# Parasitism of Diamondback Moth (Lepidoptera: Plutellidae) Larvae by *Cotesia plutellae* (Hymenoptera: Braconidae) and *Diadegma insulare* (Hymenoptera: Ichneumonidae) in Cabbage Fields after Inundative Releases of *C. plutellae*[1,2]

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**Abstract** Cocoons of *Cotesia plutellae* (Kurdjumov) were released for nine consecutive wk along the margins of two commercial cabbage (*Brassica oleracea* var. *bravo* L.) fields near Bunnell, Flagler Co., FL, in spring 1996. The larval parasitism of diamondback moth, *Plutella xylostella* (L.), by *C. plutellae* and by the native parasitoid *Diadegma insulare* (Cresson) was evaluated in release fields and in nearby cabbage fields using two methods-sentinel collard (*Brassica oleracea* var. *acephala* L.) or sentinel cabbage plants and non-sentinel plants. Total parasitism of diamondback moth larvae on sentinel plants in the release and adjacent fields was 35.7%. There were no significant differences in the level of parasitism by *C. plutellae* among sentinel plant locations within the release fields. In non-release fields, parasitoids spread as far as 1,500 m from the nearest release site during the release period, but parasitism of larvae on sentinel plants decreased as the distance from the release area increased. Parasitism of diamondback moth larvae by *D. insulare* was 8.3% in *C. plutellae* release and adjacent fields, but 14.6% in the nearby fields. Sampling of non-sentinel cabbage plants for diamondback moth larvae demonstrated a total of 37.4% larval parasitism by *C. plutellae* in the release and adjacent fields, similar to that recorded on sentinel plants. However, *C. plutellae* were detected only as far as 800 m from the release site on non-sentinel cabbage plants, and total parasitism in the dispersal fields also was very low. *Diadegma insulare* contributed only 1.1% parasitism of larvae sampled from non-sentinel plants in all cabbage fields. *Cotesia plutellae* was more effective than *D. insulare* in attacking diamondback moth larvae in this study where field populations of diamondback moth were low (<0.1 larva per cabbage plant).

**Key Words** *Plutella xylostella,* biological control, parasitoids, dispersal.

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most destructive pest of cabbage and other crucifers throughout the world. The annual cost for control of this pest is estimated at US $1 billion (Talekar and Shelton 1993). The diamondback moth typically has been controlled using pesticides (Shelton et al. 1993b); however, it has become resistant to the synthetic insecticides used in

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many countries (Shelton et al. 1993b, Talekar and Shelton 1993). In the United States, control failures have occurred in several states (Shelton et al. 1993a). Therefore, a biological control-integrated pest management system was developed for diamondback moth by Biever et al. (1994). The program consists of three elements: regular scouting of the crop to estimate plant damage and larval infestations; application of pesticides only when needed with more reliance upon *Bacillus thuringiensis (Bt)*-based insecticides, and; preservation of natural enemies combined with periodic releases of parasitoids.

Many attempts have been made to introduce parasitoids for control of the diamondback moth and other cruciferous pests. *Cotesia plutellae* (Kurdjumov) (Hymenoptera: Braconidae) is frequently mentioned as a possible biological control agent for diamondback moth (Talekar and Shelton 1993). There have been sporadic releases of this parasitoid in Florida (Frank and McCoy 1993) with no evidence of establishment. In 1993-1994, a parasitism of 10.9% of diamondback moth larvae was obtained after releases of *C. plutellae* in cabbage fields (Mitchell et al. 1997b).

The objectives of this investigation were to evaluate parasitism of diamondback larvae following inundative releases of *C. plutellae* in the presence of the naturally-occurring parasitoid *Diadegma insulare* (Cresson) (Hymenoptera: Ichneumonidae) and to monitor dispersal of the released parasitoids into nearby fields.

## Materials and Methods

**Parasitoid source.** *Cotesia plutellae* used in this study were purchased from Biofac, Inc. (Mathis, TX). Cocoons established on paper towels (about 1,000 each) were placed in plastic bags, wrapped with old newsprint, inserted into styrofoam containers, and shipped to Gainesville, FL. Upon arrival, 100 cocoons from each shipment were randomly selected and placed into an incubator maintained at 25 to 27°C, 50 to 60% RH and 14:10 (L: D) h photoperiod to await emergence. Data on percent emergence and sex ratio from these subsamples were collected. The remaining cocoons (about 8,900) were divided to meet the experimental needs and transported to the fields for release.

**Field releases.** The cabbage (*Brassica oleracea* var. *bravo* L.) fields used in the study were located in an agricultural area devoted to the production of cabbage and potatoes. The elevation of this area is approximately 6.1 m above sea level. Typically, the wind blows from southwest to northeast for almost the entire winter-spring season. A total of seven fields was used for this study, two (A and B) of which were used for release and the other five for monitoring dispersal of the parasitoids (Fig. 1). The release fields totaled 21.6 ha and were separated only by an irrigation ditch. They were bordered on the east by woods, on the south and west by drainage ditches with the banks populated by various weeds and grasses, and on the north by an irrigation ditch and a driveway. The areas of Fields C, D, E, F, and G were 5, 5.3, 3.6, 12.1, and 12.1 ha, respectively (Fig. 1).

Fields A, B, and C were sprayed with Lannate® (methyl N-[methylamino) carbonyl] oxylethanimidothioate, DuPont Agricultural Products, Wilmington, DE) on 18 February, Mattch® (a blend of encapsulated *B.t.* var. *aizawai* and *B.t.* var. *kurstaki,* Mycogen, San Diego, CA) on 3 March, and Agree® (a transconjugated strain of *B.t.* var. *aizawai,* Novartis Crop Protection, Greensboro, NC) and Larvin® (thiodicarb, Rhone-Poulenc Agricultural Co., Research Triangle Park, NC) on 1 April. Fields D, E, and F were sprayed with Asana® (fenvalerate, DuPont Agricultural Products, Wilmington,

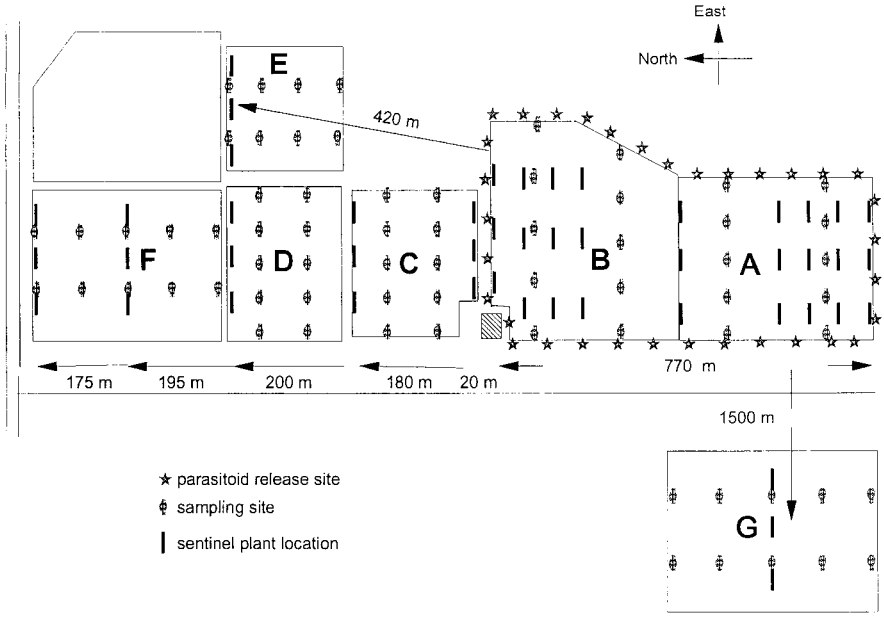


Fig. 1. Schematic of cabbage experimental fields, Bunnell, Flagler Co., FL, showing arrangement of parasitoid release stations, sentinel plant locations, and non-sentinel sampling sites.

DE) and Mattch on 18 March, Asana and Agree on 22 March, and Asana and Mattch on 26 March. Field G was sprayed with Monitor® (methamidophos, Valent USA Corp., Walnut Creek, CA) on 3 March, Monitor and Mattch on 28 March, Thiodan® (endosulfan, FMC Corp., AG Chemicals Group, Philadelphia, PA) and XenTari® (*B.t.* var. *aizawai,* Abbott Laboratory, Chemical and Agricultural Products Division, North Chicago, IL) on 27 March, Larvin and Mattch on 4 April, and Asana and Mattch on 17 April 1996.

Based on a previous study (Hu et al. 1997), the initial sites for diamondback moth invasion to cabbage fields were the field margins. Therefore, release stations were arranged at 34 sites along the edges of Fields A and B with about 60 m between stations (Fig. 1). The release of *C. plutellae* at the periphery of the cabbage fields where diamondback moth larvae were the most abundant (Hu et al. 1997) was to facilitate parasitoids finding their hosts, increasing their numbers, and spreading toward the interior of cabbage fields as they searched for new hosts (Alam 1992).

Cocoons of *C. plutellae* were released in Fields A and B at a target number of 412/ha per week for 9 consecutive wk beginning 20 February 1996. However, the actual release number was an estimated 324/ha because of 21.5 ± 3.63% (SE) pupal mortality per shipment. The sex ratio of females: males was 1:1.18 ± 0.06 per shipment. The cocoons established on paper towels were divided and counted in the laboratory, transported to the fields in plastic Petri dishes (13.9 cm diam × 1.9 cm high), and released from styrofoam buckets (Lifoam, Baltimore, MD; Fig. 2). The

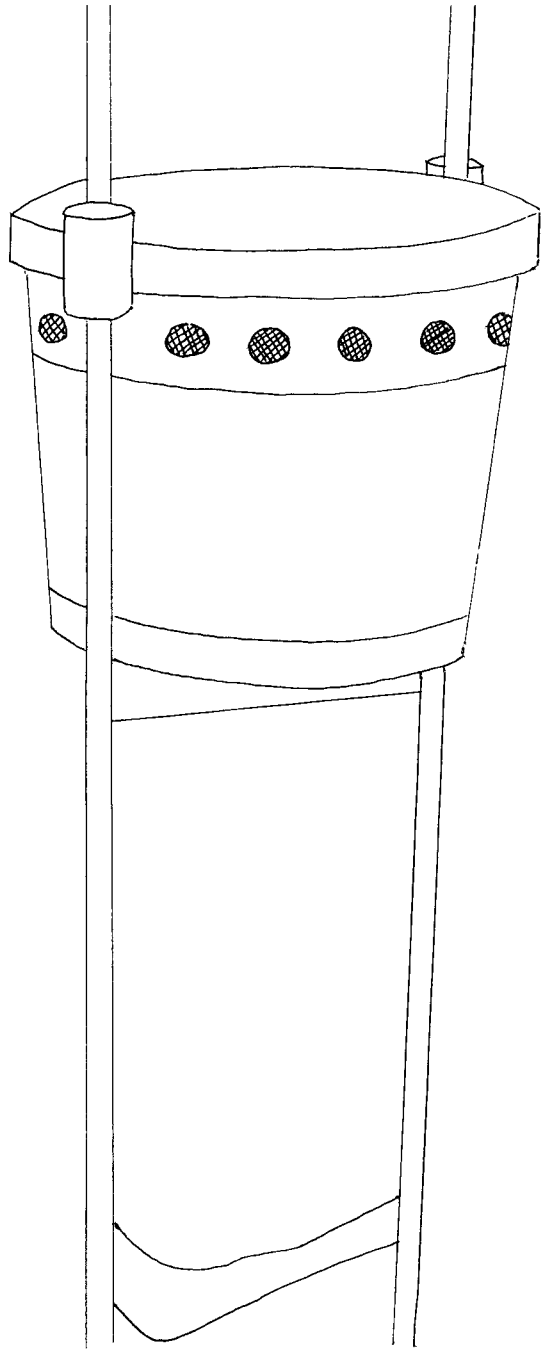

Fig. 2. Release bucket for *Cotesia plutellae*. Cocoons of the parasitoids established on paper towels were placed on the bottom of the bucket, and collard leaves with diamondback moth larvae were placed on a metal wire screen above the cocoons.

bucket was 21.5 cm high with a top diam of 22.5 cm and a bottom diam of 19.5 cm. Holes (diam 2.5 cm) were perforated through the side walls and the bottom for ventilation and covered with nylon screen (mesh size 1.5 mm) to prevent invasions by other insects, but permitting *C. plutellae* to exit from the bucket. The buckets were supported by two posts and stood about 1 m above the ground. Amdro® (American Cyanamid, Parsipanny, NJ) was applied on the ground around the posts to prevent fire ants, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), from attacking the parasitoids. Collard (*Brassica oleracea* var. *acephala* L.) leaves with diamondback moth larvae were placed on a metal wire screen above the parasitoid cocoons. The emerged wasps possibly contacted the plant-larvae complexes and even stung the larvae before they moved from the buckets to the field. According to the studies by Turlings et al. (1989, 1990), *C. marginiventris* (Cresson) located their host more efficiently if they were exposed first to plant-larval complexes. Similar behavior was observed with *C. plutellae* in flight-tunnel tests using collard or cabbage plants infested with diamondback moth larvae as the source (unpubl. data). Because greenhouse-grown collard plants were available, collard leaves were used for conditioning *C. plutellae* to be released in the fields.

**Sentinel plant set up and collection.** During the release period, an average of five sentinel plants was chosen at each site, and each plant was infested with an estimated 40 to 60 first-instar diamondback larvae weekly. The larvae provided by J. E. Carpenter (USDA-ARS, Tifton, GA) were sterile because they hatched from eggs produced by mating irradiated diamondback moth males with non-irradiated females. Sterile larvae were used to prevent a possible increase in field populations of diamondback moth as a result of released larvae that avoided parasitism. After 7 d, the larvae remaining on the sentinel plants were collected, transferred to the laboratory, and dissected for parasitoids (Day 1994). Eggs and larvae of *C. plutellae* and *D. insulare* (native species) dissected from the host were distinguishable under a stereo microscope. Both eggs are transparent and elongated. The egg of *Diadegma* is banana-shaped and slightly bigger than that of *Cotesia;* the egg of *Cotesia* is tapered toward one end. *Diadegma* larvae are slender with a pointed tail, and the digestive tract contains yellowish pigments after 1 d old; *Cotesia* larvae have a bubble-shaped tail (unpubl. data).

Five new plants were chosen for diamondback moth larval infestation at each site and each sampling date to avoid heavy defoliation of the plants caused by diamondback moth larvae. In the release Fields (A and B), 9 vertical rows (running east-west) of sentinel plants were designated, each consisting of three sites (Fig. 1). To monitor the spread of the released parasitoids, sentinel plant sites also were established in Fields C, D, E, F, and G (Fig. 1). Fewer sentinel plant sites were established in Fields C-G, as those fields were used to detect dispersal of the released parasitoid. The average distance of the sentinel plant sites ranged from 20 to 1500 m from the nearest release site.

**Sampling procedure.** Non-sentinel cabbage plants were inspected weekly for diamondback moth larvae. The numbers of plants sampled at each site decreased from 65 per 15 m row-length in the first week to 13 per 3.5 m row-length in the week of harvest as the plants increased in size. All leaves of the plants at different ages were searched in the same way for larvae and cocoons of the diamondback moth. Two sampling rows were established across each field (Fig. 1), each with five sampling sites (Field E had 4 sites) arranged as follows: along each end, in the middle, and halfway between the middle and each end (50 to 70 m from ends). All the larvae

diamondback moth larvae on the sentinel plants were collected and transported to the laboratory in a styrofoam cooler, dissected under a dissecting microscope, and examined for the presence of parasitoids (Day 1994).

**Statistical analyses.** For comparison of parasitism of diamondback moth larvae among the sentinel plant locations across the season, a two-way analysis of variance (ANOVA) was used to test for overall differences by sites, and Duncan's Multiple Range Test was used to separate the means (SAS Institute 1990). Before performing these analyses, percent parasitism was transformed to angles, arcsine [sqrt (n + 1)], to meet the assumption of the ANOVA procedure (Marks 1990).

## Results

**Sentinel plants in the release field.** A total of 1,495 diamondback moth larvae was collected from the sentinel plants in Fields A and B, of which 43.6% contained parasitoid eggs and/or larvae. Parasitism by *C. plutellae* was 36.3% and by *D. insulare,* 7.3%.

No significant differences were detected between sentinel plant locations across the field ($F = 0.04$; df = 2, 16; $P > 0.05$; Fig. 3) and locations along the length of rows ($F = 0.74$; df = 8, 16; $P > 0.05$). Even though the release stations were arranged along

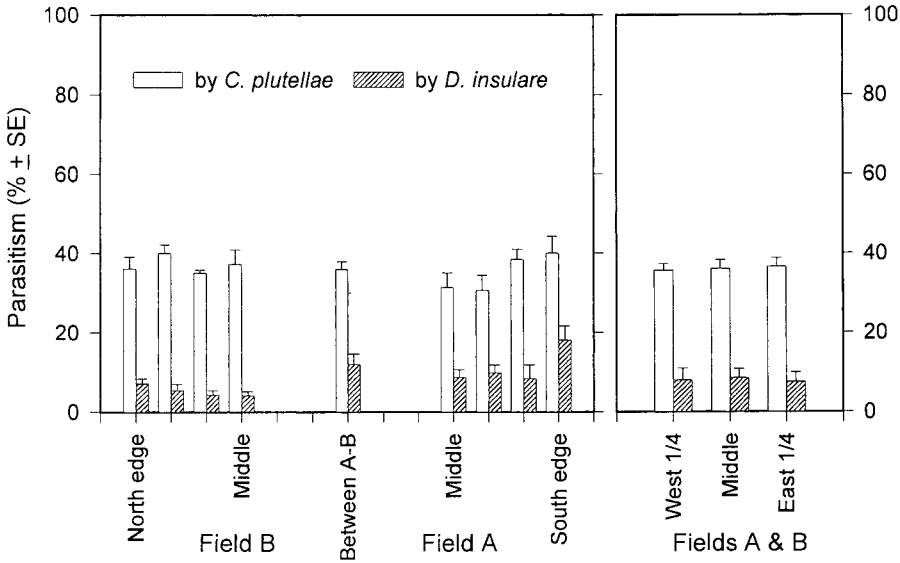


Fig. 3. Seasonal average percentage parasitism of diamondback moth larvae on sentinel plants by *Cotesia plutellae* and *Diadegma insulare* in release Fields A and B. Positions of sentinel plants are shown in Fig. 1. Each set of two bars on the left graph represent the average parasitism of three sampling sites along each cabbage row (east-west). Each set of two bars on the right graph show the average parasitism of the sample sites along the west end, east end and across the field middle (north-south), respectively.

the field edges, *C. plutellae* was collected throughout the fields. Parasitism caused by *D. insulare* also showed no significant differences among locations across the field ($F = 0.08$; df = 8, 16; $P > 0.05$) or among positions along the length of rows ($F = 1.53$; df = 2 16; $P > 0.05$).

**Sentinel plants in non-release fields.** Field C was adjacent to Field B and had three sentinel plant locations on the north and south sides (Fig. 1). A total of 448 diamondback moth larvae was examined, and 33.5% contained *C. plutellae* and 8.4% contained *D. insulare*. Total parasitism in Field C was 41.9%. Parasitism caused by either species of the parasitoids in Field C was similar to that in Fields A and B (Fig. 4). Combined data from Fields A-C showed that parasitism by *C. plutellae* was 35.7% and by *D. insulare,* 8.3%. The total pooled parasitism in Fields A-C was 44%.

Sentinel plants in Field D were about 400 m away from the nearest release site in Field B (Fig. 1). Of 211 diamondback moth larvae examined, 24.6% contained *C. plutellae* and 16.6% contained *D. insulare.* Total parasitism in Field D was 41.2%.

The average distance of the sentinel plants in Field E from the nearest release

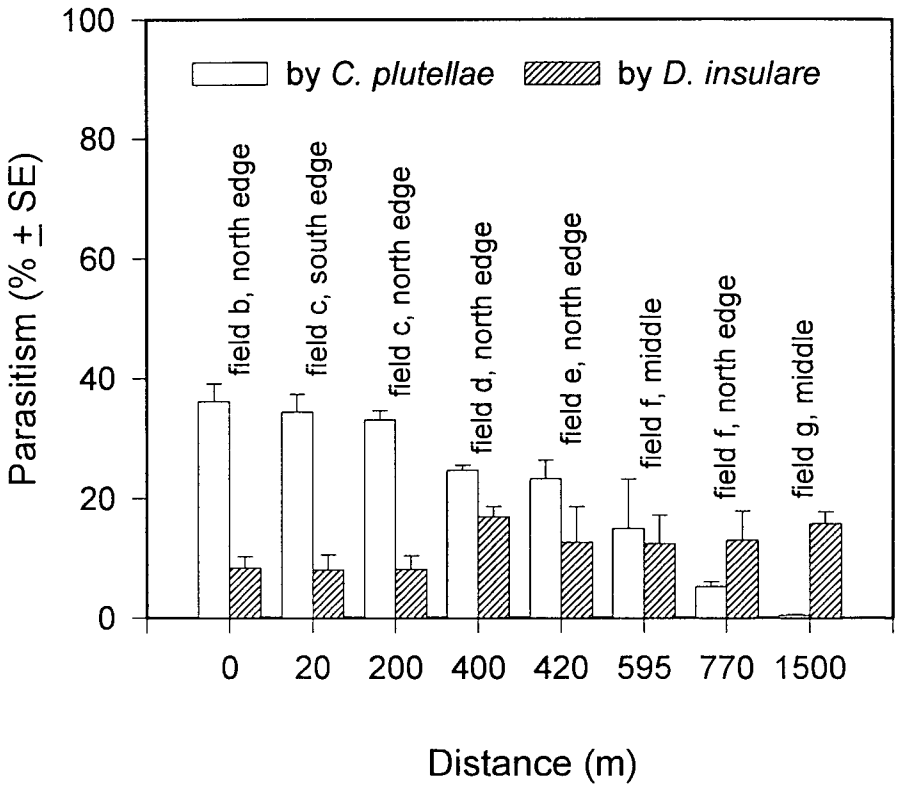


Fig. 4. Seasonal average percentage parasitism of diamondback moth larvae on sentinel plants located different distances from the nearest release site. (See Fig. 1 for position of sentinel plants and release sites).

sites was about 420 m. Of 111 larvae examined, 23.4% contained *C. plutellae* and 12.7% contained *D. insulare.* Total parasitism in Field E was 36.1%.

In Field F, sentinel plants were located along the middle and north end of the field (Fig. 1). Of 213 larvae examined from plants in the field middle (about 600 m from the nearest release site), 15% contained *C. plutellae* and 12.6% contained *D. insulare.* Of 370 examined larvae from plants in the north end (about 770 m from the release site), 5.1% contained *C. plutellae* and 13.2% contained *D. insulare.* For all of Field F, *C. plutellae* and *D. insulare* caused 7% and 13% parasitism, respectively. Total parasitism was 21%.

In Field G, the distance of sentinel plants from the nearest release site was about 1,500 m. Of 248 larvae examined, only 0.81% contained *C. plutellae* but 15.8% contained *D. insulare.* Total parasitism in Field G was 16.6%.

Results from the non-release fields showed that *C. plutellae* generally dispersed in a northerly direction from the release area, possibly carried by prevailing winds which typically are from the southwest to northeast during the winter-spring growing season. Parasitism by *C. plutellae* in the non-release areas decreased as the average distance of the sentinel plant locations increased. Parasitism by *D. insulare* in the release and adjacent Fields (A-C) was lower than the nearby Fields (D-G) (Fig. 4), indicating *C. plutellae* possibly replaces *D. insulare.* The combined parasitism by both species decreased as the distance from the release area increased ($Y = 44.21 - 0.021\ X$; $r^2 = 0.80$; $df = 6$, $P < 0.01$).

There was no increase in parasitism over time by *C. plutellae* in diamondback larvae collected from sentinel plants in the release Fields (A and B) or in the dispersal Fields (C-G). Actually, Fig. 5 showed a decrease of parasitism by *P. plutellae* in the *Cotesia* release fields, and an increase of parasitism by *D. insulare* over time in both the release and dispersal areas.

A follow-up investigation was conducted in Fields A, B, F, and G using sentinel plants, infested with diamondback moth larvae after the cabbage was harvested at the end of April 1996 in order to determine the existence of the parasitoid in that area. No *C. plutellae* or *D. insulare* were detected after 11 July 1996 although sampling continued until 15 October 1996. After no parasitoids were found in the larvae on sentinel plants, collard plants without diamondback moth larvae were set in Fields A, B, F, and G weekly from 25 July 1996 to monitor for diamondback moth occurrence. No diamondback larvae were found on these plants until 5 November 1996.

**Non-sentinel plants from all fields.** A total of 363 diamondback moth larvae collected from non-sentinel plants, of which 14.6% contained *C. plutellae.* The data were not separated by sampling sites within a field because of the low populations (less than 0.2 larva per plant) of diamondback larvae (less than 0.2 larva per plant in the fields during the experimental period. Parasitism by *C. plutellae* was 37.4% in Fields A-C (release and adjacent fields), 10.3% in Field D, 2.4% in Field E, 1.9% in Field F, and none in Field G. Only 1.1% of the 363 larvae examined contained *D. insulare;* two of them were from Fields A-C, one from Field E, and one from Field G.

## Discussion

The introduction of *C. plutellae* into different areas of the world has yielded inconsistent results with regard to suppression of diamondback moth (Talekar and Shelton 1993). In the western hemisphere, *C. plutellae* reportedly flourished after introduc-

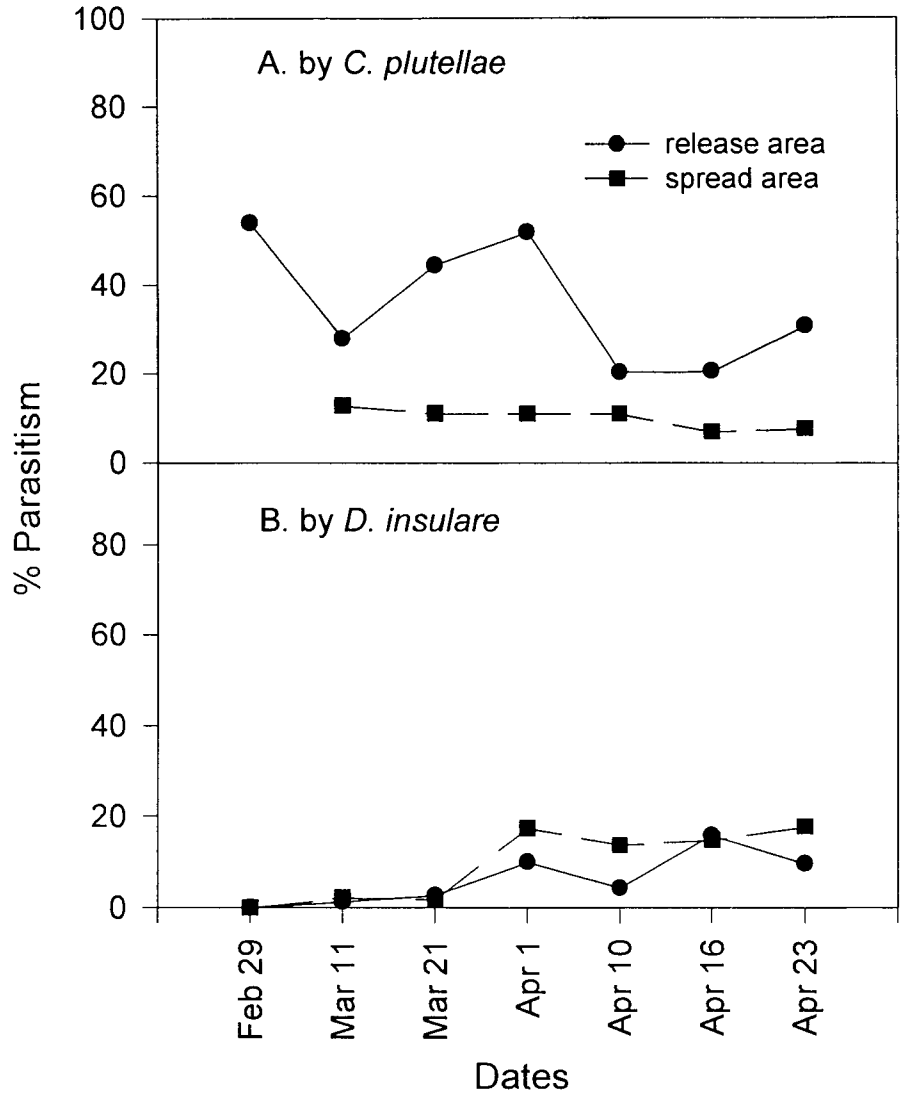


Fig. 5. Seasonal distribution of percentage parasitism of diamondback moth larvae on sentinel plants in the release and dispersal fields.

tions into Barbados and Jamaica, resulting in significant control of the diamondback moth (Alam 1992). However, attempts to introduce this parasitoid into Honduras, Belize, and Costa Rica have not resulted in suppression of the pest (Andrews et al. 1992). A previous study in the same cabbage production area showed 6.6 to 15.0% parasitism of diamondback moth larvae (Mitchell et al. 1997b). The present study showed 35.7% and 37.4% parasitism of diamondback moth larvae from the sentinel

plants and non-sentinel plants, respectively, by *C. plutellae* in release and adjacent fields.

During investigations of the diamondback moth and its parasitoids in the fall-winter season of 1996-1997 in different cabbage fields in the area, two *C. plutellae* and 14 *D. insulare* were found in December 1996 and in January and February 1997. This shows that a small number of these parasitoids survived summer 1996 and attacked diamondback moth larvae during the 1996-1997 winter season. However, a previous study by Mitchell et al (1997b) was unable to show that *C. plutellae* became established in the area despite the release of >124,000 parasitoids over a 2-yr period. One possible explanation for survival of *C. plutellae* from releases made in spring 1996 to the fall-winter season of 1996-1997 was changes in weather. Typically, the summer and fall seasons in this area are characterized by extremely heavy rainfall. For example, in fall of 1995, >1.19 m of rain was recorded from September through December, and cabbage growers often had to replant due to flooded fields. In contrast, <0.5 m of rain fell during the same period in 1996. The fall-winter season in 1996 also experienced unseasonably warm temperatures. Thus, it is possible that the atypical combination of low rainfall and warm temperatures through the fall-winter seasons of 1996-1997 was conducive to survival of *C. plutellae* parasitoids from releases made in spring 1996. However, it is unclear as to why diamondback moth and *C. plutellae* were not detected during summer 1996. Perhaps an exhaustive survey of diamondback moth host plants in the area would have resulted in detection of both species.

The parasitoids released along the edges of Fields A and B dispersed evenly throughout these fields. Feeding damage resulting from a relatively high density of diamondback moth larvae on sentinel plants may have produced a high concentration of allelochemicals that attracted the parasitoids towards the plant-larval complex (Turlings et al. 1989, 1990), and *C. plutellae* parasitoids correspondingly searched for diamondback moth larvae in the vicinity of the sentinel plants. Parasitism by *C. plutellae* was about the same for the field populations of diamondback moth larvae even though larval densities were very low. This parasitism rate may be a result of a moderate, season-long infestation of cabbage looper, *Trichoplusia ni* (Hübner) (Lepidoptera: Noctuidae). Laboratory tests by the authors have shown that *C. plutellae* and *D. insulare* also are attracted to collard and cabbage plants with feeding cabbage looper larvae (unpubl. data). Feeding damage caused by cabbage looper larvae probably attracted *C. plutellae* to the plants and stimulated the parasitoids to continue their search for diamondback moth larval hosts.

Parasitism of diamondback moth larvae by *D. insulare* in the non-sentinel field cabbage was much lower than on sentinel plants. Laboratory studies have shown that *D. insulare* is highly attracted to cabbage and collard plants damaged by feeding diamondback larvae (unpubl. data). Sentinel plants bearing numerous diamondback larvae probably produced a higher concentration of kairomones than non-sentinel cabbage plants infested with very few diamondback larvae which would account for the difference in parasitism between the two.

Following releases in the field, *C. plutellae* did not increase their numbers due perhaps to low host populations, application of pesticides, and unfavorable weather. *Cotesia plutellae* is highly density-dependent with the level of parasitism increasing with the increase in host populations (Alam 1992, Ooi 1992, Rowell et al. 1992). *Diadegma insulare* also is highly density-dependent (Mitchell et al. 1997a; Ooi 1992).

The low parasitism of diamondback moth larvae during the entire season in all the fields probably was due to low host populations in the fields.

Results from a previous study by Mitchell et al. (1997b) suggest that inundative release of *C. plutellae* in cabbage fields in the numbers used here (about 3,082/ha over the season) probably will not result in economic control of diamondback moth. However, it might be feasible to combine releases of *C. plutellae* with other control tactics such as pheromone for mating disruption (McLaughlin et al. 1994), trap crops (Mitchell et al. 1997a), and *B.t.* pesticides that are not harmful to these parasitoids (Kao and Tzeng 1992, Morallo-Rejesus and Sayaboc 1992). Such a combination would provide a highly desirable integrated approach to managing diamondback moth in cabbage grown in Florida (Leibee 1996).

The displacement of *D. insulare* by *C. plutellae* may be due to responses of the species to host stages. *C. plutellae* prefers early instars of the host larvae, but *D. insulare* prefers the late instars (Mitchell et al. unpl. data). Higher density of *C. plutellae* in the fields may be another factor for the replacement.

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