Effect of Insecticides and *Plutella xylostella* (Lepidoptera: Plutellidae) Genotype on a Predator and Parasitoid and Implications for the Evolution of Insecticide Resistance

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ABSTRACT In the laboratory and in cages in the greenhouse, we evaluated the toxicity of two insecticides (lambda-cyhalothrin and spinosad) on the parasitoid, *Diadegma insulare* (Cresson), and the predator, *Coleomegilla maculate* (DeGeer), both natural enemies of the diamondback moth, *Plutella xylostella* (L.). Lambda-cyhalothrin was very toxic to both natural enemies. Spinosad was less toxic to *C. maculata* adults and larvae, and slightly toxic to *D. insulare*. Both natural enemies suppressed *P. xylostella* populations in cages with 80% spinosad-treated and 20% nontreated plants; such suppression was not seen when lambda-cyhalothrin was used. Using broccoli, *Brassica oleracea* L. variety *italica*, a common host for *P. xylostella*, we also studied direct and indirect effects of both natural enemies in the presence and absence of the two insecticides and to different *P. xylostella* genotypes: resistant to the insecticide, susceptible, or heterozygous. Neither natural enemy could distinguish host genotype if *P. xylostella* were feeding on nontreated plants. They could also not distinguish between larvae feeding on spinosad-treated plants and nontreated plants, but *D. insulare* could distinguish between larvae feeding on lambda-cyhalothrin treated and nontreated plants. Our studies suggest that lambda-cyhalothrin has direct toxicity to these two natural enemies, can affect their host foraging and acceptance of *P. xylostella* and consequently would not be compatible in conserving these natural enemies in a program for suppression of *P. xylostella*. In contrast, our studies suggest that treatment with spinosad has much less effect on these natural enemies and would allow them to help suppress populations of *P. xylostella*. These findings are discussed in relation to the evolution of insecticide resistance and suppression of the pest populations.

KEY WORDS insecticide, resistance, host genotype, diamondback moth, biological control

The diamondback moth, *Plutella xylostella* (L.) (Lepidoptera: Plutellidae), is the most destructive insect pest of brassica crops in many parts of the world (Talekar and Shelton 1993, Grzywacz et al. 2010). This insect is now present wherever brassica crops are grown and is considered the most widely distributed of all Lepidoptera (Shelton 2004). Use of insecticides remains the main control strategy for *P. xylostella* because insecticides are easy to apply and often are cost-effective (Talekar and Shelton 1993). Unfortunately, some *P. xylostella* populations have evolved resistance to almost every insecticide class applied in the field, including pyrethroids, carbamates, organophosphates, spinosyns, avermectins, neonicotinoids, pyrazoles, and oxadiazines (Zhao et al. 2006, Grzywacz et al. 2010).

Biological control agents can be an integral component of integrated pest management (IPM) programs because they help suppress insect pest populations in agricultural ecosystems (Tillman and Mulrooney 2000, Sarfraz et al. 2005). Identification and conservation of such natural enemies has been identified as a key strategy needed for control of *P. xylostella* (Grzywacz et al. 2010). It has been reported that before 1917 in the United States and Europe (Marsh 1917, Mustata 1992), *P. xylostella* populations were suppressed to a level below economic thresholds solely by natural enemies.

Diadegma insulare (Cresson) (Hymenoptera: Ichneumonidae) is a solitary larval endoparasitoid of *P. xylostella* and is one of its most important biocontrol agents (Shelton et al. 2002). In North America, parasitism by *D. insulare* often exceeds 70% in the field (Xu et al. 2001, Hutchison et al. 2004). *Coleomegilla maculata* DeGeer (Coleoptera: Coccinellidae) is a common predator of aphids, thrips and eggs, and larvae of Lep-

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idoptera in many cropping systems throughout the United States (Roger et al. 2000). Larvae of all four instars, as well as adults, have been used widely in biological control programs (Rondon et al. 2006).

Insecticides remain important components of IPM programs for *P. xylostella* (Talekar and Shelton 1993, Grzywacz et al. 2010), despite the occurrence of resistant *P. xylostella* populations in some areas. Some newer insecticides are promoted as being softer on natural enemies and may be incorporated into IPM programs more readily. For example, spinosad is in the naturalyte class of insecticides and classified as a reduced risk insecticide (Williams et al. 2003). It is primarily absorbed in the gut and kills by causing rapid excitation of the insect nervous system (Salgado 1998). It has been registered for use on over 180 crops in the United States and in over 35 countries for control of caterpillars, beetles, leafminers, and thrips (Zhao et al. 2002). In contrast, lambda-cyhalothrin is a broad-spectrum pyrethroid insecticide with a long residual activity against many economically important agricultural pests, especially Lepidoptera (Liu et al. 1981).

Although these insecticides are used for managing *P. xylostella* populations in the field, they may also be detrimental to natural enemies. Although spinosad is generally considered nontoxic to natural enemies, some studies have reported it may be toxic to parasitoids (Murray and Lloyd 1997, Ruberson and Tillman 1999, Cossentine et al. 2010). In contrast, lambda-cyhalothrin is generally considered very toxic to beneficial insects (Tillman 1995, Ruberson and Tillman 1999) and has been shown to be toxic to *D. insulare* (Xu et al. 2001).

In this study, we compared the direct and indirect toxicity of the two insecticides, spinosad and lambda-cyhalothrin, on *D. insulare* and *C. maculata.* We also investigated the potential for more subtle effects by examining the host foraging and host acceptance behavior of both natural enemies in the presence or absence of the insecticides. This was done by determining whether either natural enemy could discriminate between different genotypes of *P. xylostella* (insecticide resistant [RR], susceptible [SS], or heterozygous [RS] individuals) and insecticide-treated or nontreated plants. Additionally, we conducted a series of large cage experiments in greenhouses over three generations of *P. xylostella* to determine how these insecticides, combined with *D. insulare* or *C. maculata*, affected the evolution of resistance to the insecticide.

Materials and Methods

Insects. Five strains of *P. xylostella* were used: 1) Pearl-RR strain, which is resistant to spinosad (Zhao et al. 2006); 2) Waipio-RR strain, which is resistant to lambda-cyhalothrin (Chen et al. 2008); 3) Geneva 88-SS strain, a susceptible strain that has been maintained on a wheat germ-casein artificial diet for over 500 generations (Shelton et al. 1991) without any changes in susceptibility; 4) Pearl-RS strain, which was created by crossing Pearl-RR with Geneva 88-SS, and: 5) Waipo-RS strain, which was created by crossing Waipo-RR with Geneva 88-SS. All strains were reared on an artificial diet and maintained in a climatic chamber at 27 ± 1°C, 50 ± 10% RH, and 16:8 L:D. The *C. maculata* originated from a laboratory population maintained by Pioneer Hi-Bred International, Inc. (Johnston, IA) and were reared on decapsulated eggs of brine shrimp, *Artemia franciscana* (Brine Shrimp Direct, Ogden UT) and a 1.5% agar solution provided separately. All stages of the insects were maintained in a climatic chamber at 27 ± 1°C, 50 ± 10% RH and 16:8 L:D. The population of *D. insulare* was originally field-collected in Florida and subsequently reared for >30 generations in our greenhouse, according to the procedures of Xu et al. (2001).

Insecticides and Plants. Commercial formulations of two insecticides were used: spinosad (SpinTor 2 SC, DowAgroSciences, Indianapolis, IN) and lambda-cyhalothrin (Warrior T, Syngenta Crop Protection, Greensboro, NC). Plants were treated with labeled field rates, converted to parts per million (90 ppm for spinosad and 80 ppm for lambda-cyhalothrin). To detect changes in resistance allele frequencies, diagnostic doses of 10 ppm for spinosad (Zhao et al. 2002) and 20 ppm for lambda-cyhalothrin (based on our preliminary experiment) were used. Potted broccoli plants, *Brassica oleracea* L. variety *italic* cultivar 'Packman,' with eight true leaves were sprayed with a small hand-held sprayer at 280 liter/ha.

Discrimination by *D. insulare* and *C. maculata* on Different Genotypes of *P. xylostella*. To determine whether the natural enemy could differentiate the genotype (related to insecticide susceptibility) of its host, choice tests were conducted in 1 × 1 × 1 m netted cages. Fifty *P. xylostella* second instars (either Pearl-RR, RS, and SS or Waipio-RR, RS, and SS) of a single genotype were placed on an untreated broccoli leaf, with its petiole inserted in a 100 ml flask filled with water 1 d before being exposed to each natural enemy. For each set of experiments (only one insecticide was examined per set) three flasks, each with a different genotype of *P. xylostella* (RR, RS, or SS), were placed in a triangle with equal distance (80 cm) between the flasks. Based on the results of our preliminary experiments, we decided to use two pairs of newly emerged *D. insulare* adults which were put into a Plexiglas cylinder cage (10 × 10 × 20 cm) with sugar water and allowed to mate for 3 d. Then the two pairs of 3-d-old adult *D. insulare* were released in the center of the triangle in each cage. A flask of 10% sugar solution with a cotton wick was placed in the center of the triangle in each cage as a food source for *D. insulare.* After 48 h, all *P. xylostella* larvae were removed from the cage and transferred to cups containing artificial diet (Shelton et al. 1991) and allowed to develop into *P. xylostella* adults or *D. insulare* adults. All 50 larvae from one leaf were placed in one cup. Parasitism rates [% parasitism = (number of *D. insulare*/ (number of *D. insulare* + number of *P. xylostella*) *100] associated with *D. insulare* on each genotype of *P. xylostella* were recorded. Each treatment (cage) was repli-

cated five times for the Pearl strains and four times for Waipio strains.

A similar choice experiment was conducted for *C. maculata.* Forty *P. xylostella* second instars (either Pearl-RR, RS, and SS or Waipio-RR, RS, and SS) of a single genotype were placed on an unsprayed broccoli leaf, with its petiole inserted in a 100 ml flask filled with water 1 d before being exposed to each natural enemy. One 30-ml cup with two 5-d old *C. maculata* adults (one female and one male) or three third-instars was placed in the center of the cage. Then the cup's cover was removed to free the *C. maculata* within the cage. Three cages with 40 *P. xylostella* second instars, but without *C. maculata*, served as controls to determine the number of missing or dead *P. xylostella* larvae. After 48 h, the number of remaining *P. xylostella* larvae were counted in the cages. Predation rates [% predation = (initial number of *P. xylostella* – number of remaining larvae – number of missing or dead larvae) / initial number of *P. xylostella* × 100] were recorded. The treatment for *C. maculata* larvae was replicated six times and the adult treatment four times.

Discrimination by *D. insulare* on Different Genotypes of *P. xylostella* on Lambda-Cyhalothrin or Spinosad-Treated Plants Hosting Different Genotypes of *P. xylostella.* To evaluate whether the parasitoids could discriminate between insecticide-treated plants hosting different genotypes of *P. xylostella*, a 2 × 3 design [(plant types: insecticide treated or not) × (*P. xylostella* genotypes: RR, RS, and SS)] was used. Individual leaves were removed from the plant and treated with an insecticide or left untreated as a control. Each cage (1 × 1 × 1 m) had six treatments: Pearl-RR, Pearl- RS, SS on a spinosad-treated leaf and the same genotypes on untreated leaves. Each treatment had 50 *P. xylostella* second instars (RR, RS, or SS) on each leaf. The six leaves in flasks were placed randomly in a rectangle with equal distance (50 cm) between the flasks. The *P. xylostella* larvae were allowed to feed for 1 d before being exposed to *D. insulare.* Four pairs of newly emerged *D. insulare* adults were put into a Plexiglas cylinder cage (10 × 10 × 20 cm) with sugar water and allowed to mate for 3 d. Then the four pairs of 3-d-old *D. insulare* were released into the center of each cage. After 48 h, *P. xylostella* larvae were recovered and transferred to cups containing artificial diet and allowed to develop into *P. xylostella* adults or *D. insulare* adults, as described above. Parasitism rates (%) associated with *D. insulare* on each genotype of *P. xylsotella* were recorded. The six treatments were replicated six times.

A similar experiment was conducted with lambda-cyhalothrin. Each cage (1 × 1 × 1 m) contained six treatments: Waipio - RR, Waipio - RS, SS on lambda-cyhalothrin-treated leaves and untreated leaves, respectively. Each treatment had 50 *P. xylostella* second instars (RR, RS, or SS) on each leaf and four pairs of 3-d-old *D. insulare* were released into the cage. Parasitism rates on each genotype of *P. xylostella* were recorded. The six treatments were replicated six times.

Discrimination by *C. maculata* on Spinosad-Treated Plants or Untreated Plants Hosting Resistant Genotypes of *P. xylostella.* Forty *P. xylostella* Pearl-RR second instars were placed on an untreated broccoli leaf and another 40 *P. xylostella* Pearl-RR second instars were placed on a spinosad-treated leaf. Each leaf petiole was inserted into a 100 ml flask filled with water 1 d before being exposed to a predator. One 30-ml cup with two *C. maculata* adults (one female and one male) or two third-instars was put in the center of the cage, as described above. Then the cup's cover was removed to free the *C. maculata* within the cage. After 48 h, the number of remaining larvae was counted. Three cages with 40 *P. xylostella* second instars, but without *C. maculata*, served as controls to determine the number of missing or dead *P. xylostella* larvae. Predation rates [% predation = (initial number of *P. xylostella* – number of remaining larvae – number of missing or dead larvae) / initial number of *P. xylostella* × 100] were recorded. The experiment was replicated six times.

Contact Toxicity of Spinosad and Lambda-Cyhalothrin to *D. insulare* and *C. maculata.* A broccoli leaf was dipped into a solution of spinosad at 90 ppm or lambda-cyhalothrin at 80 ppm and its petiole was inserted into a 100 ml flask filled with water and air-dried for 1 h. Then the treated leaf was placed into a Plexiglas cylinder cage (10 × 10 × 20 cm) and ten 2- to 3-d-old *D. insulare* females or ten 7-d-old *C. maculata* males or females were released into the cylinder cage. A broccoli leaf dipped into a nonionic surfactant served as the control. Corrected mortality (=(mortality rate of treated parasitoids or predators – mortality rate of control) / (1-mortality rate of control) was calculated after 24 h (Abbott 1925).

Effect of Spinosad-Treated and Lambda-Cyhalothrin Treated Plants on *D. insulare* and *C. maculata* Populations in a Greenhouse Cage Trial. The experiment was conducted in cages in greenhouses with the temperature ranging from 20° to 27°C during the course of the experiment. The experiment was conducted over three generations of *P. xylostella* or around 70 d. Each cage was 1.8 m long × 0.9 m wide × 1.7 m high and constructed of nylon netting. Four treatments were included: 1) 80% spinosad-treated broccoli and 20% untreated broccoli plants with *D. insulare;* 2) 80% spinosad-treated broccoli and 20% untreated broccoli plants with *C. maculata;* 3) 80% lambda-cyhalothrin treated broccoli and 20% untreated broccoli plants with *D. insulare;* 4) 80% lambda-cyhalothrin treated broccoli and 20% untreated broccoli plants with *C. maculata.* The 20% untreated plants represented a refuge for *P. xylostella* and the natural enemies. Such refuges occur naturally in an agricultural setting because of poor spray coverage or weed hosts, or could be deliberately incorporated as a tactic to preserve natural enemies or susceptible alleles in the pest population.

The goal of this study was to assess *P. xylostella, D. insulare*, and *C. maculata* population abundance in the cages with insecticide-treated and untreated plants and to follow the evolution of insecticide resistance in the *P. xylostella* populations. Therefore, two populations of *P. xylostella*, each with a known insecticide

Table 1. Parasitism by *D. insulare* and predation by *C. maculata* on different genotypes of *P. xylostella* feeding on untreated broccoli

	Pearl			Waipio		
	RR	RS	SS	RR	RS	SS
Parasitism %	35.6 ± 12.93a	48.5 ± 8.45a	48.9 ± 9.01a	38.5 ± 12.53a	39.6 ± 13.56a	49.5 ± 11.72a
Predation %						
Adult	58.1 ± 6.56a	61.9 ± 7.10a	44.4 ± 2.37a	59.4 ± 6.24a	45.6 ± 3.29a	41.3 ± 5.25a
Larvae	67.8 ± 4.36a	51.1 ± 6.25a	50.6 ± 6.29a	66.7 ± 4.13a	60.0 ± 8.12a	62.2 ± 7.63a

Means (±SEM) within the same row followed by a same letter are not significantly different ($P > 0.05$; Tukey test); Pearl and Waipio RR strains are resistant to spinosad and lambda-cyhalothrin, respectively. The SS populations are the Geneva 88 strains, whereas the RS strains resulted from the cross of a RR strain and the Geneva SS strain.

resistance allele frequency, were needed and these synthetic populations were developed in a manner similar to that described by Zhao et al. (2005). One population was created by releasing 80 Pearl-RS moths and 120 G88 (S) moths into a Plexiglas cylinder cage ($10 \times 10 \times 20$ cm) to prepare a population for treatments 1 and 2. The other was created by releasing 80 Waipio RS and 120 G88 moths into a similar Plexiglas cylinder cage to prepare a population for treatments 3 and 4. The total number of moths was 200 with a 1:1 ratio of female and male moths for each population. Eggs were collected from the cylinder cage and put on artificial diet to rear F1 larvae. We used ≈1,000 F_1- F_3 moths to produce F_2-F_4 eggs of the synthetic population. The expected resistant allele frequency of both populations was 0.2. The actual survival of unselected F_3 larvae was 0.95% on spinosad-treated plants and 0.67% on lambda-cyhalothrin treated plants. Based on the actual survival, the actual initial allele frequency (square root of survival rate) at the start of the experiment was estimated to be 0.08 for spinosad resistance and 0.10 for lambda-cyhalothrin resistance. Two hundred F_4 pupae of each synthetic *P. xylostella* population were released into each cage. According to results from our preliminary experiments, we used three pairs of newly emerged *D. insulare* adults that we placed into a Plexiglas cylinder cage ($10 \times 10 \times 20$ cm) with sugar water to mate for 3 d. Then the three pairs of *D. insulare* or three pairs of *C. maculata* that had emerged within the week were released into each cage when *P. xylostella* larvae were mostly second instars.

There were three replications (=cages) for each treatment for a total of 12 cages, and each cage contained 15 plants (12 insecticide-treated plants plus three untreated plants). Individual plants were replaced at each insect generation or when the plants were severely defoliated and did not provide sufficient food for the *P. xylostella* population. The number of *C. maculata* and *P. xylostella* larvae (primarily third or fourth instars) and pupae on the broccoli plants was counted every generation when larval and pupal densities reached a plateau. The number of *D. insulare* pupae on broccoli plants was counted 3 d after most of *P. xylostella* pupated.

To document changes in resistance allele frequency, ≈25 larvae from the untreated plants were collected from each cage at the third generation and reared on artificial diet (Shelton et al. 1991) to the adult stage. The F_1 offspring of the adults were reared on artificial diet to the second-instar. Then they were bioassayed in 30-ml plastic cups using insecticide-treated cabbage leaf disks with the respective discriminating doses of 10 ppm for spinosad and 20 ppm for lambda-cyhalothrin. Untreated cabbage leaf disks were used as controls. In total, 100 larvae were tested (10 replications, 10 larvae/rep) for each cage. Survival was determined after 24 h at 27 ± 1°C.

Statistical Analysis. Percent parasitism and predation rates were transformed by using the arcsine square-root, then were analyzed using one-way ANOVA, and differences between treatment means were separated using Tukey's test at a 5% level of significance. The statistical analyses were conducted using SPSS 17.0 Windows (1998) (SPSS, Chicago, IL).

Populations of *P. xylostella*, *D. insulare*, and *C. maculata* were transformed by log ($x + 1$) and analyzed using repeated measure analysis of variance (ANOVA) in the SAS LIFETEST procedure, and difference between spinosad-treated cages and lambda-cyhalothrin treated cages were separated based on *t*-test ($P < 0.05$ or $P < 0.01$) using SAS version six package (SAS Institute 2001).

Results

Discrimination by *D. insulare* and *C. maculata* on Different Genotypes of *P. xylostella*. For the Pearl and Waipio strains, parasitism rates associated with *D. insulare* with RR, RS, and SS genotypes on untreated broccoli plants were not significantly different (Pearl: $F = 0.543$, df = 12, $P = 0.595$; Waipio: $F = 0.229$, df = 9, $P = 0.800$; Table 1). For both strains, there were no significant differences in predation rates associated with *C. maculata* adults (Pearl: $F = 2.570$, df = 9, $P = 0.131$; Waipio: $F = 3.470$, df = 9, $P = 0.076$) or larvae (Pearl: $F = 2.944$, df = 15, $P = 0.083$; Waipio: $F = 0.245$, df = 15, $P = 0.786$).

Discrimination by *D. insulare* on Different Genotypes of *P. xylostella* on Lambda-Cyhalothrin or Spinosad-Treated Plants Hosting Different Genotypes of *P. xylostella*. Because RS and SS larvae did not survive on spinosad-treated or lambda-cyhalothrin treated broccoli plants, no parasitoids emerged. However, parasitism rates because of *D. insulare* were similar when RR larvae (62.9 ± 7.5%) fed on spinosad-treated broccoli, and RR (67.0 ± 4.7%), RS (62.7 ± 11.5%), and SS (66.2 ± 3.6%) larvae fed on untreated broccoli plants ($F = 0.088$; df = 12; $P = 0.965$). In contrast, for lambda-cyhalothrin, parasitism rates were 62.1 ± 6.4,

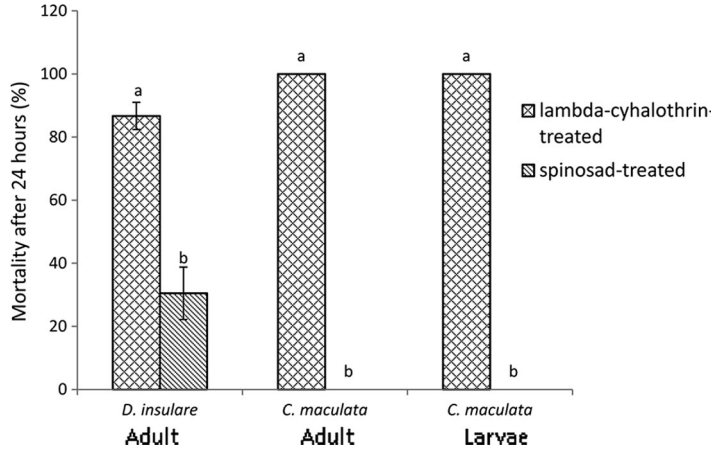

Fig. 1. Direct mortality of *D. insulare* and *C. maculata* treated with spinosad or lambda-cyhalothrin after 24 h. Means (±SE) with different letters are significantly different based on Tukey's test ($P < 0.05$).

60.2 ± 3.9, and 59.8 ± 6.9% when Waipio-RR, Waipio-RS, and SS larvae fed on untreated broccoli, but 20.3 ± 5.8% when Waipio-RR larvae fed on treated broccoli. There were significant differences among the six treatments ($F = 23.930$; df = 30; $P < 0.0001$).

Discrimination by *C. maculata* on Spinosad-Treated Plants or Untreated Plants Hosting Resistant Genotypes of *P. xylostella*. Predation by *C. maculata* adult was 57.7 ± 6.4% and 55.4 ± 3.2% when they fed on RR *P. xylostella* reared on spinosad-treated or untreated plants, respectively. Predation by *C. maculata* larvae was 67.9 ± 3.2% and 60.0 ± 5.8%, respectively. There were no significant differences in predation by *C. maculata* adults and larvae on sprayed or unsprayed broccoli plants (adult: $t = 0.415$, df = 5, $P = 0.695$; larvae: $t = 1.327$, df = 5, $P = 0.242$).

Contact Toxicity of Spinosad and Lambda-Cyhalothrin to *D. insulare* and *C. maculata*. Lambda-cyhalothrin was toxic to *D. insulare* and *C. maculata* with mortality rates of 86.7 ± 4.5% for *D. insulare* and 100% for *C. maculata* adults and larvae (Fig. 1). The mortality rate for *D. insulare* was 30.5 ± 8.7% when they were exposed to a spinosad-treated leaf. There was no mortality of *C. maculata* adults and larvae when exposed to spinosad.

Effect of Spinosad-Treated and Lambda-Cyhalothrin Treated Plants on *D. insulare* and *C. maculata* Populations in a Greenhouse Cage Trial. The *P. xylostella* populations in the lambda-cyhalothrin treated cages with *D. insulare* were significantly higher than in the spinosad-treated cages for the first generation ($t = -4.00$; $P = 0.0317$), but there were no significant differences ($F = 3.25$; df = 4; $P = 0.1458$) in the second and third generations (Fig. 2A). However, *P. xylostella* populations for both treatments decreased significantly with increasing generations (generations: $F = 41.62$; df = 8; $P < 0.0001$). The *D. insulare* population in the spinosad-treated cages was significantly higher than in the lambda-cyhalothrin treated cages in the second generation ($F = 10.10$, df = 4, $P = 0.0336$; Fig. 2B). There were significant differences in the ratio of *D. insulara*/*P. xylostella* between spinosad treated and lambda-cyhalothrin treated cages ($F = 38.99$, df = 4, $P = 0.0034$; Fig. 2C), again suggesting that spinosad was less harmful than lambda-cyhalothrin to *D. insulare*. The ratios increased significantly with increasing generations ($F = 106.77$; df = 8; $P < 0.0001$), suggesting longer-term conservation of this parasitoid in the system.

Populations of *P. xylostella* in the lambda-cyhalothrin treated cages with *C. maculata* were significantly higher ($F = 113.48$; df = 4; $P = 0.0004$) than in the spinosad-treated cages in the first three generations (Fig. 3A). No *C. maculata* survived in the lambda-cyhalothrin treated cages (Fig. 3B). However, *C. maculata* survived in the spinosad-treated cages and were significantly higher ($F = 17.02$; df = 8; $P = 0.0013$) in all three generations. The ratio of *C. maculata*/*P. xylostella* populations significantly increased ($F = 123.11$; df = 8; $P < 0.0001$) with increasing generations (Fig. 3C), again indicating the safety of spinosad to *C. maculata*.

Evolution of insecticide resistance in *P. xylostella* population was also assessed in the large cage experiment. Survival of *P. xylostella* was 1.0 ± 0.7% on spinosad-dipped cabbage leaves after three generations of selection in the spinosad-treated cages with *C. maculata*. Because 85% of the collected larvae were parasitized by *D. insulare* in the spinosad-treated cages, only 12 *P. xylostella* pupae were found. Survival of the offspring larvae was 0% on spinosad-dipped cabbage leaves. Spinosad resistance allele frequency in *P. xylostella* was around 0.1, almost the same as in the beginning of the experiment. In contrast, larval survival was 17.0 ± 3.9% and 12.5 ± 3.3% on lambda-cyhalothrin-dipped cabbage leaves in the lambda-cyhalothrin treated cages with *D. insulare* and lambda-cyhalothrin treated with *C. maculata*, respectively. Resistance allele frequency of *P. xylostella* for lambda-cyhalothrin resistance was 0.36 in the cages with *D. insulare* and 0.30 with *C. maculata*, which was a significant ($F = 11.247$; df = 2; $P < 0.0001$) increase over three generations of selection.

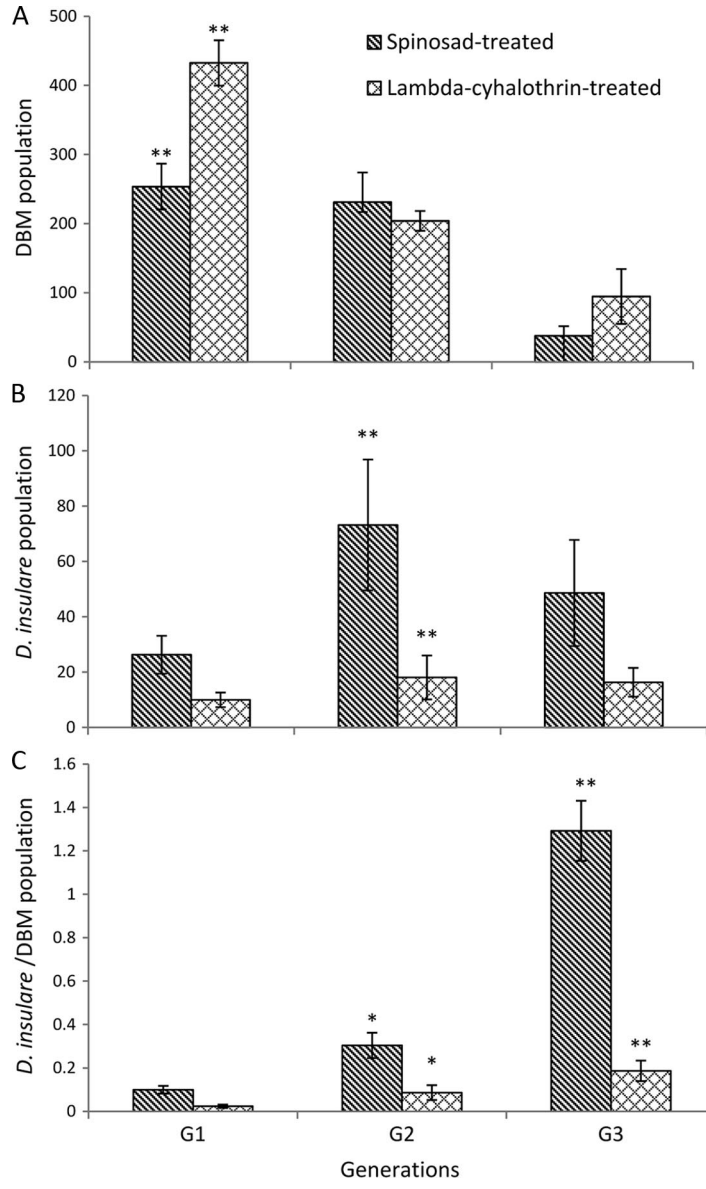

Fig. 2. (A) *P. xylostella* (DBM) population abundance, (B) *D. insulare* population abundance, (C) ratio of *D. insulare*/DBM population abundance. Means (±SE) marked with "*" or "**" are significantly different between spinosad-treated cages and lambda-cyhalothrin treated cages based on *t*-test ($P < 0.05$; $P < 0.01$).

Discussion

Insecticides and biological control can be effective in reducing insect pest populations. Our current study focused on direct and indirect effects of two important insecticides on natural enemies of *P. xylostella*, *D. insulare*, and *C. maculata.* However, our study also investigated potential interactions between resistant genotypes and natural enemies. Such effects could alter resistance evolution if natural enemies displayed a preference for a particular genotype. In our current study, resistance allele frequencies in the spinosad-treated cages did not change appreciably over three generations of selection, but resistance significantly increased in the lambda-cyhalothrin treated cages. We conclude that resistance evolution in the lambda-cyhalothrin sprayed treatment occurred faster than in the spinosad treatment because of its deleterious effects on natural enemies. Further studies over additional generations should be conducted to verify this trend.

Because field populations of *P. xylostella* have been challenged by many insecticides and developed resistance to some, one would expect to find resistant, heterozygous and susceptible larvae in the field at any

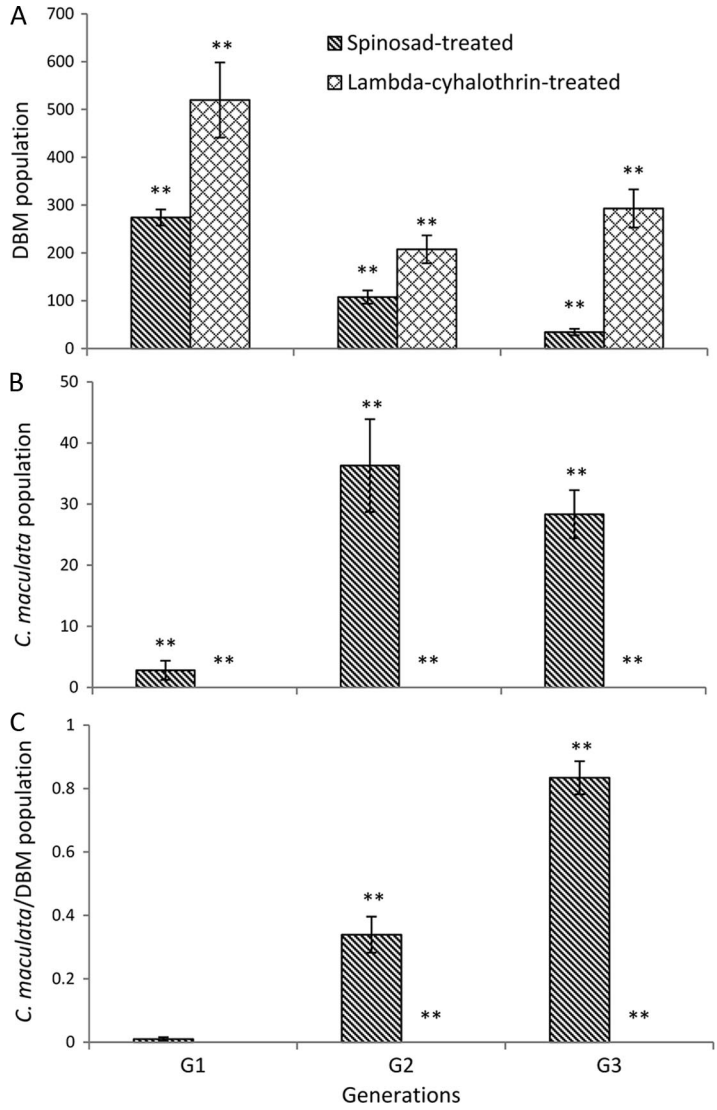

Fig. 3. (A) *P. xylostella* (DBM) population abundance, (B) *C. maculata* population abundance, (C) ratio of *C. maculata*/DBM population abundance. Means (±SE) marked with "**" are significantly different between spinosad-treated cages and lambda-cyhalothrin treated cages based *t*-test ($P < 0.01$).

given time. If natural enemies have a preference for resistant individuals, then this would reduce the likelihood of the population becoming resistant to the insecticide (Gould et al. 1991). Our results indicate that parasitism rates of *D. insulare* and predation rates of *C. maculata* are not significantly different regardless of genotype (Table 1). Therefore, we conclude that both natural enemies do not discriminate between different host genotypes. If they had preferred one genotype, this would have altered resistance evolution (Onstad 2008, p. 219).

In this study, we determined the direct and indirect effects of two insecticides on *D. insulare.* For the spinosad-treated leaves, parasitism rates were not significantly different when resistant *P. xylostella* fed on treated broccoli leaves or when RR, RS, and SS hosts fed on untreated broccoli leaves. In fact, no parasitoids emerged when RS and SS *P. xylostella* fed on treated broccoli leaves because the host (*P. xylostella*) did not survive. For lambda-cyhalothrin treated leaves, parasitism was significantly lower when RR *P. xylostella* fed on treated broccoli, compared with RR, RS, and SS *P. xylostella* that fed on untreated broccoli. Furthermore, there were no interactions for treatment and *P. xylostella* genotypes on the spinosad-treated leaves, but the parasitoid could discriminate between treated and untreated lambda-cyhalothrin leaves. We believe this is the first study to address the effects of insecticides on the foraging behavior and host acceptance of *D. insulare.* For *C. maculata*, adults and larvae did not show any preference between spinosad-treated and untreated leaves.

Spinosad is classified as an environmentally and toxicologically reduced-risk insecticide by the United States Environmental Protection Agency (EPA 1997). Our results showed that spinosad was nontoxic to *C. maculata* adults and larvae, but inhibited survival of *D. insulare* when the parasitoid contacted the broccoli treated with spinosad. Galvan et al. (2005a) reported that spinosad decreased survival of first instars of the ladybird *Harmonia axyridis* (Pallas), extended their development time, decreased their weight gain, and reduced oviposition under laboratory conditions. However, they found the density of *H. axyridis* larvae in sweet corn plots treated with spinosad did not significantly differ from untreated plots in the field (Galvan et al. 2005b).

Research has focused on the direct or indirect effects of insecticides, including spinosad, on *D. insulare* (Hill and Foster 2000, 2003; Xu et al. 2001, 2004; Cordero et al. 2007; Cossentine et al. 2010). Xu et al. (2004) showed that spinosad caused 100% mortality of *D. insulare* adults, but did not reduce adult emergence when *D. insulare* pupae were treated. Cordero et al. (2007) observed ≈80% mortality of *D. insulare* after 24 h at the field-use rates of spinosad. Hill and Foster (2000) reported spinosad caused 100% mortality of *D. insulare* 8 h after treatment under laboratory conditions. However, in a follow-up study, parasitism of *P. xylostella* larvae was not affected by spinosad application under field conditions (Hill and Foster 2003). In general, it appears that hymenopteran parasitoids are more susceptible to spinosad than predatory insects (Williams et al. 2003). Most of the above laboratory-based experiments were conducted in petri dishes, but we used larger cages ($10 \times 10 \times 20$ cm) that may have allowed higher survivorship. In our greenhouse experiments with spinosad-sprayed plants and 20% refuge plants, $>$ 60 *D. insulare* pupae were found in the second generation, although we released only three pairs of *D. insulare* adults at the beginning of the experiment. This again suggests the relative safety of spinosad to *D. insulare.*

That broad-spectrum pyrethroids are generally toxic to beneficial insects has been well documented by previous research (Tillman 1995; Ruberson and Tillman 1999; Tillman and Mulrooney 2000; Galvan et al. 2005a,b). We found the pyrethroid lambda-cyhalothrin to be highly toxic to both *C. maculata* and *D. insulare.* In the cages with 80% lambda-cyhalothrin treated plants and 20% refuge plants, no *C. maculata* were found for three generations. Although >80% adult mortality of *D. insulare* was observed in the laboratory on lambda-cyhalothrin treated plants, some *D. insulare* adults survived and reproduced in the cages with 80% lambda-cyhalothrin treated plants and 20% refuge plants. More than 20 *D. insulare* adults were present at the second and third generations, but all were on refuge plants. Based on the above facts and low parasitism rates when RR *P. xylostella* fed on lambda-cyhalothrin treated plants, it may be that *D. insulare* could discriminate between lambda-cyhalothrin treated and untreated plants and avoid the former.

P. xylostella remains a pervasive and intransigent pest to manage using either synthetic insecticides or biological control alone (Shelton 2004). Our results (Fig. 3) indicate that *P. xylostella* populations remained high at the third generation with 80% lambda-cyhalothrin treated plants and 20% untreated plants because all *C. maculata* were killed by lambda-cyhalothrin. However, *P. xylostella* population growth was reduced in the cages with 80% spinosad-treated plants and 20% nontreated plants when *C. maculata* were included. We found some *D. insulare* survived on refuge plants although lambda-cyhalothrin was highly toxic to the parasitoid (Fig. 2). Thus, a refuge to maintain natural enemies may be helpful when insecticides are used as a component of IPM.

In summary, both natural enemies could not distinguish host genotype if *P. xylostella* fed on untreated plants so this would not be a factor influencing the evolution of resistance in this system. Furthermore, they could not distinguish between spinosad-treated and untreated plants, but *D. insulare* could avoid lambda-cyhalothrin treated plants, which may influence the evolution of resistance. In general, we concluded that lambda-cyhalothrin was more disruptive to these two important natural enemies, and that appropriate treatment with spinosad can control *P. xylostella* and still be compatible with biological control by these two natural enemies. This in turn may help slow the evolution of resistance to spinosad.

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