Comparison of *Diadegma insulare* (Hymenoptera: Ichneumonidae) and *Microplitis plutellae* (Hymenoptera: Braconidae) as Biological Control Agents of *Plutella xylostella* (Lepidoptera: Plutellidae): Field Parasitism, Insecticide Susceptibility, and Host-Searching

JIANXIANG XU,[1,2] ANTHONY M. SHELTON,[1] AND XIANIAN CHENG[3]

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ABSTRACT Parasitism of *Plutella xylostella* (L.) third and fourth instars was evaluated in a cabbage field in Geneva, NY, in 1999. Over the entire season, average parasitism was 33.6% for third instars and 53.6% for fourth instars, and the main parasitoids were *Diadegma insulare* (Cresson) and *Microplitis plutellae* Muesbeck. In the early season, total parasitism was low, and mainly caused by *D. insulare.* However, later in the season, parasitism reached >80% for the fouth instars and 50% for the third instars. Our survey indicated that *M. plutellae* heavily parasitized *P. xylostella,* and provided higher parasitism rates than *D. insulare* in the late season. Comparison of these two species in laboratory bioassays indicated there were no significant differences in susceptibility to four insecticides commonly used in crucifer fields. For both parasitoids, an experience with *P. xylostella* on a damaged leaf increased their host-searching efficacy. Compared with *M. plutellae, D. insulare* was a better host-searcher both for the naive and the experienced adults. Although both parasitoids can cause high mortality rates of *P. xylostella, D. insulare* may be more suitable to be released in fields to enhance natural control against *P. xylostella.*

KEY WORDS *Diadegma insulare, Microplitis plutellae, Plutella xylostella,* parasitism, searching behavior

DIAMONDBACK MOTH, *Plutella xylostella* (L.), is the most destructive pest of economically important crucifer crops worldwide (Talekar and Shelton 1993). In Southeast Asia, for example, major outbreaks of *P. xylostella* sometimes cause >90% crop losses (Verkerk and Wright 1996). As a result of intensive insecticide use, the insect has developed resistance to nearly all classes of insecticides used against it (Shelton et al. 1993). Worldwide there are now increased efforts to develop biological control-based integrated pest management programs, in which one of the important elements is preservation or augmentation of natural enemies (Talekar and Shelton 1993, Biever et al. 1994).

Although many species of predators and pathogens of *P. xylostella* have been recorded (Yamada and Yamaguchi 1985, Ibrahim and Low 1993, Usha et al. 1997), their effects are often not as important as those of parasitoids in reducing field populations of *P. xylostella.* Marsh (1917) pointed out that *P. xylostella* was a striking example of a potentially serious pest normally held in control naturally by parasitoids. In Europe, it has been reported that parasitoids alone keep the population under control (Mustata 1992).

There are >90 parasitoid species reported for *P. xylostella* and ≈60 of them appear to be important (including 38 larval parasitoids and 13 pupal parasitoids) (Goodwin 1979, Lim 1986). Talekar and Shelton (1993) suggested that larval parasitoids have the greatest control potential, and Lim (1986) suggested that the major ones, in order of decreasing importance, belong largely to the genera *Diadegma, Cotesia,* and *Microplitis.* In North America, the two most important parasitoids of *P. xylostella* are *Diadegma insulare* (Cresson) and *Microplitis plutellae* Muesbeck (Pimentel 1961, Harcourt 1986, Horn 1987, Zhao et al. 1992, Godin and Boivin 1998). Although parasitism may exceed 80% at times, *P. xylostella* may still not be adequately controlled in some years. Releasing parasitoids may be an effective tactic to improve biological control of *P. xylostella.* Compared with introducing exotic species, augmenting an established parasitoid may be an easier approach, and *D. insulare* and *M. plutellae* are two potential candidates.

Although both species parasitize the first three instars of *P. xylostella* (parasitism of first instars is very low), *M. plutellae* kills and emerges from fourth instars, whereas *D. insulare* kills and emerges from the prepupal stage (Harcourt 1960, Putnam 1968). Comparisons of the two species indicated that the number of eggs produced per day is similar, but *M. plutellae* lives for a shorter period of time, resulting in an overall higher fecundity rate for *D. insulare* (Putnam 1968, Bolter and Laing 1983). Experiments in the laboratory

[1] Department of Entomology, Cornell University/NYSAES, Geneva, NY 14456.
[2] Department of Plant Protection, Yangzhou University, Yangzhou, 225009, P. R. China.
[3] Department of Plant Protection, Nanjing Agricultural University, Nanjing, 210095, P. R. China.

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demonstrated that two females, one of each species (*D. insulare* and *M. plutellae*), produced more total progeny than two of the same species (Putnam 1968), suggesting that these two species can coexist in fields and their co-existence may yield a more effective result against *P. xylostella.* When eggs of *D. insulare* and *M. plutellae* were deposited in the same larvae, the first instar of *M. plutellae* always survived by physically attacking the larva of *D. insulare*, unless the egg of *D. insulare* was oviposited ≥12 h earlier. These findings indicate that *M. plutellae* may be intrinsically superior to *D. insulare*, despite the ability of *D. insulare* to discriminate parasitized hosts better (Bolter and Laing 1983). When adults of the two species were released on a damaged cabbage leaf with feeding larvae, the time taken by *M. plutellae* to find a larva was significantly less than by *D. insulare.* Based on those results, Bolter and Laing (1983) suggested that *M. plutellae* may also be extrinsically superior.

More information is needed to compare and evaluate these two species for augmentative release. In this study, we monitored natural parasitism dynamics of *P. xylostella* in a cabbage field in Geneva, NY, compared the susceptibility of *D. insulare* and *M. plutellae* to several commonly used insecticides, and evaluated the host-searching behavior of the two species.

Materials and Methods

Parasitism of *P. xylostella*. The experiment was conducted in 1999 at the Fruit and Vegetable Crops Research Farm of the New York State Agricultural Experiment Station in Geneva, NY. Experimental plots were established during the second week of May, and fourth weeks of June and July by transplanting common cabbage (*Brassica oleracea capitata* L. 'Vantage Point'). Each plot was 20 by 60 m with plants spaced at 90 cm between rows and 45 cm within rows. Throughout the growing season, no insecticides were applied to the plants. For each plot, starting at 3–4 wk after transplanting, we sampled for 6–7 wk. The first sampling was conducted on 7 June. To avoid possible edge effects, plants within 1 m of the edge of the plot were not sampled. All third and fourth instars of *P. xylostella* were collected from >30 randomly selected cabbage plants at each sampling time, and then taken to the laboratory. The larvae were reared with cabbage leaves in plastic 30-ml clear plastic cups (Kabri-Kal, Kalamazoo, MI; 10 larvae in each cup) with clear lids until pupation. After pupation they were transferred individually to 96-well ELISA plates and covered by parafilm until moth or wasp emergence. Incubation conditions were maintained at 27 ± 1°C, 35 ± 2% RH, and a photoperiod of 16:8 (L:D) h. Species and numbers of parasitoids were recorded and parasitism rates of third and fourth instars of *P. xylostella* were calculated for each sample date and each parasitoid species. The last sampling was conducted on 18 October. Only parasitism of fourth instars was calculated for the last two samples because of a limited number of third instars.

Susceptibility of *D. insulare* and *M. plutellae* to Insecticides Commonly Used in Crucifer Crops. *Diadegma insulare* and *M. plutellae* collected from the Research Farm of NYSAES on 19 and 26 July 1999 were maintained on *P. xylostella* from a laboratory colony (Geneva 88 strain) (Shelton et al. 1991). *Plutella xylostella* eggs on aluminum foil oviposition strips were placed on oilseed rape (*Brassica napus* L.) seedlings in wooden cages (45 by 50 by 76 cm). When host larvae reached second to third instars, six to eight pairs of mated wasps (*D. insulare* or *M. plutellae*) were transferred into the cage. The rearing cages were kept in a greenhouse at 25 ± 5°C and a photoperiod of 14:10 (L:D) h. Natural light was supplemented by fluorescent lights. A 10% sugar solution was provided as food for wasps.

Four insecticides were selected based on their current use for control of key insect pests of crucifers: carbaryl (Sevin XLR Plus, Rhone-Poulenc, Research Triangle Park, NC), diazinon (Diazinon 4E, Ciba, Greensboro, NC), dimethoate (Dimethoate 4E, Helena Chemical, Memphis, TN), and permethrin (Ambush 2E, Zeneca, Wilmington, DE).

Susceptibility of *D. insulare* and *M. plutellae* adults to insecticides was assessed using a direct contact residual method with glass vials (2.5 cm diameter by 9.5 cm long). Adult (F_4–F_5) *D. insulare* and *M. plutellae* were used in the bioassay. Preliminary tests were carried out on small groups of parasitoids before the formal tests so that an appropriate range of concentrations (10–90% mortality range) for each insecticide could be selected for a full-scale test. Six to seven concentrations of each insecticide were prepared, including a control, and five or six vials were treated with each concentration. A 5-ml solution of the insecticide was poured into each vial and the vial was swirled for 10–20 s. The excess was poured off and the residue was air-dried inside a fume hood for 4–5 h. Distilled water was used as a control. After being anesthetized with CO_2 for ≈3 s, six randomly selected 1- to 2-d-old *D. insulare* or *M. plutellae* adults were placed into each of the treated vials. Each vial was capped with nylon gauze and a cotton wick, soaked with 10% sugar solution, and pinned to the gauze inside the vial to provide food for the wasps. The test vials were then placed in an environmental chamber at 27 ± 1°C, 35 ± 2% RH, and a photoperiod of 16:8 (L:D) h. Mortality was recorded after 24 h. Individuals were considered dead if they were unable to maintain a normal posture or walk normally, covering at least 1 mm/s. Based on preliminary test results, there were no significant differences in susceptibility to insecticides between males and females of either species, so sexes were not separated in the formal bioassay.

Concentration-mortality regressions were estimated by probit analysis using POLO (LeOra Software 1997). Differences in susceptibility were considered significant when 95% fiducial limits (FL) for LC_{50}s did not overlap.

Host-Searching Behavior of *D. insulare* and *M. plutellae* Adults. Newly emerged *D. insulare* or *M. plutellae* adults were collected from the rearing cages

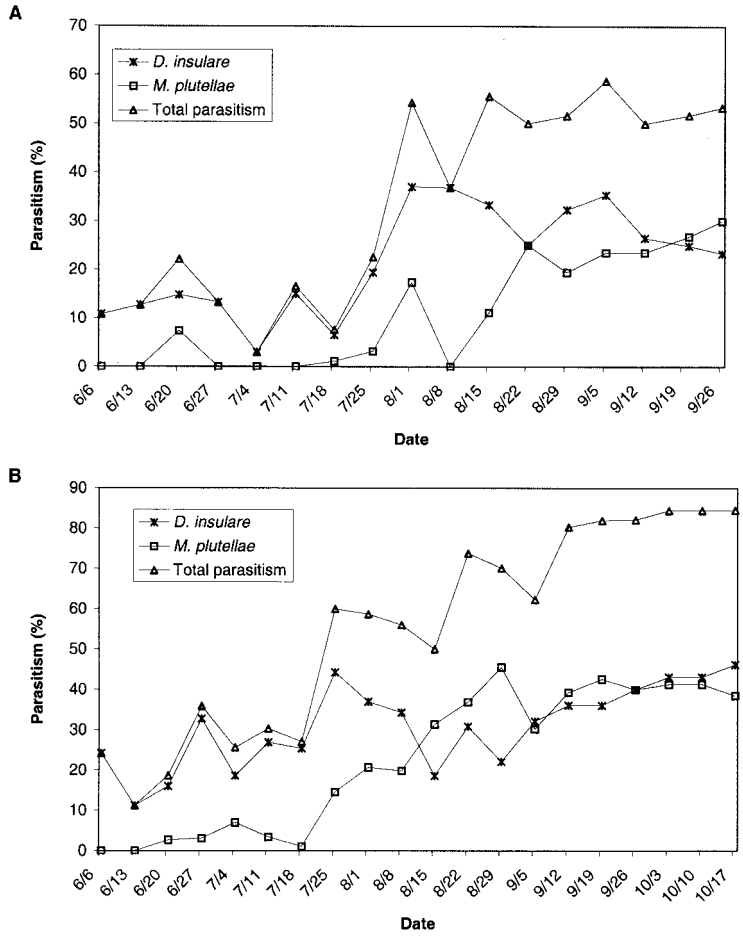

Fig. 1. Parasitism dynamics of *P. xylostella* in cabbage field in Geneva, NY, 1999. (A) Third-instars. (B) Fourth-instars.

and transferred into clear plastic cylinder cages (12 cm diameter by 15 cm tall) to mate for 24 h before the host-searching bioassay. Naive and experienced females were used in the bioassay. For the naive females, once they emerged, the wasps were given no chance to contact either host insects or plants before the bioassay. For the experienced females, after 24 h in the mating cages, the wasps were released on cabbage plants (8–10 true leaves) that had been infested with 10 second- or third-instar *P. xylostella* 24 h earlier, and then collected again within 6–10 s after they had contacted feeding larvae. The cabbage plants (Vantage Point) were grown individually in 15.2-cm plastic pots in a greenhouse at 25 ± 5°C and a photoperiod of 14:10 (L:D) h.

A dual choice bioassay was conducted in a 45 by 50 by 76 cm wood frame cage with nylon screen-covered openings on three sides and a removable wooden door on the fourth side containing two 18-cm-diameter holes with cloth sleeves. The top of the cage was clear glass. Two cabbage plants, one intact and the other damaged by an infestation of second- or third-instar *P. xylostella* placed on the plants 24 h before the bioassay, were placed at the two ends of the cage. In the bioassay for naive parasitoids, two levels of *P. xylostella* larval infestation were used for the damaged cabbage: low infestation with two to five larvae, and high infestation with 20 larvae. In the bioassay with experienced wasps, the plants were infested with 8–10 larvae for 24 h. A mated female was released from a glass vial at the center point between the two cabbage plants. The first plant on which the female landed and the time it took before landing on the plant were recorded. For the naive females, we observed for 20 min or until the wasp landed on a plant, whereas for the experienced females, we observed for 5 min. This difference in observation time was determined after preliminary tests indicated experienced wasps were faster at locating hosts. All females were used only once. Plants were switched from one side to the other after five wasps were tested. The bioassay was carried out in the greenhouse between 0930 and 1500 hours. Temperature was 25 ± 5°C and the natural light was supplemented with fluorescent lighting. Results of all dual choice tests were analyzed with chi-square tests to evaluate whether there were any significant differ-

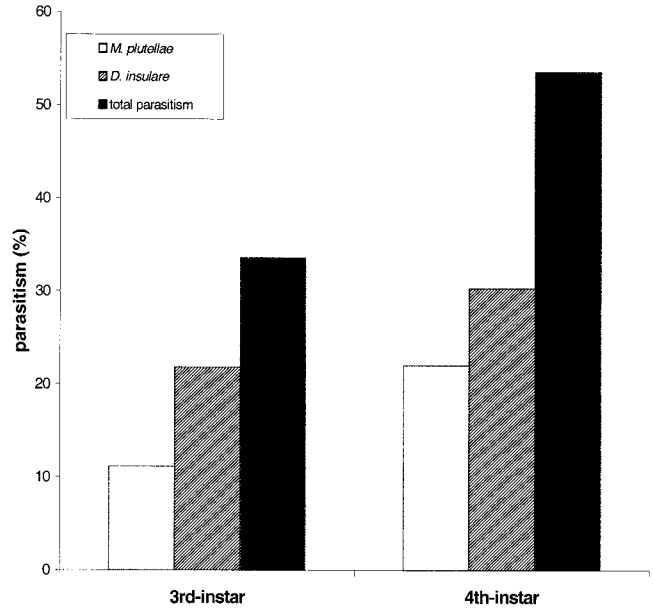

Fig. 2. Average percent parasitism of third- and fourth-instar *P. xylostella* over the season in Geneva, NY, 1999.

ences in preference for wasps to land on damaged or undamaged cabbage (Zar 1984).

Results and Discussion

Parasitism of *P. xylostella*. During the growing season (from 7 June to 18 October) a total of 2,512 *P. xylostella* larvae was collected, among which 1,040 were parasitized. In the early season (May and June), total parasitism of *P. xylostella* larvae was low, *D. insulare* was the dominant parasitoid, and parasitism by *M. plutellae* was <10% (Fig. 1). Beginning in mid-August, parasitism of *P. xylostella* larvae by *M. plutellae* was often as high, or higher than, that by *D. insulare*, and total parasitism was >50% for third instars and >80% for fourth instars. For the whole season, the average parasitism by both parasitoids was 33.6% for the third instars and 53.6% for the fourth instars; and 64.9% of the parasitism of third instars was caused by *D. insulare* and 33.0% by *M. plutellae.* For fourth instars, 56.5% of the parasitism was caused by *D. insulare*, whereas 41.7% was caused by *M. plutellae* (Fig. 2). The remaining parasitism was caused by *Oomyzus sokolowskii* (Kurdjumov) and an unidentified pteromalid species. A previously published report from a 2-yr field survey in Ithaca, NY (Pimentel 1961), indicated a much lower rate of parasitism of *P. xylostella* by *D. insulare* (35–52%) and *M. plutellae* (2–3%).

Susceptibility of *D. insulare* and *M. plutellae* Adults to Insecticides Commonly Used in Crucifer Crops. Among the four insecticides tested, *D. insulare* and *M. plutellae* adults were most sensitive to permethrin and diazinon, and least sensitive to carbaryl (Table 1). There were no significant differences between *D. insulare* and *M. plutellae* in susceptibility to the four insecticides tested, based on the overlap of the 95% FL values.

Some parasitoids are more tolerant of pyrethroid than organophosphate and carbamate insecticides (Plapp and Campanhola 1986), but this was not the case in our laboratory bioassay with *D. insulare* and *M. plutellae.* Using the Sevin XLR PLUS formulation of carbaryl in our bioassay may explain this phenomenon. A previous report showed that this formulation is less hazardous to honey bees than other carbaryl formulations when direct application to bees is avoided and the spray residues have dried (Hanny and Harvey 1982). So the formulation may also be important when

Table 1. Susceptibilities of *D. insulare* and *M. plutellae* adults to four insecticides

Insecticides	Species	*n*	Slope ± SE	LC_{50} (ppm [AI])	95% FL	χ^2
Permethrin	*D. insulare*	204	4.96 ± 0.65	4.87	4.40–5.40	0.77
	M. plutellae	204	4.25 ± 0.61	4.05	3.56–4.54	1.37
Carbaryl	*D. insulare*	204	1.99 ± 0.25	1,138	880–1,478	3.83
	M. plutellae	175	2.68 ± 0.36	721	578–897	0.94
Diazinon	*D. insulare*	210	5.29 ± 0.68	4.22	3.81–4.65	2.26
	M. plutellae	210	6.28 ± 0.80	3.91	3.56–4.26	1.89
Dimethoate	*D. insulare*	210	5.07 ± 0.65	10.90	9.87–12.08	1.15
	M. plutellae	210	5.69 ± 0.74	12.68	11.57–14.03	1.52

Table 2. The response of *D. insulare* and *M. plutellae* naïve and experienced females in dual-choice bioassay (intact cabbage versus damaged cabbage)

Treatment	No. of wasps tested	*D. insulare*		*M. plutellae*	
		No. landing on damaged plant	No. landing on intact plant	No. landing on damaged plant	No. landing on intact plant
Naive wasps	80	30 NS	20 NS	12 NS	5 NS
Experienced wasps	40	26***	3***	14**	2**

Asterisks indicate statistically significant preferences within the bioassay for species, $^{**}P < 0.01$; $^{***}P < 0.001$; NS, no significant preference for damaged or intact plant ($P > 0.05$).

evaluating the effects of insecticides on natural enemies.

Lower susceptibility to insecticides can be an important advantage for parasitoids in crops in which insecticides are still widely used. Differential susceptibility may also affect parasitoid establishment in fields and fulfillment of their biological control potential. In the Cameron Highlands of Malaysia, for example, the slow establishment of the intrinsically superior *Didegma semiclausum* Hellen relative to *Cotesia plutellae* (Kurdjumov) may have been due to its greater susceptibility to certain insecticides in use there (Chua and Ooi 1986, Ooi 1992, Furlong and Wright 1993, Wright and Verkerk 1995). For *D. insulare* and *M. plutellae* adults, no significant differences in their susceptibility to the four insecticides were detected with dose–response bioassays. Considering that insecticides can cause complicated sublethal effects on parasitoids (e.g., Kao and Tzeng 1990, Longley and Jepson 1996), more detailed research may be needed to evaluate subtle differences of the response of these two species to insecticides.

Host searching behavior of *D. insulare* and *M. plutellae*. There were no significant differences in the responses of the naive wasps between the plants damaged by two to five larvae and those damaged by 20 larvae (*D. insulare*, $\chi^2 = 1.933$, df = 2, $P = 0.380$; *M. plutellae*, $\chi^2 = 1.930$, df = 2, $P = 0.381$). Data from the two damage levels were therefore combined for subsequent analysis. For the naive wasps, *D. insulare* was much more active than *M. plutellae* (Table 2). Of the 80 naive *D. insulare* tested, 62.5% landed on one of the cabbage plants (either on the damaged plant or on the intact plant) within the 20 min observation period compared with only 21.2% of the *M. plutellae* ($\chi^2 = 27.963$, df = 1, $P = 0.001$). An even greater percentage of the experienced wasps showed a general response to the cabbage plants by landing on either the damaged or intact plant, with 72.5% of the experienced *D. insulare* and 40.0% the *M. plutellae* landing on a plant. For the experienced wasps, this difference between species was also highly significant ($\chi^2 = 8.584$, df = 1, $P = 0.003$). Of the wasps that landed on a plant, in all cases more selected the damaged than the undamaged plant (Table 2). However, the preference for damaged plants was statistically significant only for the experienced wasps (naive *D. insulare*, $\chi^2 = 2.00$, df = 1, $P = 0.157$; naive *M. plutellae*, $\chi^2 = 2.882$, df = 1, $P = 0.090$; experienced *D. insulare*, $\chi^2 = 18.241$, df = 1, $P = 0.001$; experienced *M. plutellae*, $\chi^2 = 9.000$, df = 1, $P = 0.003$). There was no significant difference between the species in the proportion of those wasps landing on plants that selected the damaged over the undamaged plant (naive wasps, $\chi^2 = 0.608$, df = 1, $P = 0.436$: experienced wasps, $\chi^2 = 0.048$, df = 1, $P = 0.826$). Therefore, the two species appeared to differ in their attraction to cabbage plants, but of those individuals that are attracted to plants, the two species appeared equally able to select damaged over undamaged plants.

Observations of the wasps indicated that those landing on damaged leaves continued to search for a host on the leaf, whereas >80% of wasps landing on leaves of intact cabbage flew away within 10 s. Our results demonstrated that some factors from the damaged cabbage-larvae complex affected the searching behavior of both *D. insulare* and *M. plutellae.* But these cues were not strong enough to permit naive wasps to locate their host within a short time, especially in the case of *M. plutellae.* Extended observations showed that >90% of the *D. insulare* searched around or on the damaged cabbage 2–3 h after release; whereas for the *M. plutellae*, >50% of the wasps still remained on the walls of the cage.

Bolter and Laing (1983) found that *M. plutellae* was able to locate and parasitize larvae on a cabbage leaf much faster than *D. insulare.* In their bioassay, they placed a cabbage leaf in a container and then female parasitoids were introduced. However, in our bioassay, most of the *D. insulare* landing on damaged leaves (both naive and experienced wasps) located the host successfully within 8–10 s and our data did not show that *D. insulare* took a longer time to locate their host. Differences between their results and ours may be due to the different methods. Our results indicated that *D. insulare* was better both in locating a damaged plant and in locating its host.

Important characteristics of effective parasitoids include high fecundity, high searching efficiency, adaptation to the environment, compatibility with other parasitoids, synchronization with the host (van Lenteren and Woets 1988), and low susceptibility to insecticides. Based on our field collections in 1999, *D. insulare* and *M. plutellae* provided high rates of parasitism of *P. xylostella.* Both species are present in the area. However, from our laboratory results, although no significant differences existed in susceptibility to insecticides between these two parasitoids, *D. insulare* appeared to be more efficient at locating its host. With its higher fecundity as shown in earlier studies (Putnam 1968, Bolter and Laing 1983) and better searching

ability as demonstrated here, *D. insulare* may be a better candidate for inoculative or inundative releases.

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