

## PERFORMANCE OF NATURAL ENEMIES REARED ON ARTIFICIAL DIETS

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### INTRODUCTION

Augmentative or inundative releases of parasitoids or predators as a tactic to suppress pest species is often constrained by the costs of insect rearing (Greany *et al.*, 1989). For most natural enemies, current production methods require rearing the host species on artificial diet and using these insects to rear the natural enemy. As such, additional expenses are incurred because several insect colonies must be reared simultaneously and because additional laboratory space, labor, and materials are required.

*Diapetimorpha introita* (Cresson) (Hymenoptera: Ichneumonidae) is a native pupal ectoparasitoid of the fall armyworm, *Spodoptera frugiperda* (J.E. Smith), and the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (Pair and Gross, 1984). The biology and laboratory rearing of *D. introita* were reviewed by Pair (1995). In nature, female parasitoids search the soil surface until they locate a host pupation site. Kairomones associated with the mandibular silk used by the host larva to construct the cocoon facilitate the location and identification of suitable oviposition sites by *D. introita* females (J.E.C., unpublished data). Once the wasps determine the boundaries of the host pupation site, they insert their ovipositor through the soil and lay an egg in the proximity of the host pupa. Upon hatching, the parasitoid larva locates the pupa and begins feeding through its integument. Later instars invade the host pupa. Once larval development is completed, the parasitoid pupates in the host pupal gallery. Adult wasps exit the gallery through the soil cap.

Greany and Carpenter (1996) developed an artificial medium devoid of insect components for laboratory rearing of *D. introita*, and Carpenter and Greany (1998) showed that medium-reared *D. introita* searched for and parasitized host pupae in field-cage experiments. These authors suggested that the ability to rear *D. introita* on an inexpensive artificial medium significantly enhanced the possibility of mass rearing this parasitoid for use in inundative releases against *Spodoptera* spp., especially against low density populations in overwintering habitats.

Host location and host acceptance by female parasitoids can be influenced by maternal factors such as age (Doutt, 1959), physiological state (Vinson, 1976), and previous experience (Vet and Groenewold, 1990), and by host factors such as host species, host age, and host size (Price, 1970; Schmidt, 1974; Vinson, 1976). Many studies have examined the role of kairomones on the location and acceptance of hosts by female parasitoids. However, until the development of effective artificial rearing systems, laboratory-reared parasitoids completely lacking in host kairomone conditioning, both as developing immatures and as adults, were not available.

In the present study we examined the role of kairomone exposure on host acceptance and selection by *D. introita* females. Naïve and kairomone-exposed *D. introita* females were given a choice of oviposition sites, and comparisons were made between oviposition on various natural host species and between the natural hosts and a nonhost species, the corn earworm, *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae). Kairomone exposure was accomplished by either rearing the parasitoid *in vivo* on host pupae (preimaginal), or by placing host kairomones in the cage where

the parasitoids were emerging (imaginal), while exposure to kairomones was prevented by rearing the parasitoids on artificial medium.

## Materials and Methods

*Diapetimorpha introita*. The laboratory colony was started in 1992 from field-collected material and wasps were routinely reared on pupae of the fall armyworm, *S. frugiperda*. Adult wasps were maintained in screen cages (29 x 27.2 x 16.3 cm) at  $22 \pm 0.2^\circ\text{C}$ , ca 40% relative humidity, and ambient photoperiod. Cages were supplied with water and a 10% honey solution. Fall armyworm pupae in soil were provided for oviposition. *Diapetimorpha introita* larvae were reared in a growth chamber at  $29^\circ\text{C}$ , 70% r. h. and 0L: 24D photoperiod. Wasp pupae were placed in the screen cages before adult wasp emergence.

Before the initiation of our studies, colony eggs of *D. introita* were placed in rearing cages in the proximity of fall armyworm or beet armyworm pupae, or directly on host-free artificial medium (Greany and Carpenter, 1996) and reared through to adulthood for one generation. Four-day old *D. introita* females reared on fall armyworm, beet armyworm, or artificial medium were collected and exposed to host kairomone treatments as shown in Table 1.

**Table 1.** Methods of rearing and host kairomone exposure for *Diapetimorpha introita* larvae and adults.

| Species to whose kairomone the adult parasitoid was exposed | Species or Medium on which Larval Parasitoids were Reared |                    |                   |
|---|---|--------------------|-------------------|
|   | Fall Armyworm Host  | Beet Armyworm Host | Artificial Medium |
| Fall Armyworm (A)   | A1  | A2                 | A3                |
| Beet Armyworm (B)   | B1  | B2                 | B3                |
| Corn Earworm (C)  | -   | -                  | C3                |
| None (D)  | D1  | D2                 | D3                |

Subgroup A females were exposed to fall armyworm kairomones by exposing adults to mandibular silk of fall armyworm for 24 h. Subgroup B females were exposed to beet armyworm kairomones and subgroup C females were exposed to corn earworm kairomones for 24 h in the same manner. Subgroup D females were left naïve for 24 h, that is, they received no post-emergence exposure to host kairomones. Wasps from selected treatment groups were used in laboratory and field studies as described below.

*Lepidoptera*. The four species of Noctuidae used in these studies came from laboratory-adapted colonies maintained on a pinto bean diet similar to the one described in Burton (1969) for rearing *Heliothis* spp. Mature larvae of the fall armyworm, beet armyworm, corn earworm, and tobacco budworm (*Heliothis virescens* [F.]), were used as described below.

### Experiment 1. Larval Trail Study

Laboratory observations were made to determine the ability of *D. introita* females to follow host larval trails and detect host pupation sites for several noctuid species. A large plastic container (530 × 380 × 130 cm) was filled with sifted sterilized topsoil to a depth of 80 cm and covered with Plexiglas. For each trial a single fifth instar larva of fall armyworm, beet armyworm, corn earworm, or tobacco budworm was placed on top of the soil in the center of the container. A black marker was used to trace larval movements on the Plexiglas until the larva burrowed into the soil to pupate. Two days after the larva burrowed into the soil, a mated, five-day old *D. introita* female reared on fall armyworm was placed in the center of the container and allowed to search the soil surface. Parasitoid movement, antennation, and searching behaviors were recorded on the Plexiglas with a marker pen. The observation period for each parasitoid ended when oviposition occurred or when the wasp became inactive.

### Experiment 2. Host Preference Laboratory Study

A plastic container (20 × 20 × 4.5 cm) was fitted with a stiff cardboard lid with two circular openings spaced 4 cm from each other to hold small plastic cups (30 ml). Non-waxed butcher paper was used to cover the cardboard and was changed at the end of each observation period. A second plastic container of the same dimensions was inverted on top of the cardboard lid and used as an observation chamber. Plastic cups were filled with sifted sterilized topsoil, and mature larvae of fall armyworm, beet armyworm, and corn earworm were placed on the soil and allowed to pupate. One-day old pupae were used in the host preference study according to treatment requirements. The species combinations used were (1) fall armyworm vs. beet armyworm; (2) fall armyworm vs. corn earworm pupae. In the first combination, fall armyworm is the preferred and beet armyworm the less preferred host of *D. introita*. In the second combination corn earworm is a nonhost species for *D. introita*. A total of 20 five-day old *D. introita* females from each of the 10 treatments in Table 1 were released individually into the observation chamber and observed until oviposition occurred. Wasp behaviors recorded included antennation at pupation sites and sequence of sites visited by each parasitoid during each observation period. Once oviposition occurred, female parasitoids were collected and cups with pupae were examined to verify whether oviposition had taken place. New cups with pupae were used for each replicate.

### Experiment 3. Field-Cage Study

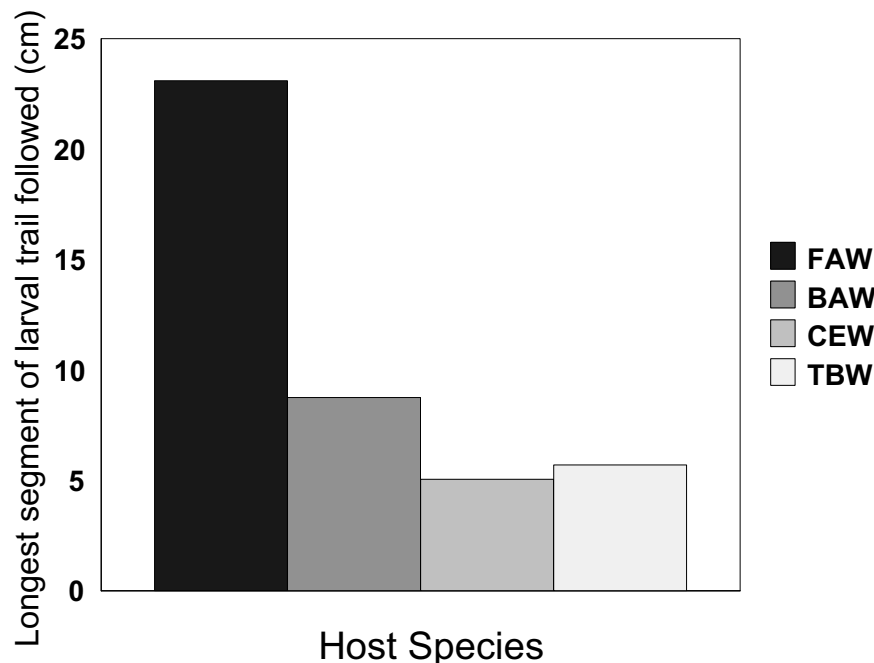
A 2 ha field at the USDA station in Tifton, Georgia was planted with corn, *Zea mays* (L.) (Pioneer 3167) in April 1999 and again in April 2000. When corn plants reached the whorl stage (1.22 m in height, ca 60 d after planting), 40 screen field-cages (1.83 × 1.83 × 1.83 cm) (Chicopee, Gainesville, Georgia) were placed in the field. Distance between each field cage was no less than 8 m. Each cage enclosed a total of 12 corn plants (six plants per row in two rows, with 5-10 cm between plants and 60 cm between rows). Cages were secured to the ground using wooden stakes and bottom edges along each side were buried in the soil. Inside the cage all weeds were removed by hand and a wooden board (0.60 × 3.65 × 7.30 m) was placed between the rows to allow easy access to the corn plants without disturbing the soil.

The six corn plants in the left row of each cage were infested with beet armyworm larvae, while those in the right row were infested with fall armyworm larvae. Three 4<sup>th</sup>-5<sup>th</sup> instar larvae of each species were placed in the whorl of each plant. Three days after initial infestation with larvae, three treatments and a control were randomly assigned to the field cages. Control cages received no other insects (n=10). Ten cages received naive medium-reared *D. introita* wasps (see Table 1, treatment D<sub>3</sub>). Ten cages received medium-reared *D. introita* that had been exposed to fall armyworm kairomones (Table 1, treatment A<sub>3</sub>) for 24 h. The last ten cages received medium-reared *D. introita* that had been exposed to beet armyworm kairomones (Table 1, treatment B<sub>3</sub>) for 24 h. Two 7-10-day old female wasps were released into each field cage according to the treatments assigned. After four days, released *D. introita* females were collected from each cage. All cages were checked daily for an additional 25 days, and emerging insects (wasps, moths, both) were collected and identified to species. The number of moths emerging in each cage was used to assess the ability of *D. introita* females reared on artificial medium to search for and parasitize fall armyworm and beet armyworm in field cages.

## RESULTS AND DISCUSSION

### Experiment 1. Larval Trail Study

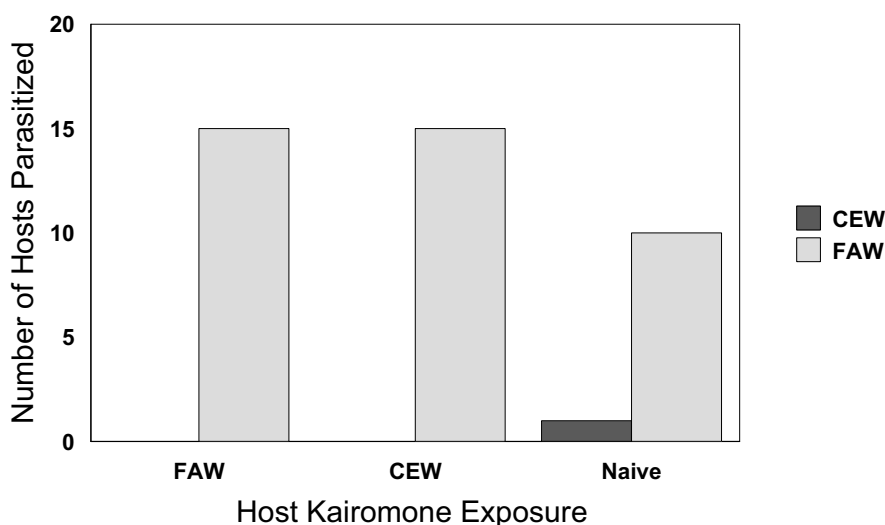
*Diapetimorpha introita* female wasps reared on fall armyworm pupae were able to detect larval trails on the soil surface and follow these trails for all of the noctuid larvae in our study. However, the percentage of *D. introita* antennations that occurred on selected larval trails was greatest for trails of fall armyworm. Also, the greatest distance followed by *D. introita* on particular larval trails occurred when the trails were those of fall armyworm, indicating that *D. introita* preferred fall armyworm over beet armyworm as a host (Fig. 1).



**Figure 1.** Mean length of longest segment of larval trail that *Diapetimorpha introita* females (reared on FAW) followed for four noctuid species: FAW (*Spodoptera frugiperda*), BAW (*Spodoptera exigua*), CEW (*Helicoverpa zea*), and TBW (*Heliothis virescens*).

## Experiment 2. Host Preference Laboratory Study

The oviposition preference of *D. introita* between a host fall armyworm and non-host corn earworm pupation site was not influenced by exposure of adult parasitoids to host or non-host kairomones (Fig. 2). Although fewer *D. introita* oviposited when they were naïve than when they were exposed to host kairomones, the host fall armyworm was still preferred over the non-host corn earworm. Likewise, the oviposition preference of *D. introita* between two hosts (fall armyworm and beet armyworm) was not influenced by preimaginal (Table 1, A<sub>1</sub>, B<sub>1</sub>, A<sub>2</sub>, B<sub>2</sub>, D<sub>1</sub>, D<sub>2</sub>) or imaginal (Table 1, A<sub>3</sub>, B<sub>3</sub>) exposure to host kairomones. *Diapetimorpha introita* female wasps reared on fall armyworm pupae, beet armyworm pupae, or artificial medium all preferred to oviposit in the fall armyworm pupation sites.



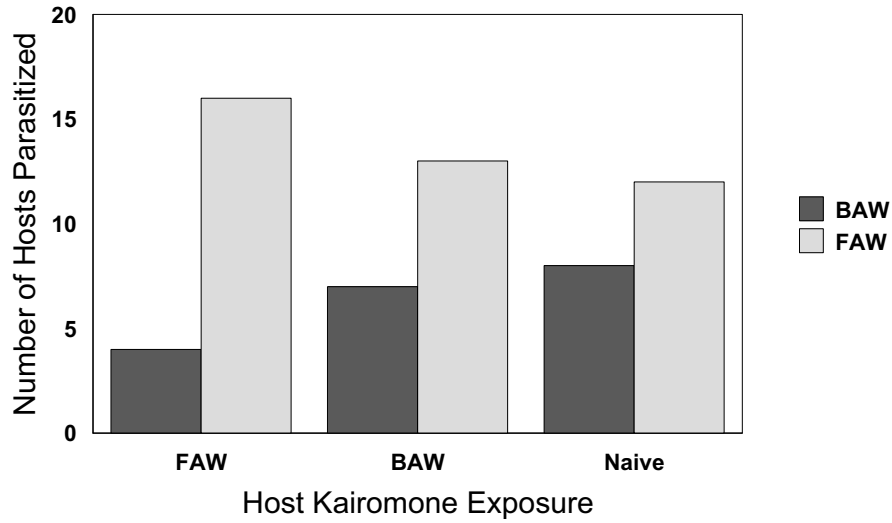
**Figure 2.** Oviposition of medium reared, naïve *Diapetimorpha introita* females versus medium reared *D. introita* with a 24 h adult parasitoid exposure to FAW or CEW kairomone, when given a choice of a preferred host *Spodoptera frugiperda* (FAW) or a non-host *Helicoverpa zea* (CEW).

This oviposition preference for fall armyworm was greatest when *D. introita* were reared on medium and exposed to fall armyworm kairomones, and least when *D. introita* were reared on medium and exposed to no kairomones (naïve) (Fig. 3).

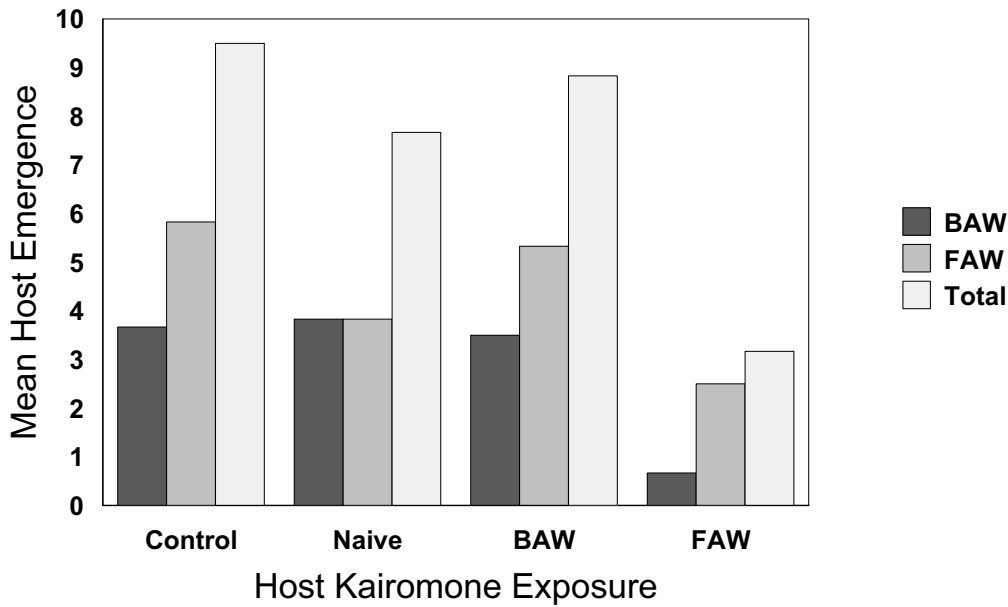
## Field-Cage Study

The influence of *D. introita* females on the emergence of fall armyworm and beet armyworm was greatest when female wasps were exposed to fall armyworm kairomones before release into the cages (Fig. 4). Naïve wasps and wasps exposed to beet armyworm kairomones were not effective in parasitizing host pupae. Wasps exposed to beet armyworm kairomones did not demonstrate an increased preference for beet armyworm as a host. However, *D. introita* exposed to fall armyworm kairomones attacked fall armyworm and beet armyworm equally, reducing the mean host emergence by more than 65%.

Hymenopteran pupal ectoparasitoids can be reared successfully on artificial diets devoid of host material and host products (Carpenter and Greany, 1998) and thereby reduce the costs required for mass production. However, it is important that diet-reared wasps exhibit normal behavior in their propensity and ability to search for and parasitize natural hosts in the field. Also,



**Figure 3** Oviposition of kairomone-exposed and naive *Diapetimorpha introita* females reared on artificial medium when given a choice between a preferred host, *Spodoptera frugiperda* (FAW), and a non-preferred host, *Spodoptera exigua* (BAW).



**Figure 4.** Numbers of moths from a preferred host, *Spodoptera frugiperda* (FAW), and a non-preferred host, *S. exigua* (BAW), emerging in field cages stocked with parasitoids with either kairomone exposure or no exposure (artificial-medium-reared *Diapetimorpha introita* females) versus moth emergence in control cages not stocked with parasitoids.

it might be useful if the host preference of parasitoids reared on artificial media could be manipulated through preimaginal and imaginal exposure to host kairomones before release. Although both preimaginal and imaginal conditioning of parasitoid can influence ovipositional responses of wasps (Thorpe, 1938; Arthur, 1981), *D. introita* host preferences were not significantly affected by the rearing medium (artificial diet or host pupae) or the host kairomones (or lack of host kairomones) to which the wasps were exposed. Also, our results indicate that there is limited opportunity to manipulate the host preference of *D. introita* by preimaginal or imaginal exposure to host or non-host kairomones. Nevertheless, wasps reared on artificial medium were effective in searching for and parasitizing hosts in field cages, and increased their rate of parasitism when they were exposed to host kairomones before release.

## REFERENCES

- Arthur, A. P. 1981. Host acceptance by parasitoids, pp. 97-120. In Nordlund, D., R. L. Jones, and W. J. Lewis (eds.). *Semiochemicals, Their Role in Pest Control*. Wiley, New York.
- Burton, R. L. 1969. Mass rearing the corn earworm in the laboratory. *USDA-Agricultural Research Services Series* 33-134.
- Carpenter, J. E. and P. D. Greany. 1998. Comparative development and performance of artificially reared versus host-reared *Diapetimorpha introita* (Cresson) (Hymenoptera: Ichneumonidae) wasps. *Biological Control* 11: 203-208.
- Doutt, R. L. 1959. The biology of parasitic Hymenoptera. *Annual Review of Entomology* 4: 161-182.
- Greany, P. D. and J. E. Carpenter. 1998. Culture medium for parasitic and predaceous insects. *U.S. Patent No. 5799607*.
- Greany, P. D., S. M. Ferkovich, and W. R. Clark. 1989. Progress towards development of an artificial diet and an *in vitro* rearing system for *Microplitis crocipes*. *Southwestern Entomologist* 12: 89-94.
- Pair, S. D. 1995. Biology and rearing of *Diapetimorpha introita* (Cresson) (Hymenoptera: Ichneumonidae) on host and non-host noctuid pupae. *Journal of Entomological Science* 30: 468-480.
- Pair, S. D. and H. R. Gross. 1984. Field mortality of pupae of the fall armyworm, *Spodoptera frugiperda* (J. E. Smith), by predators and a newly discovered parasitoid, *Diapetimorpha introita*. *Journal of Georgia Entomological Society* 19: 22-26.
- Price, P. W. 1970. Trail odors: Recognition by insects parasitic on cocoons. *Science* 170: 546-547.
- Schmidt, G. T. 1974. Host-acceptance behavior of *Campolites sonorensis* toward *Heliothis zea*. *Annals of Entomological Society of America* 67: 835-844.
- Thorpe, W. H. 1938. Further experiments on olfactory conditioning in a parasitic insect. The nature of the conditioning process. *Proceedings of the Royal Society of London, B, Biological Sciences* 126: 370-397.
- Vet, L. E. M. and A. W. Groenewold. 1990. Semiochemicals and learning in parasitoids. *Journal of Chemical Ecology* 16: 3119-3135.
- Vinson, S. B. 1976. Host selection by insect parasitoids. *Annual Review of Entomology* 21: 109-133.