Responses of *Diadegma insulare* (Hymenoptera: Ichneumonidae) to Caterpillar Feeding in a Flight Tunnel[1]

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Abstract A flight tunnel bioassay was used to evaluate attraction responses of female *Diadegma insulare* (Cresson), a host-specific parasitoid of the diamondback moth, *Plutella xylostella* (L.), to collard plants (*Brassica oleracea* var. *acephala* L.) infested with host and nonhost caterpillars. Adult female wasps showed increased responses to odors of the plant-host complex after a brief contact experience with host-infested collard leaves. Such an increase shows evidence of associative learning in this parasitoid to the odor released from the larval-plant complex. The same experimental design was also used to determine responses of the parasitoid to plants infested with nonhost larvae-cabbage looper, *Trichoplusia ni* (Hübner), and imported cabbageworm, *Pieris rapae* (L.). The female wasps conditioned to the plants infested with host larvae also showed increased responses to plants infested with larvae of cabbage looper or imported cabbageworm. These results indicate that plants damaged by host and nonhost caterpillars may release general odors that are attractive to *D. insulare.* The general damage odors caused by host and nonhost larval feeding may enhance biological control of the diamondback moth. Host-search ability of the parasitoid increases after experiencing host damage. When host populations are low in fields, plant odors caused by generalist herbivore feeding may also attract *D. insulare* parasitoids to the vicinity, thereby improving encounter chances of the parasitoid encountering diamondback moth larvae.

Key Words Diamondback moth, parasitoids, learning, cabbage looper, imported cabbageworm, biological control

Among the parasitoids attacking the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) shows the most promise as a biological control agent to minimize crop damage (Idris and Grafius 1993, Losata and Kok 1986). It is the most important parasitoid of the diamondback moth in North America (Latheef and Irwin 1983, Pimentel 1961, Harcourt 1963, Horn 1987) and has resulted in greater than 90% parasitism in untreated fields (Muckenfuss et al. 1990). *Diadegma insulare* is the most abundant parasitoid of the diamondback moth in Florida (Mitchell et al. 1997). Mass-produced *D. insulare* adults in the laboratory and field cages have been released into cabbage

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fields to suppress populations of the diamondback moth in northeast Florida (Mitchell et al., unpubl. data).

Previous investigations have shown that many species of parasitoids increase searching efficiency by learning profitable cues (Moraes et al. 1998, Turlings et al. 1992). Associative learning in parasitoids has been used in manipulating biological control agents, such as conditioning mass-reared parasitoids before their release into a target area (Lewis and Martin 1990, Papaj and Vet 1990). The objectives of this study were to determine whether (1) prior experience to host caterpillar feeding increases response of *D. insulare* to host infested plants, or (2) if experienced wasps exhibited greater responses to nonhost infested plants.

Materials and Methods

Rearing of plants and insects. Collard plants, *Brassica oleracea* var. *acephala* L., were planted in pots measuring 7 cm high, 10 cm top diam and 7.5 cm bottom diam. The plants used for tests were 6 to 8 wks old, 7 to 10 cm high, and contained 6 to 10 leaves. *Diadegma insulare* collected from Bunnell, FL, May 1996 were reared on second/third-instar diamondback moth larvae that were reared on a wheat germ-based artificial diet (Shelton et al. 1991). Parasitoid cocoons were placed in emergence cages (30 × 30 × 30 cm) without hosts and plants; the emerging adult parasitoids were considered naive with respect to stimuli from the host or the plant-host complex. Parasitoid females used in the bioassay were 3 to 6 days old. A water-soaked cotton ball and light smears of honey on the inside of the cage served as water and food sources. All *D. insulare* were maintained under a 12:12 h L: D cycle at 25°C and 50% RH. For assays, only female wasps were taken out of the cage and treated as needed.

Pre-flight experience. Half the females used for the tests were untreated and were categorized as naive. Naive females had no experience with any hosts, host products, or plant leaves. The other half of the females obtained their experience by the following procedure. Collard plants of the size described above were infested with second/third-instar diamondback moth larvae (6 to 8 larvae each plant leaf). After 12 h, the plant was placed into an emergence cage, and *Diadegma* females were added. The cage was illuminated by a 75-W Crypton light. Most of the wasps landed on the plant and started to search for diamondback moth larvae. Individual wasps observed walking on the damaged plant leaves for a maximum of 5 min or less time by stinging diamondback moth larvae at least once were removed and placed into a separate cage. Females obtained in this manner were classified as experienced wasps.

Herbivore damage. Plants used for flight tunnel assays were damaged by host or nonhost larval feeding. Six to eight second/third-instar host (diamondback moth) larvae were placed on each leaf. The nonhost were first/second-instar cabbage looper, *Trichoplusia ni* (Hübner), larvae and imported cabbageworm, *Pieris rapae* (L.), larvae. Cabbage looper and imported cabbageworm are important pests of cabbage, and their larvae were collected from cabbage crops in Bunnell cabbage fields. To obtain approximately the same amount of plant damage (estimated 2 to 3% damage to the leaf area by visual inspection), fewer (½ to ⅓) nonhost larvae were used for each leaf because they were larger and fed more rapidly than the diamondback moth larvae. The plants were infested 12 h before they were used for flight tunnel assays.

Flight tunnel assays. All free flight tests with *D. insulare* were conducted in a Plexiglas flight tunnel, 60 × 60 cm in cross section, 240 cm long, with an airflow of 0.2

m/s. Five Krypton lights (75 W) illuminated the flight tunnel with approximately 800 lux from above. A temperature of 27 ± 1°C and 75 ± 5% RH were maintained in the flight tunnel during the test. All tests were conducted between 1100 and 1500 h.

In all bioassays, individual parasitoids were placed in a horizontal polystyrene tube near the center of the downwind end of the flight tunnel. The wasps were held in the tube and exposed to the odors in the air flow from the upwind plant-larval complex and were released after 2 min of such preflight conditioning in the tube. The plant infested with caterpillars was placed at the center of upwind end of the tunnel. The wasp and the plant were at the same vertical level and the distance between them was about 1.8 m. The wasps were observed following release for non-response, dispersal, and landing. Wasps that completed the whole journey and landed onto the plant were counted as responders. Those that did not finish the complete journey and landed on the wall of the tunnel or failed to leave the release tube after 5 min were counted as nonresponders.

Three tests were conducted to document the response of *D. insulare* to the plant with caterpillar feeding. Test 1 compared the attraction responses of naive wasps with experienced wasps to collard plants infested with diamondback moth larvae. Alterations of naive and experienced wasps were made after every 10 individual releases. Test 2 compared responses to the plants infested with diamondback moth larvae with the plants infested with the cabbage looper larvae by naive and experienced wasps. Test 3 compared responses to the plants infested with diamondback moth larvae with the plants infested with imported cabbageworm larvae by naive and experienced females. Tests 2 and 3 were non-choice tests. A plant with host larval feeding and one with nonhost larval feeding were alternatively placed into the tunnel after each 10 individual releases. Each replicate involved 10 female wasps. A total of 10, 15, and 13 replicates was included for tests 1, 2, and 3, respectively.

Data analyses. The number of responders from each test was calculated for percentage landing. Mean percentage landings were analyzed using two-way analysis of variance (ANOVA) and Student's *t*-test (SAS Institute 1990). Variables were transformed using arsine of square root to meet the assumptions of ANOVA before analyses (Marks 1990).

Results and Discussion

In test 1, female wasps with prior experience to a plant-larval complex showed a significantly higher (t = 5.88, df = 9, P < 0.01; Fig. 1) response (56 ± 5.5%) when compared with naive wasps (13 ± 3.3%). Such an increase indicates associative learning in this wasp of odors released from the larva-plant complex.

Test 2 showed that 16 ± 2.5% ($\bar{x}$ ± SEM) of the naive wasps were attracted to the diamondback moth larval-infested plants and 14.7 ± 2.9% to the cabbage looper larval-infested plant; 42.7 ± 3.7% of the experienced wasps were attracted to the diamondback moth larval-infested plant and 40.7 ± 4.1% to cabbage looper larval-infested plants. The difference was significant between naive and experienced wasps (F = 35.9; df = 3, 56; P < 0.01; Fig. 2) but not between the diamondback moth larval-infested plant and the cabbage looper larval-infested plant (F = 0.60; df = 3, 56; P > 0.05). Similarly, in test 3 there was no significant difference (F = 0.18; df = 3, 48; P > 0.05) in the response of wasps to plants infested with diamondback moth larvae and the plants infested with imported cabbageworm larvae by naive (15.4 ± 4.0% vs. 13.1 ± 3.1%) and experienced females (49.2 ± 5.2% vs. 46.9 ± 5.6%). There was a

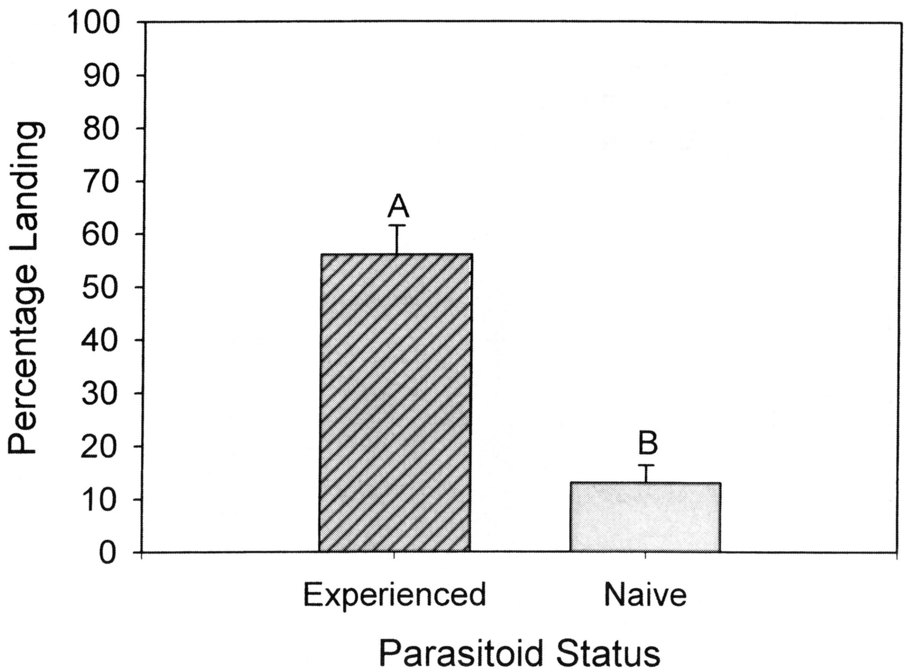

Fig. 1. Mean (±SE) percentage of female *Diadegma insulare* responding to a collard plant with upwind-oriented flight and landing on plants damaged by diamondback moth (DBM) larvae. Different letters above bars indicate significant differences at $P < 0.05$.

significant difference between naive and experienced wasps ($F = 59.9$, df = 3, 48; $P < 0.01$; Fig. 3). Further, these tests indicate associative learning in the wasp of odors from the larva-plant complex. The odor may be released from damaged plants rather than the caterpillars themselves, since the conditioned wasps were attracted to the plants infested with host larvae and nonhost larvae. The plants damaged by host and nonhost caterpillar-feeding may release common chemicals that are attractive to *D. insulare*.

Associative learning of host odors has been found in other species of wasps, such as *Cotesia marginiventris* (Cresson) (Turlings et al. 1990b, 1992), a parasitoid of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith); *C. plutellae* Kurdjumov (Hu and Mitchell, unpubl. data), a parasitoid of the diamondback moth; *C. glomerata* (L.) (Mattiacci and Dicke 1994), a parasitoid of the cabbage white caterpillars (European cabbageworm), *Pieris brassicae* (L.); and *Microplitis croceipes* (Lewis and Tumlinson 1988), a parasitoid of corn earworm, *Helicoverpa zea* (Boddie). The associative learning is due to experiencing odors from the plants after their hosts attack. These parasitoid females learn to take advantage of the volatiles after herbivore attacks to locate hosts after experiencing these volatiles in association with hosts or host products. Such synomonal plant-insect interaction, which has adaptive advantages to both the wasps and the plants, may be common in these types of tritrophic systems (Tumlinson and Lewis 1991, Turlings and Tumlinson 1991, Turlings et al. 1991).

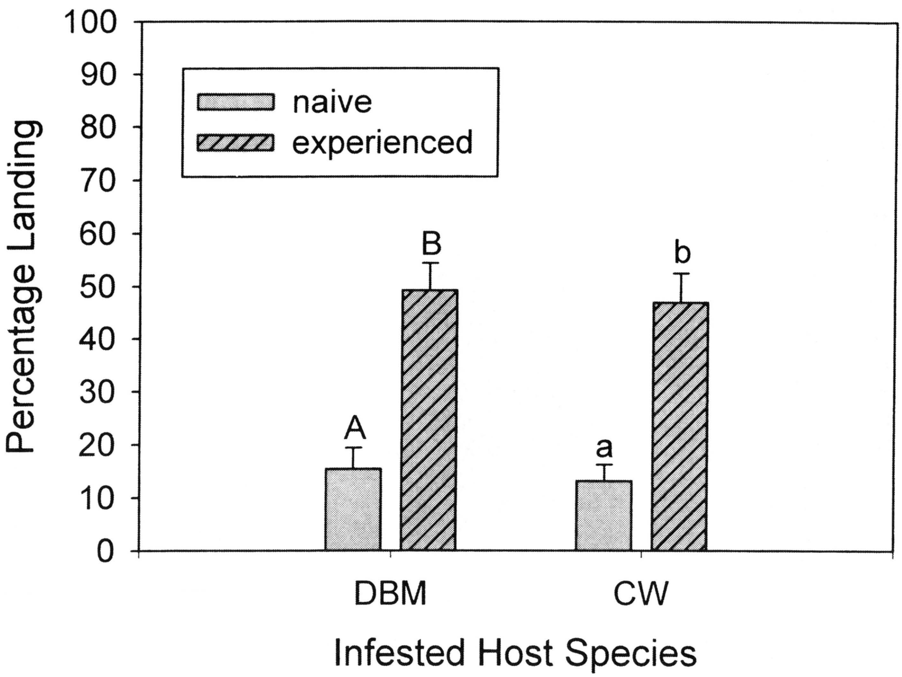

Fig. 2. Mean (±SE) percentage of female *Diadegma insulare* responding to a collard plant with upwind-oriented flight and landing on plants damaged by feeding of host (DBM) and nonhost (cabbage looper, CL) larvae. Different letters above bars indicate significant differences at $P < 0.05$).

The experience *D. insulare* receives probably is due, at least partly, to the volatile components of crucifers. Some crucifer-released chemicals have been shown to attract wasps of crucifer-feeding pests. For example, allylisothiocyanate attracts a braconid parasitoid of crucifer-feeding aphids (Tumlinson and Lewis 1991) and β-glucosidase attracts a braconid parasitoid of crucifer-feeding caterpillars (Mattiacci et al. 1995).

Associative learning of *D. insulare* could be an important component of biological control of the diamondback moth. The ability of female wasps to locate and attack hosts is a key determinant of how well a given parasitoid population performs (Lewis et al. 1990). Parasitoids reared on hosts on artificial diet have no experience with natural host odors. When they are released into fields, the wasps may not forage effectively. Experienced wasps, however, may search for their hosts immediately after release, because they have experience. Our current project includes manipulation of *D. insulare* populations for suppression of diamondback moth populations in cabbage. We have successfully colonized this parasitoid in the laboratory (Sieglaff et al. 1998) and in field cages (Hu et al. 1998). Before releasing the reared wasps into cabbage fields, we now condition adult *D. insulare* by exposing them to plant-leaves with diamondback moth feeding.

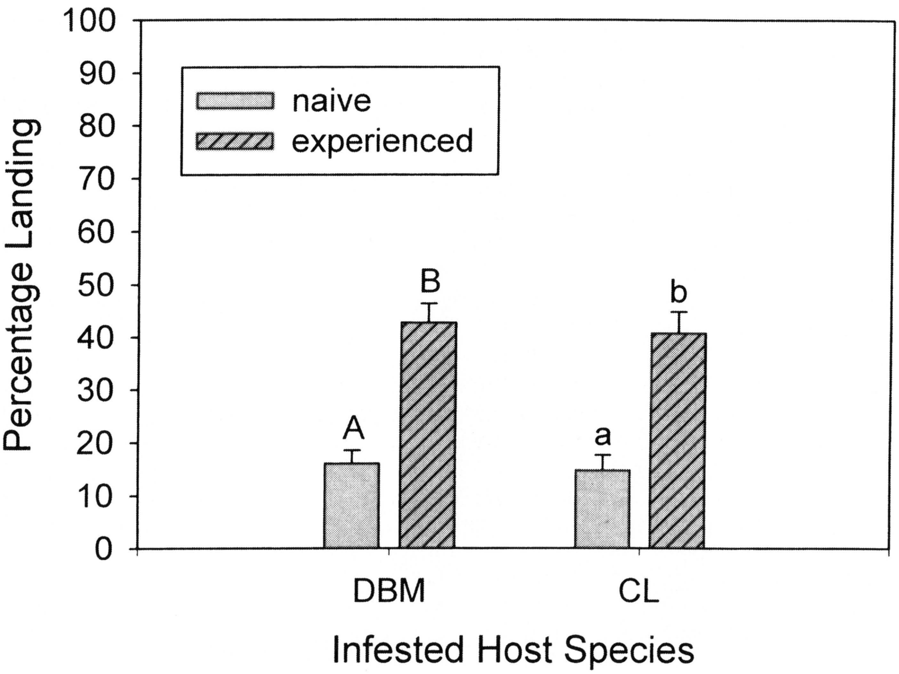

Fig. 3. Mean (±SE) percentage of female *Diadegma insulare* responding to a collard plant with upwind-oriented flight and landing on plants damaged by feeding of host (DBM) and nonhost (imported cabbageworm, CW) larvae. Different letters above bars indicate significant differences at $P < 0.05$.

Associative learning of odors from nonhost larval feeding also may provide another benefit to biological control programs. When diamondback moth populations are low in cabbage fields, the plant odor caused by nonhost larval feeding (cabbage loopers or imported cabbageworms) may prevent an immediate dispersal of *D. insulare* by eliciting searching behavior for the diamondback moth larvae in the release field.

Although the chemical nature of the host searching stimulant is unknown, we do know that we can use the odors emitted from larval infested plant material in a practical biological control program for the diamondback moth in cabbage. Further studies are under way to evaluate the potential in other natural enemies of crucifer pests.

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