

SEMIOCHEMICALS MEDIATING MICROPLITIS CROCEIPES<sup>1</sup>  
HABITAT, HOST, AND MATE FINDING BEHAVIOR

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ABSTRACT

Semiochemicals play a major role in the behavior of Microplitis croceipes Cresson. Evidence indicates that odors associated with the habitat, host, and mate guide the finding process of the parasitoid. For host location, some of the semiochemicals have been identified and their roles defined. Research is currently in progress to identify the chemicals associated with habitat and mate finding.

INTRODUCTION

Insects must successfully find habitat, food, and mates to survive. For parasitoids, chemicals are involved in all of these processes. The successful parasitoid must select and respond to the proper chemicals from amongst a milieu of chemicals which make up the search environment. It has evolved to do this after generations of exposure to those chemicals closely associated with hosts and mates. This includes not only those chemicals which are always associated with the hosts or mates, such as host plant chemicals, but also might include chemicals from plants associated with the host plant, i.e., those plants that represent the proper ecosystem. Consequently, the range of chemicals to which a parasitoid such as Microplitis croceipes Cresson responds can be very wide and not always easily rationalized.

Chemicals such as those described above which mediate interactions among organisms have been designated semiochemicals by Law and Regnier (1971). Functional definitions of several types of semiochemicals such as kairomones and allomones have been reviewed by Nordlund (1981).

HABITAT FINDING

A survey of recent literature (Juniper and Southwood 1986, Bell and Carde 1984, Ahmad 1983) reveals that a large number of plant chemicals have been identified to which herbivorous insects respond. However, only a small number have been identified which affect parasitoid behavior (Kainoh 1987).

Most plant volatiles to which insects respond are constituents of the plant cuticle, which for most plants contains literally hundreds of chemicals. For example, Kamm and Fronk (1964) identified over 95 volatile chemicals from alfalfa of which the alfalfa seed chalcid responded to 38.

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Many of the plant chemicals that affect insect behavior belong to groups of common plant volatiles such as the unsaturated 6 carbon compounds like hexenals, hexenols, and hexenol esters. Others belong to classes of chemicals that are associated with certain groups of plants such as the mustard oils with crucifers or the sulfides with onions and garlics.

Although the plant plays a significant role in the behavior of M. croceipes, no active plant chemicals have been identified. It will be interesting to discover the range of chemicals to which M. croceipes responds since its host Heliothis zea (Boddie) has such a wide range of plant hosts. For example, M. croceipes parasitizes its host, H. zea, on cotton but not corn or sorghum.

Elzen et al. (1983) demonstrated that Campoletis sonorensis (Cameron), a parasitoid of several noctuids including H. zea, is attracted to the volatiles of several different plants including cotton, tobacco, sorghum, and geranium. They (Elzen et al. 1984) later identified several chemicals from the essential oil of cotton that are responsible for the attraction. However, it is not known if these same chemicals are responsible for attraction to the other plants.

A further example of response complexity can be seen from the results of Ding et al. (unpublished results, D.C. Ding, R.L. Jones, and P.D. Swedenborg, University of Minnesota) with Macrocentrus grandii (Goidanich), a monophagous parasitoid of the European corn borer (ECB), Ostrinia nubilalis (Hubner). ECB, like Heliothis spp., has a wide variety of host plants. We proposed to examine the response of M. grandii to these host plants as well as to a wide variety of nonhost plants that represented a cross section of the higher plants. The hypothesis was that in an olfactometer, M. grandii would prefer any plant to purified air. The hypothesis proved false. Out of 51 species across 22 families tested, 6 species of plants were actually repellent (pine, cattail, duckweed, onion, basswood, lilac - all nonhost for ECB). Fifteen species were attractive, 9 ECB hosts (wheat, corn, snapbean, hollyhock, pepper, potato, lettuce and goldenrod), and 6 nonhosts (nutgrass, wild rose, alfalfa, boxelder, milkweed, and dandelion). The rationale for such diversity is not apparent. It could be coincidental (similar plant volatiles) or the nonhost plants could be closely associated with host plants in the ecosystem.

In summary, the impact of plant chemicals on behavior is very complex for insects that function at the third trophic level (Price 1981), especially those with polyphagous hosts like M. croceipes. However, the elucidation of the chemical ecology involved will greatly clarify parasitoid behavior and offer potential tools for the enhancement of biological control. For example, attractive plant chemicals could prove useful in directing M. croceipes to search for H. zea on crops to which it is minimally attracted such as sorghum. As H. zea on sorghum is exposed, parasitism rates should be very high once M. croceipes is directed to the habitat.

#### HOST FINDING AND ACCEPTANCE

M. croceipes uses methyl branched hydrocarbons contained in the epicuticle of H. zea larvae to locate and identify suitable hosts (Jones et al. 1971). These chemicals are also located in H. zea frass, and the frass trail of a feeding larvae is used by M. croceipes females to locate the host.

The most active component was identified as 13-methylhentriacontane (13 MHC) (Jones et al. 1971). In a laboratory bioassay that measured decreased orthokinesis and antennation, responses of 0, 0.2, 0.5, 2.3, and 1.7 (antennations out of 3 trials) were obtained for 150 ng of the 9, 11, 12, 13, and 15-methyl analogs of hentriacontane, respectively. Thirteen-

methyl dotriacontane and 13-methyl tritriacontane also elicited positive responses.

Tests for synergism with 13 MHC and 15 MHC indicated that the chemicals diluted each other. For example, 50 ng of each analog produced a score similar to the mean of the two chemicals tested separately. This indicates that the analogs are acting at the same receptor sites.

Further information about the mechanism of these types of chemicals can be obtained by looking at Vinson's work with Cardiochiles nigriceps (Viereck) (Vinson et al. 1975). This work demonstrated that synergism does occur when methyl alkanes of different chain lengths are tested together. Also the optimum methyl position varied with chain length. For example, optimum response was obtained with 11-methyl hentriacontane, 16-methyl dotriacontane, and 13-methyl tritriacontane. Selected mixtures of the three chain lengths increased the mean response 2.0 to 2.5X above responses to a single chemical. The optimum response of the bell shaped response curve also shifted from 5 µg to 500 ng. This is indicative of different receptor sites for chemicals of varying chain lengths. This work should be repeated using M. croceipes.

Lewis et al. (1987) have recently evaluated the response of M. croceipes to the stereoisomers of 13 MHC and have determined that females respond equally to both isomers. This indicates that the female either cannot distinguish between the two isomers or she responds to them similarly. The relative concentrations of the two isomers in host cuticle is unknown. It would be interesting to know if M. croceipes can distinguish the two isomers. Perhaps associative learning experiments with one of the isomers could clarify this.

The potential application of these chemicals in artificial rearing programs is obvious. The availability of a chemical that denotes acceptability to females makes the design of an artificial host feasible. The response of M. croceipes females to 13 MHC is strong enough to induce them to sting nonhost such as Ostrinia nubilalis (R. L. Jones, unpublished). However, the results of Tilden and Ferkovich (1988) would indicate that a peptide in the hemolymph of H. zea is sufficient to induce oviposition by M. croceipes into an artificial substrate.

The utility of these chemicals in the field has not been demonstrated. As they are not very volatile, attraction to them over a distance is not a mode of action and consequently, these chemicals would be of no use in attracting M. croceipes to a specific location. Since they act as trail substances, the first presumption is that broad application in the field would mask the host and confuse the parasitoid. However I suspect that 13 MHC applied at very low doses might serve to increase the search time and decrease habitat departure rates. At such levels, 13 MHC contained in frass or associated with the host would be in significantly higher concentration and confusion could be avoided. Such experiments should be conducted.

#### MATE FINDING

A cooperative project involving Dr. Janine Powell, Dr. Ed King and Dr. Gary Elzen of the USDA, Stoneville, MS; Dr. James Tumlinson, USDA, Gainesville, FL; Mr. Paul Swedenborg of the University of Minnesota, St. Paul, MN and me is currently underway. We have demonstrated that M. croceipes females emit a sex pheromone that attracts and elicits landing and wing fanning responses in males.

A laboratory wind tunnel constructed from an aquarium (33w x 47h x 77 cm l) is currently used to monitor pheromone identification work. In this environment, males exhibit flight initiation, upwind anemotaxis, casting, landing, wing fanning, and copulatory attempts with other males. These responses are rhythmic with the optimum responses 3-6 h post photophase

initiation.

Activity is found in a hexane rinse of the females, a collection of volatiles on charcoal, or a collection of active chemicals onto the interior of a glass flask.

Several isolation procedures have been adopted to purify the active component(s) with mixed success. Open column chromatography on Florosil and high performance liquid chromatography (HPLC) using silica, reversed phase and size exclusion columns are effective separation techniques that do not alter the pheromone activity. Results of these methods indicate that the active component(s) is of moderate polarity, similar to aldehydes, ketones, or esters. However, the use of silver nitrate impregnated columns significantly reduces activity that cannot be recovered by recombinations of fractions.

The active component(s) has proven to be particularly heat sensitive and only recently have methods been developed for collection on capillary gas chromatography (g-c). Performance on non polar g-c indicates a chemical with an equivalent chain length of 19 carbons; whereas, size exclusion columns indicate a size equivalent of 8-12 straight chain carbon equivalents. Thus, the active chemical likely contains a ring structure.

The heat sensitivity has hindered chemical tests; but the active component is hydrolyzed by acid at room temperature and deactivated by lithium aluminum hydride but not by sodium biohydride - indicative of an ester structure. The presence of unsaturation is indicated by ozonolysis.

The chemical is very active and appears to be present in the female in very small quantities. For example, 0.01 female hours (FH) produces a response in the olfactometer whereas g-c of 120 FH indicates the presence of 10 ng of material. This indicates that a female may produce only 100 pg of pheromone per hour.

The sources of sex pheromones in the parasitic Hymenoptera are variable. The literature indicates that the abdomen is the primary source in the Braconidae, Ichneumonidae, Chalcidae, Pteromalidae, and Aphelinidae. Weseloh (1976) indicated that the pheromone for Apanteles spp. is produced in the DuFour's gland. Tagawa (1977) concluded that the pheromone source was a secretory gland at the base of the second valvifer in Apanteles glomeratus L. Elzen's present work indicates that the DuFour's gland may also be the pheromone source in M. croceipes although the body washes indicate that the cuticle is contaminated with the pheromone. Our work with another braconid, M. grandii, has shown that one sex pheromone component, Z-4-tridecenal, is produced from oxidation of cuticular 9, 13-dienes, although another component, source unknown, is present and necessary for field attraction (P. Swedenborg and R. Jones, unpublished data). Previous work by Eller et al. (1984) showed that ethyl palmitoleate was the sex pheromone for Syndipinus rubiginosus Walley. Robacker and Hendry (1977) implicated neral and geranial as pheromones for Itopectis conquisitor (Say) although this was not verified in the field. Pheromone sources were not indicated in either of these latter two cases.

Parasitoid pheromones identified to date are varied in structure. Based on the work with M. grandii, some parasitoids may be in the early stages of sex pheromone evolution. The males may have adapted to respond to specific female odors, much as other insects have adapted to host produced chemicals called kairomones. The coevolutionary adaptation of a specific female gland to produce higher quantities of the attractive chemicals may not have occurred yet.

Sex pheromones should prove useful to monitor parasitoid populations. Eller et al. (1984) demonstrated that synthetic pheromone attracted males in the field. It now remains to be demonstrated that trap catches can be correlated with parasitism rates. Variable sex ratios in some species will complicate and perhaps defeat the development of this technique.

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