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ABSTRACT Augmentative release of the endoparasitoid *Cotesia plutellae* (Kurdjumov) to control diamondback moth, *Plutella* xylostella (L.), in cabbage, *Brassica oleracea* variety *capitata* (L.), would be expensive for growers if done continually during a growing season. A method for establishing released parasitoids would be very beneficial in the control of this pest. One method under consideration is to use sterile diamondback moth larvae deposited on 'nursery' collard plants as hosts for *C. plutellae* to allow the parasitoid to build up in numbers and spread into adjacent cabbage fields. Therefore, the ability of *C. plutellae* to accept and develop successfully in normal and sterile diamondback larvae was evaluated. *C. plutellae* does not discriminate between normal larvae and larvae from parents sterilized by gamma radiation, and both sets of larvae served as suitable hosts for the parasitoid. Parasitism, foliage consumption, and survivorship were similar for the 2 types of larvae. Adult female F_1 parasitoids developed from sterile diamondback larvae were as fit as those from normal larvae. In laboratory bioassays, sterile and normal diamondback larvae traversed similar distances before pupation. Field cage assays showed less distance traversed by both types of larvae compared with the laboratory studies. Survivorship for both types of larvae was very low under field conditions. Results indicate that sterile diamondback moth larvae are acceptable hosts for *C. plutellae* and suggest that the nursery approach toward establishment and build up of numbers would be a viable approach to in-field production of the parasitoid with little chance for harm to nearby cabbage because of spread of sterile larvae from the nursery plants. This approach could be a cost effective way to augment naturally occurring parasitoids and predators in diamondback moth management programs.

KEY WORDS *Plutella xylostella*, parasitoid, biological control, cabbage, insect pest management, spatial dispersion

THE DIAMONDBACK MOTH, *Plutellae xylostella* (L.), attacks brassicaceous-cruciferous crops worldwide, with annual control costs estimated at U.S. $1 billion (Talekar et al. 1986, Talekar and Shelton 1993). In Florida, serious economic damage results from their attack of cabbage (*Brassica oleracea* variety *capitata*) (Jansson and Lecrone 1988). Diamondback moth attacks cabbage from seedling to harvest stage, reducing yield quantity and quality. Plants tolerate varying amounts of damage depending on the maturity of the plant (Mahr et al. 1993); seedlings are generally much more susceptible to damage. Indigenous parasitoid populations are at low densities at the seedling stage and do not readily control early season populations of diamondback moth. Consequently, growers must resort to the use of chemicals to achieve diamondback moth control.

The development of resistance in diamondback moths to all major groups of insecticides, including insect growth regulators and *Bacillus thuringensis* ssp. *kurstaki*, is widespread (Sun et al. 1978, Miyata et al. 1982, Sun et al. 1986, Tabashnik et al. 1990, Shelton and Wyman 1992, Talekar and Shelton 1993, Tabashnik 1994). Insecticide resistance, destruction of beneficial insects, and environmental pollution from synthetic pesticides makes it important to evaluate alternative or improved management methods for diamondback moth control (Ooi and Sudderuddin 1978).

Proposed integrated pest management (IPM) strategies for diamondback moth include biological, chemical, and cultural control methods (Lim 1986, Waterhouse and Norris 1987, Biever et al. 1994). *Cotesia plutellae* (Kurdjumov) and *Diadegma insulare* (Cress) are 2 parasitoids used in diamondback moth control, with *C. plutellae* considered less effective than *D. insulare* (Parker 1971, Ru and Workman 1979, Horn 1987, Muckenfuss et al. 1992, Mitchell et al. 1997). Despite this, *C. plutellae* is a good candidate for augmentative control of diamondback moth because a mass-rearing technology has been developed for it; whereas, *D. insulare* is presently more difficult to mass rear.

The USDA-ARS has conducted field trials in Bunnell, Flagler County, FL, to evaluate the efficacy of a

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combination of control strategies including pheromones, trap cropping and augmentative release of *C. plutellae* to control diamondback moth in cabbage. To enhance the build up of released parasitoids in or near cabbage fields, a reservoir of diamondback moth larvae could be established on separate plantings of collard, *Brassica oleracea* variety *ocephala* (L.), just before the augmentative release of this parasitoid. This reservoir of diamondback moth larvae would preferentially be sterile. Sterility can be induced in larvae of the diamondback moth by exposing parent moths to substerilizing doses of gamma radiation (Sutrisno and Hoedaya 1993).

In this study we evaluated the suitability of sterile diamondback moth larvae as hosts, in terms of number of parasitoid progeny that successfully developed from the sterile and normal larvae when exposed to *C. plutellae*, and the fecundity of parasitoid F_1 offspring developed in sterile larvae. We also measured the distance moved by diamondback moth larvae under both laboratory and field conditions.

Materials and Methods

Normal Larvae. Diamondback moth larvae used were reared at the USDA–ARS facilities in Gainesville, FL, on modified pinto bean diet as described by Guy et al. (1985). The colony was 13 mo old.

Sterile Diamondback Moth Larvae. Sterile diamondback moth larvae were produced from the laboratory colony at the USDA facility in Tifton, GA, which originated from the Gainesville, FL, colony. To obtain sterile eggs, larvae were sexed and reared separately on 300 ml meridic diet in plastic boxes (20 by 20 by 8 cm) (Burton and Perkins 1989). Male moths (<24 h old) were exposed to 150 Gy radiation at 20°C using a well-type ^{60}Co source (Gammarad irradiator, model GR-12, U.S. Nuclear Division, Irvine, CA) delivering ≈46 Gy/min ± 5% (X-ray monitor probe calibration). Unirradiated virgin females were mated with irradiated males in Plexiglas cages. Grooved aluminum foils (12 by 12 cm) dipped in cabbage extract and air dried served as oviposition substrates. The egg foils were stored in a cooled Styrofoam container and shipped overnight to the USDA–ARS facilities in Gainesville, FL. The sterile larvae were cultured as described for the nonsterile larvae. All the larvae were maintained at a photoperiod of 14:10 (L:D) h, 22 ± 1°C, and 40–50% RH.

Parasitoid Rearing. *C. plutellae* (2–3 d old) were allowed to oviposit into diamondback moth 2nd instars. Parasitoid cocoons were placed in emergence cages in the absence of hosts or plants, so that the eclosing adults were unexposed to host or plant kairomones. Food, in the form of smeared honey and water soaked cotton balls, was provided for eclosing parasitoids.

Bioassays. *Oviposition Response of C. plutellae to Sterile and Normal Diamondback Moth Larvae.* The acceptability and suitability of sterile diamondback moth larvae as hosts for *C. plutellae* were examined. Five individually potted collard plants were each infested with either 20 normal or sterile 2nd instars. Collard plants are the preferred alternative host of diamondback moth. The plants each had 5–9 leaves. Larval transfers were done in an outdoor screened field cage (2.7 by 2.3 m) and infested plants were randomly arranged 30 cm apart in the cage and left for 6 h to allow larvae to acclimatize. After acclimatization, larvae were recounted and missing larvae replaced so that each plant harbored 20 larvae. Twenty-five mating pairs of *C. plutellae* (3 d old) were introduced into the cage for 48 h. Honey smeared on plastic petri dishes and water soaked cotton balls were provided for the parasitoids. After the 48-h oviposition period, larvae were counted, collected, and brought into the laboratory, where they were allowed to complete development. Foliage consumption was estimated by measuring leaf area consumed with a plastic mm^2 grid (Okine et al. 1996).

In the laboratory, larvae from each treatment were put into separate Plexiglas cages (25 by 25 by 25 cm) and held at 21 ± 3°C, 50–60% RH, and a photoperiod of 12:12 [L:D] h. The larvae were fed fresh collard leaves every 2 d until pupation of diamondback moth or *C. plutellae*. Mortality and proportion parasitized were recorded. Dead larvae were dissected to check for encapsulated parasitoids. There were 5 replicates per treatment, and the experiment was repeated 5 times.

Measurement of Leaf Consumption. The total amount of foliage consumed by diamondback moth larvae from hatching to pupation was estimated by transferring 20 newly hatched sterile and normal larvae onto individual potted collard plants that were maintained within the field cage. Foliage consumption was calculated using a mm^2 plastic grid under a dissecting microscope (Okine et al. 1996). This was repeated 10 times.

Evaluating Emergence Rate and Fitness of F_1 Parasitoids. Preeclosion time, percent eclosion, and sex ratio of parasitoids that developed from normal or sterile hosts were compared.

Fitness was determined in the following manner: 3 F_1 mated *C. plutellae* females from sterile and normal hosts were allowed to oviposit for 24 h into 20 late 2nd instars maintained on pinto bean diet in a modified paper cup. The proportion parasitized was obtained by counting the number of parasitoid pupae that developed. Parasitoid pupae obtained were allowed to eclose and adults emerging were sexed. This was repeated 10 times for each type of larvae.

Dispersal Pattern and Survival of Diamondback Moth Larvae in the Laboratory. The potential of sterile and normal diamondback moth larvae to migrate onto cabbage from collard plants was investigated. This was evaluated by measuring the local movement of diamondback moth larvae from hatch to pupation under laboratory and field conditions. Survival was calculated by dividing the number of pupae obtained by the initial egg number. Under laboratory conditions, a sheet of diamondback moth eggs was attached to the outermost leaf of a potted collard plant, and this plant was placed adjacent to 3 other collard plants to

form a continuum of leaves spanning 45–57 cm. Both sterile and nonsterile larvae were tested. The farthest distance moved by an individual larva was measured and recorded at 2-d intervals until pupation. The number of larvae that spun down on silk or fell from the plants were recorded. Percent survival also was determined. The trial was repeated 5 times for each type of larvae.

Dispersal and Survival in Field Cage Trials. Collard transplants were planted 50 cm apart in rows. The rows were separated by 105 cm. Three rows of plants, each with 22 plants per row, were enclosed within a fine mesh nylon screen supported by metal frames. One hundred sterile and normal larvae were used for each trial. The outermost leaf on each row was used as the point of infestation. The collard plants and larvae were monitored as described for the laboratory trial. This was repeated 2 times.

Data Analysis. Proportions of host parasitized per treatment were transformed by the arcsine transformation before analysis of variance (ANOVA) testing (SAS Institute 1988).

Results

Oviposition Response. There was no significant difference in proportion parasitism ($F = 0.17$, df = 1, 9; $P = 0.689$) between sterile larvae (37.6 ± 1.72% [mean ± SE]) and normal larvae (38.0 ± 1.38%). The proportion of larvae that survived did not differ significantly between the 2 groups for the 48-h period ($F = 0.24$, df = 1, 9; $P = 0.636$). Mortality was <10% per pot for both types of larvae. None of the dissected dead larvae contained an encapsulated parasitoid.

Foliage Consumption. Larval leaf consumption did not differ between sterile and normal larvae during the 48-h stinging period ($F = 0.16$, df = 1, 49; $P = 0.997$). Sterile larvae consumed 60 ± 0.68 mm^2 and normal larvae consumed 59 ± 4.7 mm^2.

Leaf consumption from hatch to pupation in insects unexposed to *C. plutellae* was significantly different between types of larvae ($F = 22.92$, df = 1, 19; $F < 0.001$). Sterile larvae consumed less foliage (387.9 ± 15.74 mm^2) than the normal larvae (475.3 ± 14.7 mm^2). Mortality of sterile larvae was significantly higher compared with normal larvae 45 ± 5.8% versus 33.5 ± 7.4% ($F = 31.17$, df = 1, 19; $P < 0.001$).

Emergence Rate and Fitness of F_1 Parasitoids. The period of development from hatch to pupation was 15 d for both types of larvae. The preeclosion time for *C. plutellae* pupae in both sets of larvae was 4 d, and percent adult emergence was 80.5 ± 8.8% for sterile larvae and 79.2 ± 6.3% for normal larvae ($F = 0.28$, df = 1, 9; $P = 0.611$). The sex ratio was 1.4:1 (females:males) for sterile larvae and 1.1:1 (females:males) for normal larvae. Percent parasitism obtained from F_1 *C. plutellae* was not significantly different between sterile and nonsterile larvae ($F = 0.11$, df = 1, 19; $P = 0.741$).

Dispersal Pattern and Survivability of Diamondback Moth Larvae. Under laboratory conditions the distance moved from point of egg attachment until pupation was not significantly different between sterile and normal larvae ($F = 0.16$, df = 1, 9; $P = 0.997$) under laboratory conditions. The maximum movement was 14 ± 1.7 cm and 12 ± 1.3 cm for sterile and normal larvae, respectively. Percent survival was not significantly different between normal (47 ± 1.2%) and sterile (43 ± 1.3%) larvae ($F = 0.28$, df = 1, 8; $P = 0.611$).

Under field conditions, the distance moved by sterile and normal larvae was 9 ± 1.3 cm and 13 ± 1.6 cm, respectively. Percent survival was 6 ± 1.0% and 15 ± 1.3% for sterile and normal larvae.

Discussion

The absence of a significant difference in parasitization indicates that sterile and normal larvae of the diamondback moth are equally acceptable to *C. plutellae* parasitoids. Both provided adequate nutritional and physiological conditions for parasitoid development. The success of the sterile larvae as host depended on their ability to attract and sustain parasitoid development within them. Host selection is a critical factor in biological control influencing fecundity (Hagvar and Hofsvang 1991). Sterile larvae apparently satisfied the nutritional and physiological requirements needed for growth and development of the parasitoid (Sequeira and Mackauer 1993). This similarity in parasitization of sterile and normal larvae also has been reported for *C. marginiventris* in *Spodoptera exigua* (Hübner) (Carpenter et al. 1996).

The relatively low percent parasitism obtained even under caged conditions is consistent with the levels of parasitism reported by Alam (1992), Wakisaka et al. (1992) and Mitchell et al. (1997) for *C. plutellae*. Despite the reported low parasitism rate, *C. plutellae* is considered the most promising parasitoid in the Oceanic Pacific region (Waterhouse and Norris 1987). Success of an augmentative release of a natural enemy is strongly influenced by the density of the host population at the time of release (Knipling 1992). *C. plutellae* displays highly density-dependent mortality, and parasitism levels rise with an increase in diamondback moth populations (Alam 1992). This quality could prove problematic if IPM works, because pest populations would be low and might not be able to sustain the parasitoid population as reported by Mitchell et al. (1997).

The similarity in foliage consumption during the 48-h stinging period is important because feeding produces chemicals that are used by natural enemies to locate prey (Dicke et al. 1990; Turlings et al. 1990, 1991; Dicke and Tokabayashi 1991). Foraging female *Cotesia glomerata* (L.) are strongly attracted to cabbage plants with *Pieris brassicae* (L.) feeding damage (Steinberg et al. 1992). Mitchell and co-workers have observed similar behavioral responses by *C. plutellae* to cabbage and collard plants infested with different larval pests including diamondback moth, cabbage looper [*Trichoplusia ni* (Hübner)], and imported cabbageworm [*P. rapae* (L.)] (unpublished data).

There was a higher consumption of foliage by normal larvae compared with sterile larvae when larvae

were allowed to pupate. This was attributed to a higher mortality rate among sterile larvae over their entire developmental period.

The distance traversed by diamondback moth larvae from hatch to pupation was not significantly different between the 2 types of larvae under laboratory conditions. Moreover, the survival rate of sterile and normal larvae also was not significantly different. The developmental time for pupation was the same for both sets of larvae. Mortality in the laboratory was due primarily to the 3rd and 4th instars spinning down on silk threads and landing in the underlying water trays.

Under field conditions, sterile larvae traversed a shorter distance than normal larvae. Even though developmental time from egg to pupation was similar, there was reduced survivorship of sterile larvae compared with normal larvae. There was high mortality among 1st instars of the diamondback moth for both sets of larvae, but this was more pronounced with sterile larvae. It is possible that sterile larvae were less tolerant of the higher temperatures experienced under field conditions than normal larvae. These results corroborate prior work that showed low larval survival rates and reduced egg hatch when parent generations of pink bollworm [*Pectinophora gossypiella* (Saunders)], sugarcane borer [*Diatraea saccharalis* (F.)], and fall armyworm [*Spodoptera frugiperda* (J. E. Smith)] were irradiated with sub-sterilizing doses of radiation (LaChance 1985, Arthur et al. 1990, Arthur et al. 1991). The overall low survivorship in the field trial indicates that large numbers of eggs would need to be used to have sufficient numbers of larvae to serve as hosts for *C. plutellae.*

Foliar damage was confined to a single collard plant under field conditions for both types of larvae while the damage in the laboratory trial covered 2 plants. Thus, there would be virtually no risk of sterile diamondback moth larvae moving from collards planted adjacent to cabbage in the field.

The results demonstrate that percent parasitism is not affected by sterilization of the larvae. Augmentative release can play an important role in biological control programs with collards serving as release sites for diamondback moth larvae and the parasitoid *C. plutellae.* The use of sterile diamondback moth larvae in a biological control program would provide the following 2 benefits: (1) a host for augmentative release of parasitoids, and (2) reduced pest populations through a F_1 sterility principle (Carpenter 1993).

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