EVALUATION OF REARING METHODS FOR *DIADEGMA INSULARE* (HYMENOPTERA: ICHNEUMONIDAE), AN ENDOPARASITOID OF THE DIAMONDBACK MOTH (LEPIDOPTERA: PLUTELLIDAE)

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The diamondback moth, *Plutella xylostella* (L.), is the most important insect pest of cruciferous plants worldwide (Talekar & Shelton 1993). In North America, the diamondback moth typically has been controlled by using synthetic insecticides, but with resistance to commonly used insecticides developing in the mid-1980's (Shelton et al. 1993, Talekar & Shelton 1993), IPM practices for controlling this pest have been adopted (Biever et al. 1994, Leibee 1996). The augmentative release of natural enemies can be an important component to any IPM program, and parasitoids are considered to be essential to any such program for diamondback moth management (Talekar & Shelton 1993). The release of parasitoids for control of diamondback moth has been conducted in many geographic regions (Talekar & Shelton 1993), including North America (Biever et al. 1994, Leibee 1996, Mitchell et al. 1997b). The larval endoparasitoid, *Diadegma insulare* (Cresson), can be a very important natural control of diamondback moth in North America (Lasota & Kok 1986, Idris & Grafius 1993, Muckenfuss et al. 1992) and offers the greatest potential as a biocontrol agent among larval parasitoids of this pest (Idris & Grafius 1993).

Our laboratory has been developing various strategies to combat diamondback moth populations in cabbage growing regions of Florida (Adams 1994), and *D. insulare* could be an important addition to this program. However, *D. insulare* has been exceedingly difficult to maintain in culture. The purpose of the current study was to develop an effective and economically feasible rearing method using the natural insect host coupled with an artificial diet to produce *D. insulare* in sufficient quantities for field release.

Diamondback moth (originally received from Juliet Tang, Cornell University, New York) was reared on a wheat germ-based artificial diet (Shelton et al. 1991), in 0.23 liter paper cups (Sweetheart VS508, Chicago, IL) covered with a Kimwipe (Kimberly-Clark EX-L, Atlanta, GA) and overlaid with a perforated plastic lid (Sweetheart, LS8). Adult diamondback moths were maintained in 30 × 30 × 30 cm mating cages, fed a 10% honey-water solution in small paper cups provisioned with a saturated cotton ball, and provided with several pieces of wrinkled aluminum foil (12.7 × 8.89 cm) treated with collard extract (see description below) as oviposition substrates. The diamondback moth prefers uneven surfaces on which to oviposit. The diamondback moth colony was maintained at 25°C, 50% RH and a photoperiod of 12:12 h (L:D).

D. insulare (collected from Bunnell, Florida, May 1996) were reared on 2 - 3rd instar diamondback moth larvae that had been placed on freshly harvested collard leaves. Following 24 h of exposure to *D. insulare*, host larvae were transferred to an 88 × 30 × 17 cm plastic pan and fed fresh collard leaves ad libitum until pupation. Leaves harboring pupae were placed into emergence cages (30 × 30 × 30 cm), and eclosed *D. insulare* were fed 10% honey-water solution. All *D. insulare* were maintained under a 12:12 h L:D cycle at 25° C and 50% RH.

To prepare collard extract (CE), collard leaves were harvested from a small field plot maintained at our laboratory, blended with water at the rate of 0.071 g/ml using a household blender (Hamilton Beach®, St. Louis, MO.), and filtered through a fine organdy mesh. The CE was used to produce "treated" aluminum foil oviposition substrate, "sting-cups" and amended artificial diet. Aluminum foil was crumpled to create ridges and depressions, straightened, dipped into CE, and allowed to dry overnight to produce "treated" aluminum foil for use as oviposition substrates for diamondback moth and as a possible stimulant source for *D. insulare*. CE also was mixed into cool (< 110° C) wheat germ-based artificial diet to produce 10 and 23% CE diet. "Sting-cups" were produced by impregnating 0.47—liter paper cups (James River 2186, Norwalk, CT) with CE. The cups were filled with 500 ml CE, held for 5 min, emptied-out, and allowed to dry overnight. All substrates were exposed to UV radiation for approximately one hour to sterilize their surfaces.

To determine if the CE would stimulate parasitism by *D. insulare*, five treatments were compared: artificial diet, artificial diet contiguous with "treated" aluminum foil, artificial diet with 10 and 23% CE, and a collard leaf control (as described above for *D. insulare* rearing). Each diet cake (7.6 cm diam and 2.5 cm thickness) was infested with 2nd-3rd instar diamondback moth larvae (200-450) taken from our laboratory colony. The larvae were placed on top surface of the diet cake from where they then spread to all the surfaces. The "treated" aluminum foil was created as previously described except that they were circular (14 cm diam) in shape rather than rectangular. A diet cake without CE was attached over a piece of circular foil to one leg of an A-frame structure (15 × 13 cm) made from five-mm hardware cloth. This arrangement allowed for exposure of all sides of the cake to diamondback larvae and *D. insulare* as the ridges in the foil permitted larvae and parasitoids to crawl between the two surfaces. The A-frames with larvae were placed individually in 5 liter plastic containers (Tristate Plastics 289 NL, Dixon, KY) and covered with a fine organdy mesh. The collard leaf control was also set-up on A-frames with an equivalent number of diamondback moth larvae (this treatment was meant to mimic current rearing procedures). The number of diamondback moth larvae in each treatment was determined, and a diamondback moth larvae: *D. insulare* ratio of 50:1 was used for each treatment (♂:♀ *D. insulare* ratio of 2:1, 24-48 h old). The *D. insulare* were aspirated into each treatment and allowed to oviposit for 24 h. To determine if a longer period of exposure to parasitism would enhance parasitoid production, two additional treatments were tested. Artificial diet cakes without CE were placed atop a U-shaped hardware cloth (5 mm) frame in 0.47 liter paper cups (James River 2186). One set of cups was untreated, i.e., without CE, and the other set was treated by impregnating the interior surface with CE as described for "sting cups." Each cup was infested with larvae as described, and the cups were exposed to *D. insulare* for 48 h. All treatments were maintained at 25° C, 50% RH and 12:12 h (L:D), and each had 11 replications over time.

Measurements were standardized because the number of diamondback moth larvae differed among replicates. The measurements were total *D. insulare* adults produced per *D.* insulare, ♀ *D. insulare* adults produced per *D. insulare*, percentage parasitism (i.e., total emerged *D. insulare* / [total emerged *a D. insulare* + total emerged diamondback moth adults] × 100), and sex ratio. Numbers produced were transformed by square root (n+1) and percentage parasitism was transformed by the square root of the arcsine before analysis. The data were subjected to analysis of variance, and means were separated by Duncan's multiple range test (SAS Institute 1989).

The collard leaf control produced more *D. insulare* adults and had a higher percentage parasitism than any other treatment (Table 1). Among all treatments using artificial diet, the 48 h artificial diet + "sting-cups" and 10% collard extract diet produced

TABLE 1. PRODUCTION OF PARASITOIDS PER DIADEGMA INSULARE _ AND PERCENTAGE PARASITISM OF DIAMONDBACK MOTH LARVAE (= TOTAL % *D. INSULARE* ON VARIOUS DIETS.[1]

Treatment	Total *D. insulare* / *D. insulare* ♀ (SE)	♀ *D. insulare* / *D. insulare*♀ (SE)	Total % *D. insulare* (SE)	Sex Ratio ♂: ♀
Artificial diet	11.6 (3.0) bc	3.1 (1.1) bc	19.6 (4.6) c	3.9:1
Artificial diet + "treated" foil	12.7 (2.5) bc	3.2 (0.7) abc	28.6 (4.6) bc	3.5:1
10% Collard extract diet	15.3 (2.4) b	3.9 (1.2) abc	38.2 (4.8) bc	3.3:1
23% Collard extract diet	6.8 (2.0) c	1.2 (0.6) c	22.9 (4.4) bc	5.4:1
48 hr artificial diet	11.1 (2.2) bc	3.8 (0.9) abc	27.1 (5.0) bc	2.3:1
48 hr artificial diet + "sting-cup"	19.7 (4.3) b	7.7 (2.0) a	46.6 (8.8) b	2.3:1
Collard leaf control	32.0 (3.8) a	5.2 (1.6) ab	77.2 (4.5) a	4.6:1

[1]Column numbers with the same letter are not significantly different ($P < 0.05$, Duncan's multiple range test).

more *D. insulare*, and the 48 h artificial diet + "sting-cups" had a higher percentage parasitism. However, with the exception of 23% CE, the collard leaf control did not produce more ♀*D. insulare* adults than any other treatment. This suggests that a longer exposure period coupled with the use of collard extract may enhance parasitoid production. Therefore, diet treated with collard extract plus a longer exposure of the parasitoid and in larger chambers may be the best combination for mass-production of *D. insulare*, especially when the plant material is not continuously supplied.

The sex ratio of *D. insulare* varied among treatments (i.e., 2.3:1 - 5.4:1 (♂:♀)) (Table 1). The treatments with longer exposure of the parasitoid to the diet had a sex ratio of 2.3:1 which is similar to that usually found in our rearing facility (2:1 (♂:♀)), where parasitoids are allowed to fly freely about in a 3.04 m long × 2.13 m wide × 2.13 m high incubation chamber. The other treatments, however, produced undesirable ratio of ♂:♀ *D. insulare* (3.3:1-5.4:1) which is different from that reported to occur in field populations (Idris & Grafius 1993, Mitchell et al. 1997a) and our rearing facility. Lower parasitism of diamondback moth larvae by *D. insulare* compared with the standard rearing using plant materials may be due to the larvae tunneling into the diet cake preventing attack by the parasitoid. Host quality also may be a factor responsible for reduced reproduction of *D. insulare* females. For example, *D. insulare* responds to sub-optimal nitrogen levels in their host's diet with reduced levels of parasitism and highly male-biased sex ratios (Fox et al. 1990). Sex ratios in other ichneumonids are affected by various factors, such as host size and density (Sandlan, 1979), which also may be important in the biology of *D. insulare.* Further studies of these factors in the *D. insulare* rearing program is currently under way in efforts to improve the rearing of this parasitoid for augmentative release.

Summary

Experiments showed potential for rearing *D. insulare* on diamondback moth-infested artificial diet cakes when a possible stimulant, collard extract, was provided along with allowing adequate time for parasitism to occur. Parasitism increased from 19% in the artificial diet treatment to 46% in the 48 h artificial diet plus collard extract impregnated sting-cup treatment. However, this still was not as productive as using larvae placed on collard leaves (77%). The treatments with longer exposure time (48 h) of the parasitoid to diet produced a sex ratio similar to that found in our rearing facility (about 2:1 ♂:♀). The sex ratio of *D. insulare* produced from the other treatments were heavily biased towards males, a situation that is much different from that which occurs in nature.

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References Cited

ADAMS, S. 1994. Assault on the senses: pheromone overload is first in three-pronged attack on crop pests. Agr. Res. July 1994: 4-7.

BIEVER, K. D., D. L. HOSTETTER, AND J. R. KERN. 1994. Evolution and implementation of a biological control-IPM system for crucifers: a 24-year case study. American Entomol. 40: 103-108.

FOX, L. R., D. K. LETOURNEAU, J. EISENBACH, AND S. VAN NOUHUYS. 1990. Parasitism rates and sex ratios of a parasitoid wasp: effects of herbivore and plant quality. Oecologia 83: 414-419.

IDRIS, A. B., AND E. GRAFIUS. 1993. Field studies on the impact of pesticides on the diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) and para-

sitism by *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae). J. Econ. Entomol. 86: 1196-1202.

LASOTA, J. A., AND L. T. KOK. 1986. *Diadegma insularis* (Hymenoptera: Ichneumonidae) parasitism of the diamondback moth (Lepidoptera: Plutellidae) in Southwest Virginia. J. Entomol. Sci. 21: 237-242.

LEIBEE, G. L. 1996. Insect pest management in crucifers in Florida, pp. 413-422 *In* D. Rosen, F. D. Bennett and J. L. Capinera [eds.] Pest Management in the subtropics: integrated pest management - a Florida perspective. Intercept Limited, Andover, United Kingdom.

MITCHELL, E. R., G. Y. HU, AND J. S. OKINE. 1997a. Diamondback moth (Lepidoptera: Plutellidae) infestation and parasitism by *Diadegma insulare* (Hymenoptera: Ichneumonidae) in collards and adjacent cabbage fields. Florida Entomol. 80: 54-62.

MITCHELL, E. R., F. C. TINGLE, R. C. NAVASERO-WARD, AND M. KEHAT. 1997b. Diamondback moth (Lepidoptera: Plutellidae): parasitism by *Cotesia plutellae* (Hymenoptera: Braconidae) in cabbage. Florida Entomol. 80: 477-489.

MUCKENFUSS, A. E., B. M. SHEPARD, AND E. R. FERRER. 1992. Natural mortality of diamondback moth in coastal South Carolina, pp. 27-36 In N. S. Talekar [ed.] Diamondback moth and other crucifer pests: proceedings of the second international workshop, Tainan, Taiwan, 10-14 December 1990. Asian Vegetable Research and Development Center, AVRDC Publication No. 92-368, 603 pp.

SANDLAN, K. 1979. Sex ratio regulation in *Coccygomimus turionella* Linnaeus (Hymenoptera: Ichneumonidae) and its ecological implications. Ecol. Entomol. 4: 365-378.

SAS INSTITUTE. 1989. SAS/STAT user's guide, version 6.08. SAS Institute, Cary, North Carolina.

SHELTON, A. M., R. J. COOLEY, M. K. KROENING, W. T. WILSEY, AND S. D. EIGENBRODE. 1991. Comparative analysis of two rearing procedures for diamondback moth (Lepidoptera: Plutellidae). J. Entomol. Sci. 26: 17-26.

SHELTON, A. M., J. A. WYMAN, N. L. CUSHING, K. APFELBECK, T. J. DENNEHY, S. E. R. MAHR, AND S. D. EIGENBRODE. 1993. Insecticide resistance of diamondback moth (Lepidoptera: Plutellae) in North America. J. Econ. Entomol. 86: 11-19.

TALEKAR, N. S., AND A. M. SHELTON. 1993. Biology, ecology, and management of the diamondback moth. Annv. Rev. Entomol. 38: 275-301.