually clipped from oviposition cards, placed into 8-dr plastic cups and held until emergence at 26.7 ± 1°C. (A raisin was placed in each cup to provide food and moisture before release.) At 6 sites (2 lines of 3 cups each, equidistant between the center and sides of the plot) cups were opened and placed within 48-dr screw-cap jars which had been sprayed to runoff (ca. 5 ml) with tricosane and allowed to dry. Parasites were retained ca. 5 min, then the jars were opened, allowing the parasites free movement. Unstimulated parasites were released from untreated jars. Eggs were collected within 4–5 hr after parasite release, returned to the laboratory, and dissected to determine percentage parasitization. Paired comparisons of stimulated vs. unstimulated parasites were made on each of 7 days.

In addition, an influx of *Heliothis* spp. moths into cotton near Tifton, Georgia, during mid-August, 1974, permitted us to measure the response of *T. pretiosum* to naturally oviposited eggs, after the parasites had been stimulated with moth scales of *H. zea*. Paired comparisons of stimulated vs. unstimulated *T. pretiosum* were made in 21 plots 8 rows wide by 6.1 m long as follows: 25 eggs of *H. zea* parasitized by *T. pretiosum* were placed in 16-dr plastic cups 1 day before the scheduled emergence of the parasites. A paper cap (1 mm thick) containing a 6-mm hole to permit parasite emergence was brushed with moth scales of *H. zea* on the inner surface to ensure contact by the parasite as it emerged. Unstimulated parasites were handled identically except for the absence of moth scales. 16 such 16-dr cups containing ca. 800 total parasites were placed in each plot (2 on each of 8 rows) at the base of the cotton plant. Examination of a random sample of the cups 24 hr later revealed that 95+% of the emerging parasites had left. Efforts were made the following day to collect 15 eggs/plot from the plant terminals. Eggs were taken into the laboratory and dissected to determine percentage parasitization. Separation of means in all studies was done with the Student's *t* test.

RESULTS AND DISCUSSION

Microplitis croceipes

Greenhouse Tests. Because of their innate tendency to disperse upon release, *M. croceipes* are difficult to use in experimental studies. Being

positively phototactic, they usually move upward and away from the release site. Unstimulated parasites in the greenhouse study responded accordingly, by moving upon release to the ceiling of the greenhouse where they remained without initiating search. Parasites stimulated via antennal contact with the larval frass as they exited from the vial established a host-seeking pattern and generally had little difficulty locating the dishes containing larvae despite the dissimilarity between the test area and their natural habitat. These observations therefore led us to believe that location of larvae by the parasites established within the host habitat is dependent primarily on chemical stimuli, and little, if at all, on visual perception of habitat conformation. Rates of parasitization by female *M. croceipes* stimulated at the time of release were significantly higher, 1% level of probability, than that of unstimulated parasites (Table 1). However, on each test day a higher incidence of attempted parasitization was observed than the dissections revealed and the tabular data indicate. This discrepancy, we feel, was primarily the result of misses by the parasite and the loss of larval hemolymph (and probably parasite eggs) when larvae were removed from the pins.

Attempts to stabilize *M. croceipes* with 13-methylhentriacontane were unsuccessful. Parasites were stimulated by antennal contact with this biochemical (Lewis and Jones, 1971), but they remained in the area only a short time, and no oriented flight in search of additional sources of the stimulus was observed. There appear to be components in addition to the 13-methylhentriacontane, some perhaps plant derived, which together produce the oriented searching pattern observed by stimulation with larval frass.

Field Tests. Sixteen stimulated *M. croceipes* remained and searched on potted crowder pea plants; only 1 dispersed upon release. Of the unstimulated parasites, 21 dispersed while one remained and searched. As in the greenhouse test, the stimulated parasites had no tendency to disperse; instead they performed extensive antennal examinations of the stem and leaf surfaces of

TABLE 1. COMPARATIVE PARASITIZATION OF LARVAE OF *H. zea* BY FRASS-STIMULATED AND UNSTIMULATED *M. croceipes* RELEASED IN THE GREENHOUSE

	% Larvae parasitized on indicated days[a]							
	1	2	3	4	5	6	7	Mean[b]
Stimulated	33.3	33.3	13.3	40.0	13.3	33.3	26.6	27.6
Unstimulated	0	0	0	0	0	0	0	0

[a] 15 total larvae available for parasitization on each day.
[b] Means significantly different at the 0.01 level of probability.

the plants. Only after parasites searched for several minutes without receiving additional reinforcing stimuli did they disperse.

Trichogramma achaeae

Laboratory Tests. Parasitization of *H. zea* eggs by stimulated *T. achaeae* was significantly higher, 1% level of probability, than by unstimulated parasites when evaluated in petri dishes (Table 2). It is now apparent that stimulation of *T. achaeae* with tricosane before release activates the host-seeking behavior, thus increasing the frequency and/or intensity of search for an undetermined period. We suspect, however, that oviposition may in itself act as an independent reinforcing stimulus for host seeking.

Field Tests. Observations of *T. achaeae* upon release indicated that they apparently do not have an initial tendency to unoriented dispersal but rather move short distances on the plant in search of host eggs. Nevertheless, *T. achaeae* that were stimulated with tricosane upon release produced signifi-

TABLE 2. COMPARATIVE PARASITIZATION OF EGGS OF *H. zea* BY TRICOSANE-STIMULATED AND UNSTIMULATED *T. achaeae* RELEASED IN PETRI DISHES

	% Eggs parasitized on indicated days						
	1	2	3	4	5	6[a]	Mean[b]
Stimulated	29.8	32.4	28.4	14.6	19.7	32.2	26.2
Unstimulated	14.4	15.0	18.0	18.8	5.8	14.4	14.4

[a] 20 paired comparisons on day 6.
[b] Means are significantly different at the 0.01 level of probability.

TABLE 3. COMPARATIVE PARASITIZATION OF EGGS OF *H. zea* BY TRICOSANE-STIMULATED AND UNSTIMULATED *T. achaeae* RELEASED IN FIELD PLOTS OF TASSEL-STAGE SWEET CORN

	% Eggs parasitized on indicated days							
	1	2	3	4	5	6	7	Mean[a]
Stimulated	53.8	37.6	60.3	10.0	4.3	3.6	22.1	27.4
Unstimulated	48.5	21.4	42.3	7.4	2.4	4.7	14.8	20.2

[a] Means are significantly different at the 0.01 level of probability.

cantly higher rates of parasitization in the field than did unstimulated parasites (Table 3). Instances of apparent lack of response are shown in Table 2 for day 4 and in Table 3 for day 6. However, an individual statistical analysis of data for both days indicated that the reversals were not significant. Age of *T. achaeae*, which was not standardized during the study, may eventually prove to be crucial to a favorable response by stimulated *Trichogramma*.

Data are not yet available to determine how much of the higher parasitization by stimulated *Trichogramma* is attributable to retention of larger numbers of parasites in the target area for longer periods of time and how much is caused by a more oriented and intensified search by the parasites. Our petri-dish studies and those of Lewis et al. (1975*b*) demonstrate that at least part of the increased parasitization is the result of a more efficient search.

Lewis et al. (1975*a*) demonstrated the ability of field-applied tricosane to increase rates of parasitization by *Trichogramma* spp. The data in the present text showed that tricosane stimulation of *T. achaeae* at release significantly improves their efficiency. We suspect that the effect of stimulating parasites before release is additive to the effect of field-applied kairomones, and produces additional retention of *Trichogramma* in the target area.

T. pretiosum stimulated with moth scales of *H. zea* at time of release likewise responded positively, producing higher rates of parasitization (30.5%) on naturally oviposited *Heliothis* spp. eggs than did parasites that were not stimulated (21.0%). Separation of means with the Student's *t* test indicated significance at the 0.02 level of probability.

CONCLUSIONS

An oriented host-seeking behavior is evoked in *M. croceipes*, *T. achaeae*, and *T. pretiosum* when they are stimulated at the time of release from containers by exposure to their respective host-seeking stimuli. Frass of *H. zea*, a stimulus for *M. croceipes*, prolongs the retention of the parasite in the target area, thereby improving their overall efficiency. *T. achaeae* stimulated with tricosane at time of release produced significantly higher rates of parasitization than unstimulated parasites in both petri-dish bioassays and in field plots of whorl-stage sweet corn. *T. pretiosum* stimulated with moth scales of *H. zea* at release produced significantly higher rates of parasitization on naturally occurring eggs of *Heliothis* spp. in cotton than did unstimulated parasites.

The demonstrated principle of utilizing kairomones as releasers to activate host-seeking behavior before parasite release has far-reaching implications for future parasite release programs.

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