Predation of Colorado potato beetle eggs by a polyphagous ladybeetle in the presence of alternate prey: potential impact on resistance evolution

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Abstract

The influence of prey choice on the predation of a target prey item by a polyphagous insect predator was investigated in field plot studies. The target prey consisted of eggs of the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), and the predator was the 12-spotted ladybeetle, *Coleomegilla maculata* Lengi (Coleoptera: Coccinellidae). Eggs of the European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae), and nymphs and adults of the green peach aphid, *Myzus persicae* Sulzer (Homoptera: Aphididae), comprised the alternative prey choices. The objectives of these studies were to: (1) examine predation in a multiprey scenario likely to occur in an agroecosystem, and (2) use the data to simulate the impact of predator-induced mortality on the evolution of resistance to Bt-transgenic plants in the target herbivore. Simulations of the rate of resistance evolution were carried out using a deterministic genetic model. Experiments were performed using potato field plots planted in a manner reflecting a 25% or 50% non-transgenic refuge. CPB eggs were infested so as to mimic the densities of resistant and susceptible populations that might occur in commercial Bt-transgenic plantings. Densities of predators and alternate prey species were chosen to represent those that might typically occur in potato crops in the eastern USA. Simulation results indicated that when ECB eggs were present, predation on CPB eggs either became inversely spatially density-dependent, or increased significantly in a density-dependent manner. When aphids were present, predation became positively density-dependent. Model simulations predicted that ECB egg presence is beneficial, in that resistance was delayed by up to 40 pest generations (as compared to the scenario with CPB as the only prey), while aphid presence accelerated resistance evolution by 18 generations. Results suggest that resistance management strategies should take into account the composition of prey species available to generalist predators typically present, so as to best delay pest adaptation to Bt-toxins.

Introduction

Recent advances in genetic engineering techniques have allowed the development of transgenic crops that express high levels of insecticidal toxins isolated from the bacterium *Bacillus thuringiensis* (Bt). Because current Bt toxin-expressing plants produce the toxin all season long in all plant parts there is a high level of selection for resistant pest genotypes. The use of refugia to foster the presence of susceptible pest populations near transgenic crops is part of the recommended deployment of these crops (Roush & Tabashnik, 1990; Gould et al., 1994; Tabashnik, 1994). These susceptible insects are expected to mate with any resistant insects that survive in the Bt crops and result in heterozygous progeny that cannot survive on the Bt crop.

Theoretical work has explored various aspects of the ways in which pathogens, predators, and parasitoids of

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arthropods could potentially affect resistance evolution in pests exposed to *Bt* crops (Gould et al., 1991; Gould et al., 1994). However, with a few exceptions (Johnson & Gould, 1992; Arpaia et al., 1997; Riggin-Bucci & Gould, 1997), field studies have not been conducted to confirm or refute the assumptions of this theoretical work. In one such study, Arpaia et al. (1997) found inverse spatial density-dependent predation of Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), eggs by *Coleomegilla maculata* Lengi (Coleoptera: Coccinellidae). Computer simulations based on their data indicated that predation by *C. maculata* would delay resistance evolution (by adding approximately 66 generations to the 'time' required for a hypothetical resistance allele to reach a frequency of 0.5 in a pest population).

Until now, this has been the only study to provide data from field experiments that could be used to simulate resistance evolution in the presence of an insect predator. However, these studies were inherently unrealistic, in that the field plots used did not contain herbivores such as lepidopteran larvae and aphids, which frequently occur in commercial potato fields (Obrycki & Tauber, 1985; Hazzard & Ferro, 1991; Nault & Kennedy, 1996). These insects can serve as alternative prey for generalist predators such as *C. maculata* and thus influence their consumption of CPB eggs. Furthermore, Arpaia et al. (1997) used a relatively high density of *C. maculata* (6–7 beetles per plant), a level that is more comparable to that observed in tasselling (pollinating) corn, where *C. maculata* aggregates to feed on pollen (Wright & Laing, 1980; Coll & Bottrell, 1991).

In the experiments presented here, we examined predation on CPB populations, in a multi-prey scenario that a generalist arthropod predator such as *C. maculata* would be likely to face in a *Bt*/refuge potato planting. These studies were carried out to test the hypothesis that the presence of one or more alternative prey species would alter the predation of *C. maculata* on different densities of CPB eggs in a way that would be expected to impact the rate of resistance evolution in CPB populations. The two alternative prey species we used were the European corn borer (ECB), *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae), and the green peach aphid (GPA), *Myzus persicae* Sulzer (Homoptera: Aphididae). These insects occur frequently in potato crops (Mack & Smilowitz, 1978; Obrycki & Tauber, 1985; Nault & Kennedy, 1996), and are apparently immune to the toxic effects of the Bt endotoxin expressed by CPB-resistant Bt-potatoes (Hofte & Whiteley, 1989; Hruska & Pavon, 1997). In addition to incorporating greater realism by adding prey choice for the predator involved, this study used a density of *C. maculata* within the range reported to co-occur most often with CPB populations in potato in the eastern USA (Groden et al., 1990; Hazzard et al., 1991).

Materials and methods

Insect sources

Colonies of *C. maculata* were established from shipments of adults from the USDA Mission Biocontrol Laboratory in Mission, Texas. These were supplemented with periodic field collections of adults made in cornfields in Beltsville, and in stands of aquatic weeds at the Patuxent Wildlife Research Center, Maryland. Ladybeetles were maintained in the laboratory on a diet of fruit-fly (*Anastrepha ludens* Loew) eggs mixed in with a wheat germ based artificial diet, and bee pollen. European corn borer eggs were purchased from Lee French Laboratories (Lamberton, Minnesota). The Philip Alampi Biocontrol Laboratory at the New Jersey Department of Agriculture (West Trenton, New Jersey) supplied the Colorado potato beetle eggs. Aphids were established on potato plants (cv. 'Atlantic') at the University of Maryland from colonies collected on potatoes in their greenhouses. Colonies of *C. maculata* were established from shipments of adults supplied by the USDA Mission Biocontrol Laboratory in Mission, Texas. These were supplemented with periodic field collections of adults made in cornfields in Beltsville, and in stands of aquatic weeds at the Patuxent Wildlife Research Center, Maryland. Ladybeetles were maintained in the laboratory on a diet of fruit-fly eggs mixed in with a wheat germ based artificial diet, and bee pollen. Experiments were conducted in two growing seasons, 1996 and 1997. Experiments in both years had a similar overall design. However, an important difference was that in 1996, only eggs of the European corn borer (ECB) were used as the alternate prey species, while in 1997 both ECB eggs and green peach aphids were used.

Experimental procedures and statistical analysis: 1996 experiment

Six plots of transgenic and non-transgenic potatoes (cv. 'Atlantic') were planted on one site at the Central Maryland Research and Education (CMREC) farm in Upper Marlboro, Maryland. The plots measured 4×4 m, and contained four rows of potatoes, of which two were transgenic and two were the non-transgenic sister isoline (mimicking a 50% refuge planting). There were 10 plants per row in each plot.

Large nylon-mesh field cages, $4 \times 4 \times 3$ m, were placed over the plots when the plants were 1 week old. Each plot was completely enclosed in a separate cage. On the first day of each of two trials, 35–40 *C. maculata* (a density of one per plant) were released onto the soil in the center of each cage. *Coleomegilla maculata* were starved for 24 h prior to use, and a 1 : 1 ratio of males to females was used. Experiments were terminated after 3 days, a time-span within the duration of the CPB egg stage (Ferro et al., 1985). At that

point, all the CPB egg masses were removed and examined for predation, using a stereo dissecting microscope. The presence or absence of ECB eggs as an alternate prey was treated as a 'whole-plot' treatment factor (referred to hereafter as the 'prey treatment'). CPB density was treated as a 'split-plot' treatment factor. The plots were arranged so that the experimental design was a randomized complete block, with cage location within the site treated as the blocking factor. The experiment was repeated twice, using the same plots, but with the whole-plot treatments re-randomized across the site. The second trial was performed approximately 2 weeks after the end of the first, and all the ladybeetles were removed from cages prior to the start of the second trial.

Prior to the start of a trial, all plots were surveyed for the presence of any predatory or herbivorous insects that might have colonized the plants, and these insects were removed by hand. No aphids or mites colonized any of the plots before the cages were in place, though there were some lepidopteran larvae and potato leafhoppers (*Empoasca fabae* Harris) throughout. Leafhoppers were allowed to remain in the plots, as it has been shown that they are not acceptable prey for *C. maculata*, which rejects them even in a no-choice situation (Yadava & Shaw, 1968).

Transgenic plants were infested with 0.1 CPB egg mass/plant (one egg mass per 10 plants), simulating a resistant CPB population at a 'low' density. The non-transgenic row received one egg mass per plant as the 'high' density, representing a susceptible CPB population. All egg masses were modified using fine-point dissecting tweezers to contain 40 eggs per mass. Egg masses were checked to make sure that they contained only intact eggs, to avoid the possibility that the predators might orient to volatile chemicals released from damaged eggs. In cages receiving alternate prey, ECB egg masses were placed on randomly chosen plants at a density of one egg mass for every five plants (or 0.2 egg masses per plant). Chosen ECB egg masses were between 0.5 and 1 cm in diameter. All egg masses were pinned onto the undersides of randomly chosen, fully expanded leaves using insect pins.

Since the same plots were used for both trials, data expressed as the proportion of CPB eggs eaten were analyzed using repeated-measures ANOVA [PROC MIXED in SAS (SAS Institute, 1996)]. Block (cage location) was treated as a random effect in the ANOVA model, while CPB density and prey treatment were treated as fixed effects. A variety of different covariance structures were examined for goodness-of-fit using Akaike's Information Criterion (AIC), and the one with the largest AIC value (indicating the best fit) was selected for further tests of significance for random and fixed effects in the model (Littel et al., 1998). If heteroscedascity of variances was indicated, the data were adjusted using an arcsine-square-root transformation before being subjected to ANOVA. If the ANOVA indicated a significant treatment effect, pair-wise means comparisons were made using Tukey's HSD test of significance.

Experimental procedures and statistical analysis: 1997 experiment

For this experiment, plots of transgenic and non-transgenic potatoes (cv. 'Atlantic') were planted at four sites on the CMREC farm. One to 2 days after the emergence of plants from the soil, $4 \times 4 \times 3$ m fine wire-mesh cages were placed over the plots. As in the previous season, the plots measured 4×4 m. However, in these plots, three rows of potatoes were transgenic and one was the non-transgenic sister isoline (this represented a 25% refuge). The refuge size was reduced as compared to the previous year's experiment so as to reflect the smaller refuge sizes that were being recommended for commercial plantings of Bt-transgenic potato in 1997 (F. Gould, unpubl.) Once again, one *C. maculata* adult per individual plant was released into the center of each cage (a total of 36–40 ladybeetles per cage).

Trials examining predation on the two CPB densities created in each plot, both with and without the two alternate prey species added, were repeated twice over the growing season – in July and August A new set of field plots, 2 m away from the prior planting, was used for each new experimental trial. The prey species assigned to each plot were rotated across sites for each new experiment. Thus, each new set of field plots provided replicates of the prey treatments within the overall experiment. The experimental design utilized was an incomplete randomized double-block split-plot, with CPB egg density as the split-plot factor, and prey species as the whole-plot treatment factor. The two blocking factors were site and time period (July or August). Two cages were used at each of the plots, thus providing two replicates for each prey species treatment within each time period.

In cages with ECB as an alternate prey species, one ECB egg mass for every two plants (a density of 0.5 egg masses per plant) was placed on all plants within each cage (i.e., a constant level of ECB eggs on both 'low' and 'high' CPB density plants). Despite the placement of cages over the plants as soon as they emerged from the soil, aphids (*Myzus persicae*) managed to colonize all plots during all experimental trials. Thus, for those cages assigned aphids as an alternate prey, visual counts were made of aphids on 100 leaves per row. When the numbers in all cages sampled reached 100–200 per row, the experiment was begun. In cages not assigned aphids as prey, a commercial insecticide containing botanical pyrethrum and soap (Safer, Inc.) was applied to all plants using a backpack sprayer, 48 h before

an experiment began. Visual sampling (in the manner described above) confirmed the absence of aphids in sprayed cages immediately prior to, and immediately following, each experiment. Experiments subsequently conducted in a greenhouse setting showed no significant lethal or sublethal effects of the soap spray on ladybeetles placed on plants 24 h or more after treatment (N. Mallampalli and P. Barbosa, unpubl.).

The number of predators in each row was recorded daily for each of the 3 days over which each experiment was conducted. At the end of the 3 days, all CPB egg masses were retrieved, and visual estimates of aphid populations were recorded. CPB egg masses were scored for the number of eggs eaten. Data were analyzed using the PROC MIXED procedure in SAS (SAS Institute, 1996) to conduct the ANOVA, since there were both random and fixed treatment effects in the experimental design. Site and cage replicates were treated as random effects in the ANOVA model; all other treatment factors were treated as fixed effects. Pair-wise means comparisons were made using Tukey's HSD test.

Genetic modeling of resistance evolution

Predation data from both the 1996 and 1997 experiments were used to calculate the fitness of the three possible CPB genotypes involved in the inheritance of a hypothetical resistance ('R') allele, vs. a 'susceptibility ('S') allele, where resistance and susceptibility refer to CPB sensitivity to the Bt-toxin. Assuming simple Mendelian genetic inheritance, these alleles give rise to three genotypes: RR (homozygotic resistant), RS, and SS (homozygotic susceptible). Gould's (1986) genetic model, which simulates resistance evolution iteratively, calculates the fitness of a population composed of these three genotypes in each successive generation, based on starting values of genotype fitness that are input by the user. The model computes a frequency of the R allele in each generation based on these fitness values. Thus, the model can compute the number of generations needed for the frequency of the R allele to reach 1.0 (i.e., be represented twice in every individual in the population).

By calculating the percentage of CPB eggs in 'low' and 'high' CPB density treatments that were not killed by *C. maculata*, estimates of the fitness of hypothetical resistant and susceptible genotypes were derived. For example, consider the data from the 'CPB only' prey treatment in the (1996) experiments. An average of 25% of the CPB eggs in the 'low' density area (which represent resistant genotypes), and 32% in the 'high' density area (mimic of susceptible genotypes) were eaten in treatments without the alternate prey being present. Thus, in that scenario, 75% and 68% of the CPB eggs survived, respectively. Based on these values, CPB with RR genotypes were assigned a fitness of 0.75 in Bt-potatoes and 0.68 in non-Bt potatoes, since they would not be affected by the Bt toxin but did suffer mortality from predation. RS and SS genotypes were also assigned a fitness of 0.68 in non-Bt plants. In Bt plants, however, their fitness would also be reduced by sensitivity to the Bt toxin.

The arbitrary estimates of the toxin-induced mortality of the RS and SS genotypes that were used by Arpaia et al. (1997) were also used here, for comparative purposes. These authors assumed that 0.5% of the individuals with the RS genotype, and 0.1% of those with the SS genotype, would survive in a Bt-potato field. Thus, when the predation data from the CPB-only treatments were incorporated, the fitness of the RS and SS genotypes were estimated to be $(0.005) \times (0.68) = 0.0034$ and $(0.001) \times (0.68) = 0.00068$, respectively. These fitness values were then multiplied by the proportion of Bt and non-Bt plants in the hypothetical field scenario. Simulations using the data from the 1996 experiment assumed a 50% refuge (i.e., non-Bt plants), while those using data from the 1997 experiment assumed a 25% refuge (since these were the sizes of the simulated refuge in each set of experiments).

Results

Predation and the influence of alternate prey

The ANOVA of data on CPB egg predation from the 1996 experiment indicated a statistically significant interaction between CPB density and the presence/absence of ECB as an alternate prey ($F_{1,82} = 47.42$, $P = 0.0001$). Predation on the CPB eggs without alternative prey being available was not significantly different across CPB densities (Figure 1). However, when ECB eggs were available, the predation on CPB eggs in the 'low' density rows increased, while predation in the 'high' density rows decreased (Figure 1).

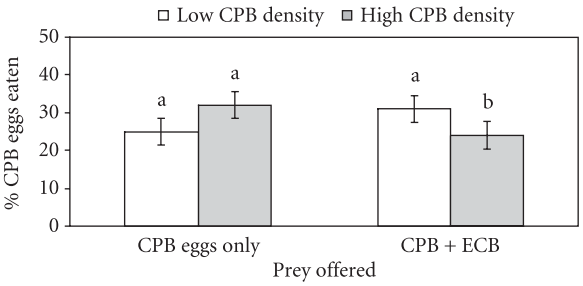


Figure 1 Mean (± 1 SE) percentage of CPB eggs eaten by *Coleomegilla maculata* in 1996 field experiments. Bars followed by the same letters are not significantly different according to Tukey's HSD test ($P>0.05$). Means comparisons shown were made within each 'prey offered' treatment combination.

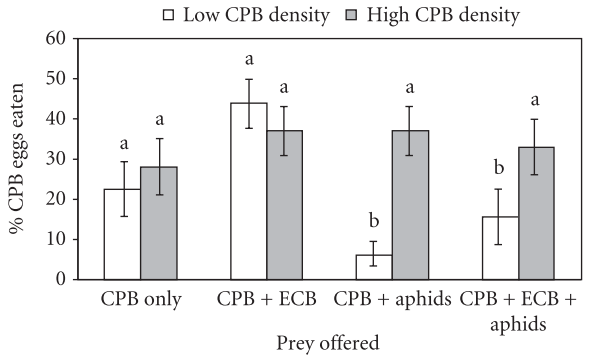


Figure 2 Mean (± 1 SE) percentage of CPB eggs eaten by *Coleomegilla maculata* in 1997 field experiments. Bars followed by the same letters are not significantly different according to Tukey's HSD test (P>0.05). Means comparisons shown were made within each 'prey offered' treatment combination.

The difference in predation on 'low' vs. 'high' CPB densities (in the treatment where ECB eggs available) was significant (P = 0.01, according to Tukey's HSD test).

Data from the 1997 experiment showed a significant heterogeneity of variances between treatments. This was corrected by using the experimental trial (July and August) as the basis for partitioning error variances for computing statistical tests of significance in subsequent mixed model ANOVA procedures. No significant effect of time period was evident when the July and August datasets were analyzed statistically ($F_{1,23} = 0.28$, P = 0.609). For data on predation of CPB eggs, the ANOVA indicated a statistically significant interaction between prey treatment and CPB density ($F_{3,23} = 4.40$, P = 0.038). An examination of the graph of these data (Figure 2) showed that, as in 1996, predation on 'low' vs. 'high' CPB densities was not significantly different when no alternate prey species were present (i.e., within the 'CPB-only' prey treatment). When only ECB eggs were present, a similar pattern in CPB predation was observed, but (overall) predation with ECB eggs present increased significantly as compared to the 'CPB-only' prey treatment (Figure 2; P = 0.02, according to Tukey's test). No other means comparisons (of overall predation) across prey treatment were significant. When aphids were present as the alternate prey, the predation became positively density-dependent. Predation in the 'low' CPB density was significantly lower than that in the 'high' CPB density. When both alternate prey species were present together, a similar pattern of positive density-dependence was observed (Figure 2).

ANOVA of data on the number of predators observed on plants, expressed as the number of *C. maculata* per row, showed no significant main effects of CPB density ($F_{1,168} = 1.07$, P = 0.304) or prey treatment ($F_{1,168} = 0.45$, P = 0.722). In contrast to the results for data on CPB egg predation, there was no significant interaction between CPB density and prey treatment ($F_{1,168} = 1.10$, P = 0.355). In addition, there were no significant effects of time or of any interaction terms involving time in the ANOVA model.

Genetic modeling of resistance evolution

Simulations using data from the 1996 experiment indicated that when CPB eggs were the only prey available, 196 generations were needed for the resistance allele (R) to reach a frequency of 1.0 (i.e., for the allele to be fixed in the population). When ECB eggs were present, it took 237 generations for the R allele to reach the same frequency (Figure 3). When the model was run with no mortality due to incorporation of predation, 275 generations were required (Figure 3).

Simulations using the 1997 data indicated that when neither alternate prey was present, 93 generations were needed for the R allele to reach a frequency of 1.0 (Figure 4A). Resistance evolved fastest when aphids were the only alternate prey present (70 generations); when the alternate prey consisted of both aphids and ECB, 80 generations were required for the R allele to reach a frequency of 1.0 (Figure 4B). The largest number of generations needed for the R allele to reach 1.0 was in simulations which assumed that ECB eggs were the only alternate prey present; here 111 generations were required (Figure 4B).

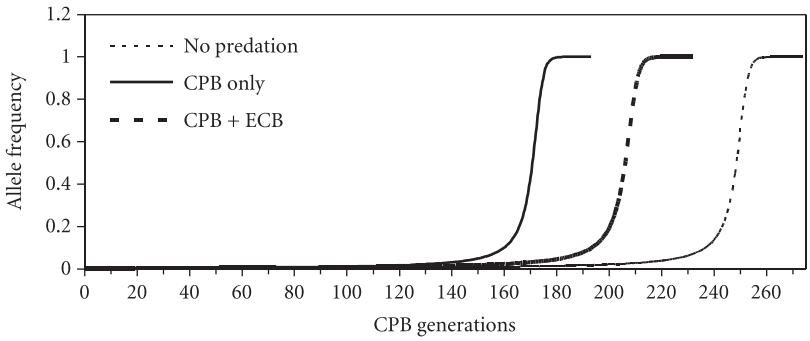
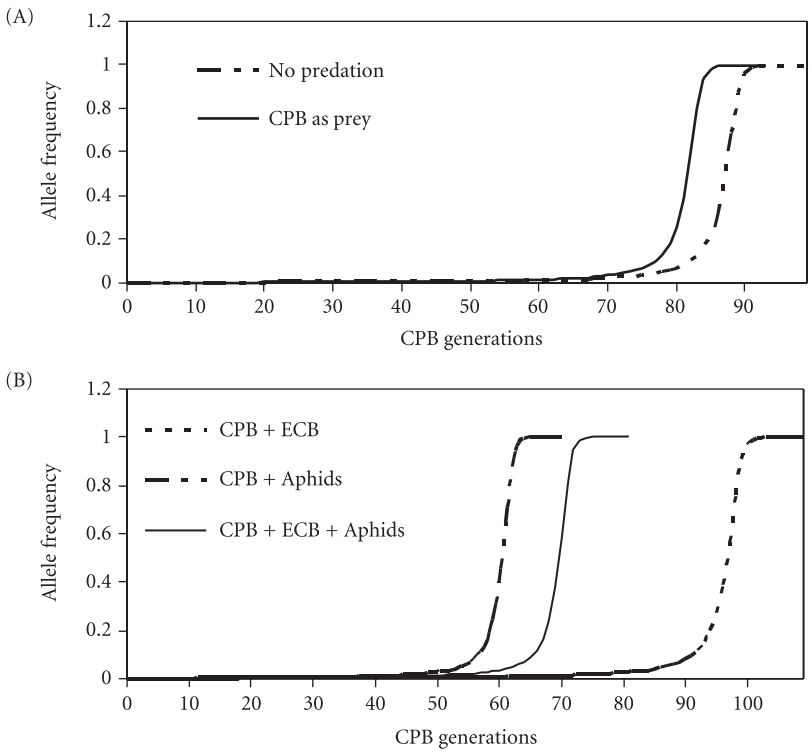


Figure 3 Results of simulation of resistance evolution based on predation data from the 1996 experiment. The Y-axis has been extended beyond 1.0 (the maximum value possible for the R allele), in order to allow the reader to easily discern the endpoints of each line.



Figures 4 Results of simulations of resistance evolution based on predation data from the 1997 experiment: (A) with no predation or CPB only as scenarios, and (B) CPB with aphids, CPB with ECB, or CPB with both ECB and aphid alternate prey as scenarios. The Y-axis has been extended beyond 1.0 (the maximum value possible for the R allele), in order to allow the reader to easily discern the endpoints of each line.

When the model was run assuming no predator-induced mortality, 100 generations were required for resistance to evolve in the 1997 simulated 25% refuge (Figure 4A). This was much lower than the number of generations needed in the comparable ('equal-predation') scenario using a 50% refuge assumption (which was used in the 1996 experiment). This is an illustration of the huge impact that varying the refuge size has on resistance evolution in organisms facing very high doses of a toxin, an issue discussed in depth by other authors (Roush & Tingey, 1991; Fischoff, 1996; Roush, 1997a; Gould, 1998).

Discussion

The results of our field studies differ from those reported by Arpaia et al. (1997), who also studied CPB egg predation by *C. maculata* in the context of Bt-transgenic potato deployment. Their study indicated that there was an inverse density-dependent predation on CPB eggs in trials conducted in small field plots and greenhouses in the absence of alternative prey. Arpaia et al. utilized their field plot data on CPB mortality to initiate resistance simulations, and the model indicated that when predators were present, resistance evolution would take much longer than when no predation was assumed (about 125 generations for R to reach a frequency of 1, as opposed to 60 generations without predation). In contrast, our results, derived from the comparable treatment (i.e., with no alternate prey present, and only CPB eggs as prey) generally led the model to predict that resistance would evolve faster when predators were present than when no predation was presumed to occur (see Figures 3 and 4a). When interpreting model results for this comparison (i.e., when CPB are the only available prey), we should note that since in both years our experiments consistently showed no statistically significant difference in predation between the low and high CPB density treatments, repetitions of our experiments might yield somewhat different mean values for predation, which in turn would change the simulation results. Nevertheless, the absence of inversely density-dependent predation in our experiments suggests that *C. maculata* predatory behavior is complex and variable, and is an aspect that merits further discussion and study.

In this context, it is noteworthy that our studies differed from the field plot trials performed by Arpaia et al. (1997) in terms of the density of *C. maculata* in experimental arenas. Arpaia et al. (1997) used 6–7 *C. maculata* adults per plant in their field studies compared to the density of one ladybeetle per plant used in our study. The density we employed was at the upper end of those observed in other studies of *C. maculata* densities in commercial potato fields (Hazzard et al., 1991; Hilbeck et al., 1997). This difference in predator densities may have influenced the prey consumption observed in each study, by changing the type

and frequency of behavioral interactions between individual predators. There is evidence that as the number of coccinellids in a given area increases, the predation efficiency decreases (Siddiqui et al., 1999), although the mechanisms underlying this phenomenon have not been investigated. It is also known that coccinellid beetles aggregate both to mate (Savoiskaya, 1965) and as a result of relatively random encounters with prey (Risch et al., 1982; Wetzler & Risch, 1984). These studies suggest that these predators often engage in other behaviors, in addition to feeding when congregated in groups, and that this may interfere with predation. It is possible that, in the study conducted by Arpaia et al. (1997), disruptive behaviors such as competition for the same prey resource or mating, may have occurred more often than in our study, thereby leading to more frequent interference of feeding in areas where predators aggregated. However, since many other experimental conditions differed between our study and that of Arpaia et al. (1997), this is only one of many possible causes.

In addition to their field plot studies, Arpaia et al. (1997) also conducted tests in large cages in greenhouses. These trials were similar to our experimental design in terms of both the total number of plants used (approximately 40), and the density of *C. maculata* deployed (one per plant). The results from these trials were similar to those from their field plot tests – inversely density-dependent predation (though weaker than what they observed in their field trials), yet with a significantly higher number of predators observed on high-CPB-density plants than on low-CPB-density plants. The authors attributed the lack of positive density-dependent predation to an insufficient spatially density dependent aggregation. We note here that our results showed no significant aggregation response to differences in CPB prey density (regardless of the presence or type of alternate prey), and no significant difference in the predation of low vs. high CPB egg populations when they were the only prey available. We speculate that the greenhouse cages used by Arpaia et al. may have been a simpler environment than our experimental arenas, to the extent that coccinellids could find one another more easily as they aggregated in the areas of higher prey density. They could then have engaged more often in the disruptive behaviors (mentioned above), rather than increasing their feeding.

Taken together, the results of our study and those of Arpaia et al. (1997) imply that inversely density-dependent predation by *C. maculata*, while optimal for delaying resistance evolution, cannot be reliably expected to occur in the field. Our results also suggest that (at least at low densities of *C. maculata*) resistance management will benefit if aphid alternate prey are eliminated or prevented from colonizing plants, since their presence reduces predation on CPB eggs and thus speeds resistance evolution. Alternatively, since the model indicates that the presence of ECB eggs actually delays resistance evolution longer than when CPB eggs are the only prey, perhaps pest managers should try to foster the availability of this species as alternate prey when low levels of *C. maculata* are expected to occur. Simply allowing low levels of oviposition could accomplish this, since the results observed in this study were obtained with non-outbreak densities of ECB (Nault & Kennedy, 1996).

In considering the implications of the results reported here, however, it should also be kept in mind that the size of the plots used in our experiments was relatively small. It is known that spatial scale may affect the observed level of aggregation by coccinellids and other natural enemies (Rosenheim et al., 1989; Ives et al., 1993). For example, in studying the aggregation of *Cocinella septempunctata* and *Hippodamia variegata* to aphids on fireweed, Ives et al. (1993) found that while the aggregating response of individual beetles on individual plant stems was weak, that of populations of beetles in large patches of fireweed stems was stronger. It has also been pointed out by other workers that environmental factors, such as the availability of more refugia for the prey to escape, may influence coccinellid predation in more complex field conditions (Hodek & Honek, 1996). Larger scale studies would be useful in resolving the impact of these factors.

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