

SEMIOCHEMICALS IN HOST AND MATE FINDING BEHAVIOR
OF *MACROCENTRUS GRANDII* GOIDANICH
(HYMENOPTERA: BRACONIDAE)

RICHARD L. JONES
Office of the Dean for Research
University of Florida, IFAS
Gainesville, FL 32611

ABSTRACT

Naive females of *Macrocentrus grandii* Goidanich, a parasitoid of the European corn borer (ECB), *Ostrinia nubilalis* Hubner were attracted to some, but not all, host plant species of the latter; attraction being exceptionally strong for *Zea mays* L., progressively weaker for potato, snapbean and pepper, neutral for sunflower, with soybean being slightly repellent. Also, extracts of volatiles from soybean were repellent. The percent of females that responded to a given ECB host plant increased progressively with age of females to a maximum at 8 days. When various plant species were damaged by feeding activity of ECB, they became highly attractive to *M. grandii* females, and, subsequently, these experienced females were attracted to undamaged plants, even of plant species that were not attractive to naive females.

Responsiveness of females to all plant species increased as a result of experience with ECB-damaged plant specimens of host species. Such experience with ECB-damaged potato increased attraction of females to sunflower by 4-fold, while experience with ECB-damaged sunflower increased attraction to potato by 2-fold. Loss of memory in females of experience with ECB-damaged plants progressed with time and was total by the ninth day. Memory was induced primarily by frass, but not because frass was derived from a particular plant species. Male *M. grandii* were not attracted to any host plants.

Female *M. grandii* emit a series of 9,13 dienes of odd-numbered 27-41 carbon atom chains, and these, when oxidized by air, produced Z-4-tridecenal, the active volatile of the pheromone. The latter when emitted at 50 ng per hr proved to be a very effective lure. Further, we found that this aldehyde is synergized significantly by the lactone, (3S,5R,6S)-3,5-dimethyl-6-(methylethyl)-3,4,5,6-tetrahydropyran-2-one. It is noteworthy that this lactone by itself is highly attractive when 1 to 500 ng are applied to the source. The lactone is produced by the mandibular gland of both males and females. Possibly the diene/aldehyde has arisen as an important intermediary evolutionary step, in a process wherein opportunistic males that recognize volatile chemicals unique to the female may have been selected. Natural selection then would favor females that produce more and more of the attractant precursor - a process that could lead eventually to selection for specialized cells and a pheromone gland.

Key Words: Tritrophic, attractants, pheromone, *Macrocentrus*, European corn borer, parasitoid, behavior.

RESUMEN

Las hembras sin experiencia de búsqueda de *Macrocentrus grandii* Goidanich, un parasitoide del barrenador europeo del maíz, *Ostrinia nubilalis* Hubner, son atraídas hacia algunas especies de plantas hospedantes del barrenador. La atracción es excepcionalmente fuerte hacia *Zemays* L., progresivamente débil para la papa, la habichuela y el pimiento, neutral para el girasol y ligeramente repelente para la soya. También los extractos volátiles de soya fueron repelentes. El porcentaje de hembras que respondió a una planta hospedante dada aumentó progresivamente con la edad

de las hembras hasta alcanzar un máximo a los 8 días. Varias especies de plantas que habían sido dañadas previamente por el barrenador se convirtieron en altamente atractivas a las hembras de *M. grandii*. Estas últimas fueron subsecuentemente atraídas por las plantas no dañadas y por las no atractivas para las hembras sin experiencia.

La respuesta a todas las especies de plantas aumentó como resultado de la experiencia de las hembras con plantas hospederas dañadas por el barrenador. La experiencia con patatas dañadas por el barrenador aumentó 4 veces la atracción de las hembras hacia el girasol, mientras que la experiencia con el girasol aumentó 2 veces la atracción hacia la patata. La pérdida de memoria en las hembras con experiencia en plantas dañadas aumentó con el tiempo y fue total el noveno día. La memoria fue inducida primariamente por las excretas, pero no porque las excretas fueran derivadas de una especie de planta en particular. Los machos de *M. grandii* no fueron atraídos por ninguna de las plantas hospederas.

La hembra de *M. grandii* emite una serie de 9,13 dienos de número impar de cadenas de carbonos de 27-41 átomos y éstos, cuando son oxidados por el aire, producen Z-4-tridecenal, el volátil activo de la feromona. Este último es un atrayente muy efectivo cuando es emitido a 50 ng/hr. Más tarde encontramos que este aldehído es sinergizado significativamente por la lactona (3S,5R,6S)-3,5-dimethyl-6-(methylethyl)-3,4,5,6-tetrahydropyran-2-uno. Es notorio que esta lactona por sí misma es altamente atractiva cuando de 1 a 500 ng son aplicados a la fuente. La lactona es producida por la glándula mandibular de machos y hembras. Posiblemente el dieno/aldehído ha creado un importante paso evolutivo intermediario, en un proceso donde han sido seleccionados los machos oportunistas que reconocen los químicos volátiles únicos de la hembra. La selección natural entonces favorecería a las hembras que producen más y más del precursor atrayente -un proceso que podría conducir eventualmente a la selección de las células especializadas y la glándula de la feromona.

This presentation provides a review of what has been learned about the role of semiochemicals in the behavior of *Macrocentrus grandii* Goidanich, a polyembryonic braconid parasitoid of the European corn borer (ECB), *Ostrinia nubilalis* Hubner. The work occurred over a period of about 10 years in the laboratory of the author at the University of Minnesota. The research reported herein encompassed two areas of behavior - the host finding behavior of the female and the mate finding behavior of the male. The semiochemicals involved in these two areas of behavior are only part of a complex of chemicals to which an insect is exposed in its natural habitat. Many insects rely heavily on semiochemicals to assess their environment and to guide their movements as well as to cue other aspects of their behavior. For insects such as a parasitoid, whose host finding involves three trophic levels, the array of significant chemicals is likely to be complex. These studies were initiated to elucidate the nature, role, and interrelationships of semiochemicals in the tritrophic interactions of *M. grandii*, its European corn borer host, and European corn borer host plants.

The initial studies focused on host finding; the results have been published by Ding et al. 1989a, 1989b, Udayagiri & Jones 1992a, 1992b, and 1993. One of the first aspects of host finding behavior addressed was the range of host plants to which *M. grandii* was attracted and to what extent this group of plants paralleled the host range of *O. nubilalis*. We proposed that tests in a y-tube olfactometer, with only air as an alternative, would reveal that *M. grandii* would be attracted to almost any green plant, regardless of species. This hypothesis proved false, but a very wide range of plants across several families were attractive, including Gramineae, Cyperaceae, Rosaceae, Leguminosae, Aceraceae, Malvaceae, Asclepiadaceae, Solanaceae and Com-

positae. Surprisingly, the most consistent attraction was among the Compositae, even more so than among the Gramineae, although the strongest attraction by far was to *Zea mays* L. A few species repelled the females. This occurred within the Pinaceae, Lemnaceae, Liliaceae, Tiliaceae, and Oleaceae families. There was no evident correlation of attractiveness to *M. grandii* with *O. nubilalis* recorded host plants.

Next, we investigated the role of learning in the attraction of *M. grandii* females to plants. Learning is a well demonstrated phenomena among insect parasitoids, but we were interested in the degree of discrimination by the female among closely related plants, the learning response to non-attractive plants, the existence of cross-attraction induced by learning, and the rate of forgetting. Naive female *M. grandii* are strongly attracted to sweetcorn, somewhat attracted to potato, neutral to sunflower, and very slightly repelled by soybean. Naive females were attracted to all four plants when the plant had been damaged by the European corn borer.

Prior exposure of female *M. grandii* to plant parts damaged by ECB larvae significantly and markedly increased responses by the female *M. grandii* to undamaged plants in the y-tube olfactometer. This was true even with sunflower and soybean. For example, experience increased attraction to potato three-fold and to sunflower four-fold. The overall level of responsiveness increased to all plant species as a result of experience with one European corn borer damaged plant species. Experience with potato increased attraction to sunflower four-fold and experience with sunflower increased the response to potato two-fold. Females experienced on potato or sunflower also strongly preferred those plants to sweet corn when offered a choice of the two plants. Similar results were obtained when leaves or stems were tested. Females also were able to discriminate between varieties of sweet corn, as a learning experience on one variety induced preference for that variety.

M. grandii females did forget the learning experience. Over a period of nine days post-experience, attraction to sunflower decreased to insignificance. It should be noted that the experience provided exposure to European corn borer damaged plants only. It did not allow for host finding, nor for parasitism. Thus, this learning does not involve a "reward" in the classical sense or a consummatory act.

Further studies were conducted to identify the specific components of the host-damaged plants that cued the learning response in the female. These studies identified the frass as the key component, but not because the plant material contained in the frass was derived from a particular plant species. For example, exposure of *M. grandii* females to sunflower in combination with frass collected from ECB larvae that had fed on sweetcorn, induced attraction to sunflower in the female, whereas exposure to sunflower alone does not.

Because the above studies were conducted in a y-tube olfactometer and, therefore, assayed a walking response, it was of interest to study female behavior in flight. Consequently, a series of experiments were conducted in a small flight chamber provided with an air flow. These studies were conducted with plant volatile extracts, and they revealed similar responses to those seen in the y-tube. Naive female *M. grandii* were most strongly attracted to sweetcorn, with decreasing attraction to potato, snapbeans, pepper, and soybean. For example, on average, 9 of 10 females landed on the sweetcorn source, 5 of 10 on potato, 2 of 10 on snapbean, 1.5 on pepper and 1 on soybean. In all tests, responses were <1 on the control source. Female response to corn increased over time when females were held unexposed. This response ranged from very little (<1/10 females) at one day of age to a maximum response (8/10 females) at eight days. This phenomena of lower threshold with increasing physiological deficit is commonly reported in hunger and oviposition studies. Male *M. grandii* were unresponsive to all plants in these studies.

The flight studies also confirmed the strong influence of experience on host choice. Bioassays of mixtures of plant volatile extracts showed that soybean significantly reduced female response to sweetcorn, whereas pepper did not. This strongly indicates a repellent chemical contained in soybean. In spite of this, experience with European corn borer damaged soybean induced females to respond positively to soybean volatiles in the flight chamber. Further studies indicated that the leaf and husk of corn contained the most active volatiles attractive to the female. These chemicals could be trapped, partially purified and still retain their activity.

Our studies revealed that *M. grandii* has a pliable behavioral system that no doubt allows it to adapt to the several plant hosts of its host, the European corn borer.

Although numerous studies have revealed the multiple roles of chemicals in the mate-finding and mate acceptance behavior of insects, very little similar information is extant for insect parasitoids. In addition to the contribution that such studies also could make to our understanding of insect behavior and its evolution, this information could lead to useful survey tools for parasitoids. Consequently, we pursued the elucidation of the *M. grandii* mating behavior scheme. These studies have been reported by Swedenborg & Jones 1992a, 1992b, Shin et al. 1993, Swedenborg et al. 1993, Swedenborg et al. 1994. Male *M. grandii* were shown to respond positively to females and extracts of females in a flight chamber by exhibiting the common behaviors of upwind anemotaxis, hovering, landing, wing fanning, and copulatory motions. These behaviors were used as the assay tool for identification of the pheromone components involved.

The first studies demonstrated that a series of dienes contained in the female cuticle elicited the mating behaviors observed in the males. It was also evident that at least two more polar components were active. Synthesis of the hydrocarbons confirmed that a series of 9,13 dienes of odd numbered 27-41 carbon atoms were active. We later demonstrated that the activity was due to air oxidation of the dienes to produce Z-4-tridecenal, the active volatile component of the pheromone. This compound, as well as the dienes, proved to be active in attracting males in the field. In slow release Hercon® wafers, a release rate of 50 ng per h was a very effective lure as it attracted twice as many males as were attracted by three virgin females. E-tridecenal was synthesized, tested, and shown to be inactive, and it was without inhibitory effects when added to Z-4-tridecenal. Further studies indicated that both the dienes and the aldehyde were significantly synergized by a polar compound found in the active extracts of females. Addition of this component to Z-4-tridecenal increased trap catch in the field two-fold. Extensive analyses and bioassays revealed this chemical to be (3S,5R,6S)-3,5-dimethyl-6-(methylethyl)-3,4,5,6-tetrahydropyran-2-one. It and its' 3R,5S,6R enantiomer were synthesized and tested. The combination of Z-4-tridecenal and the lactone proved to be a powerful lure for males in the field.

Laboratory bioassays revealed that the lactone elicited upwind anemotaxis. Males demonstrated a positive dose response to the lactone at levels of 1 ng to 500 ng applied to the source. The RSR enantiomer proved to have very slight activity, but the SRS enantiomer was 15-fold as active. When added to Z-4-tridecenal (50 ng per h release rate) in the field, 10 micrograms of the SRS enantiomer compared favorably to mixtures using the female derived materials.

The source of the lactone is the mandibular gland of both males and females. Because it occurs in the male, its primary function as a pheromone is not certain. It perhaps serves as an aggregation pheromone in the male, but this could not be demonstrated. Both male and female contain 100-300 ng of the lactone per head.

These studies represent the second report of the role of an oxidation product of hydrocarbon dienes in the pheromone scheme of an insect. A similar account was docu-

mented by Bartelt & Jones (1983) for another hymenopteran, *Pikonema alaskensis*. Because, as far as we know, the aldehyde activity is elicited entirely by the products of air oxidation of these dienes, there appears to be no specific gland and, consequently, no control of pheromone release. In both species, a potent synergist is involved, the control of which is unknown. One can readily foresee an important intermediary evolutionary step here, as this represents a case wherein opportunistic males that recognize volatile chemicals unique to the female may have been selected. Natural selection then would produce females that produce more and more of the attractant precursor - a process that could lead to selection for specialized cells and a pheromone gland.

REFERENCES CITED

- BARTELT, R. J., AND R. L. JONES. 1983. (Z)-10-nonadecenal: a pheromonally active air oxidation product of the (Z,Z)-9,19 dienes in the yellowheaded spruce sawfly. *J. Chem. Ecol.* 9: 1333-41.
- DING, D., P. D. SWEDENBORG, AND R. L. JONES. 1989a. Chemical stimuli in host-seeking behavior of *M. grandii*. *Ann. Entomol. Soc. America* 82: 232-36.
- DING, D., P. D. SWEDENBORG, AND R. L. JONES. 1989b. Plant odor preferences and learning in *M. grandii* (Hymenoptera: Braconidae), A larval parasitoid of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae). *J. Kansas Entomol. Soc.* 62: 48-61.
- SHIN, I., H. -W. ZHOU, N. L. S. QUE, H. -W. LIU, P. D. SWEDENBORG, AND R. L. JONES. 1993. A sex pheromone isolated from *Macrocentrus grandii*: Absolute stereochemistry determination and implications. *J. Org. Chem.* 58: 2923-2926.
- SWEDENBORG, P. D., AND R. L. JONES. 1992a. Multicomponent sex pheromone in *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae). *Jour. Chem. Ecol.* 18: 1901-1912.
- SWEDENBORG, P. D., AND R. L. JONES. 1992b. (Z)-4-Tridecenal, a pheromonally active air oxidation product from a series of (Z,Z)-9,13 dienes in *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae). *Jour. Chem. Ecol.* 18: 1913-1931.
- SWEDENBORG, P. D., R. L. JONES, HUNG-WEN LIU, AND T. P. KRICK. 1993. (3R*,5S*,6R*)-3,5-dimethyl-6-(methylethyl)-3,4,5,6-tetrahydropyran-2-one, a third sex pheromone component for *Macrocentrus grandii* (Goidanich) (Hymenoptera: Braconidae) and evidence for its utility at eclosion. *Jour. Chem. Ecol.* 19: 485-502.
- SWEDENBORG, P. D., R. L. JONES, HUI-QIANG ZHOU, INJAE SHIN, AND HUNG-WEN LIU. 1994. Biological activity of (3R,5S,6R)- and (3S,5R,6S)-3,5-dimethyl-6-(methylethyl)-3,4,5,6-tetrahydropyran-2-one, a pheromone of *Macrocentrus grandii* (Goidanich) (Hymenoptera: Braconidae). *Jour. Chem. Ecol.* 20: 3373-3380.
- UDAYAGIRI, S., AND R. L. JONES. 1992a. Flight behavior of *Macrocentrus grandii* Goidanich (Hymenoptera: Braconidae), a specialist parasitoid of European corn borer (Lepidoptera: Pyralidae): factors influencing response to corn volatiles. *Environ. Entomol.* 21: 1448-1456.
- UDAYAGIRI, S., AND R. L. JONES. 1992b. Role of plant odor in parasitism of European corn borer by braconid specialist parasitoid *Macrocentrus grandii* Goidanich: isolation and characterization of plant synomones eliciting parasitoid flight response. *Jour. Chem. Ecol.* 18: 1841-1855.
- UDAYAGIRI, S., AND R. L. JONES. 1993. Variation in flight response of the specialist parasitoid *Macrocentrus grandii* Goidanich to odours from food plants of its European corn borer host. *Entomol. exp. appl.* 69: 183-193.