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Potential impact of *Coleomegilla maculata* predation on adaptation of *Leptinotarsa decemlineata* to *Bt*-transgenic potatoes

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Abstract

The relationship between *Leptinotarsa decemlineata* Say egg density and *Coleomegilla maculata* DeGeer predatory behavior was investigated at different spatial scales (plant-to-plant and plot-to-plot). Both adult *C. maculata* location and daily egg consumption rates were monitored over time in greenhouse and field tests. Despite aggregation in areas of highest prey density by *C. maculata*, egg consumption was inversely related to egg mass density at the smallest and the largest spatial scales tested. The experimental data on predation rates in high and low density field treatments were included in a mathematical model to simulate impact of natural enemies on the rate of *L. decemlineata* adaptation to *Bt*-toxin-expressing transgenic potato plants when *Bt*-expressing plants are mixed at the plot-to-plot level with normal potato plants. Results showed that *C. maculata* predatory behavior could decrease the rate at which *L. decemlineata* adapted to *Bt*-toxins if plot-to-plot mixed-plantings were used.

Introduction

It has often been assumed that the effects of natural enemies and crops with host plant resistance (HPR) were so distinct that predators were not considered to be one of the factors that could affect durability of a HPR trait (for example, Bergman & Tingey, 1979). Gould et al. (1991) used computer simulations to demonstrate that the rate of predation by natural enemies could indeed affect the rate of pest adaptation to partially resistant host plants deployed in homogeneous plots. More recent simulations (Gould, 1994) indicate that natural enemies could also affect the rate of pest adaptation to plants with high levels of HPR if these plants are grown near other plants that are susceptible to the pest insect. Simulations indicate that if natural enemies prey on the pest in a density-dependent fashion, this could lead to faster adaptation by the pest to the toxin. In contrast, inverse density-dependent predation is expected to slow the rate of adaptation.

Transgenic potatoes resistant to *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) based upon expression of a toxic protein derived from *Bacillus thuringiensis* will be among the first transgenic crops deployed commercially. Several deployment strategies have been suggested to reduce the rate at which target insects may adapt to the *Bt* toxins in these potatoes (Arpaia & Ricchiuto, 1993; Gould et al., 1994). One of these strategies, planting a mixture of transgenic and susceptible potato isolines, could retard adaptation by providing refugia where susceptible beetles could survive. If, however, predation occurred in a density-dependent manner, a higher proportion of the beetles (and their eggs) in the *Bt* refugia would be killed relative to the few resistant individuals in the areas of *Bt* expression. This would have a negative impact on the utility of the refuges.

The coccinellid, *Coleomegilla maculata* (DeGeer) (Coleoptera: Coccinellidae), is an active predator of *L. decemlineata* eggs and first instar larvae. Field studies in North Carolina found egg mortality due to predation ranges from 17 to 34% in the early season, with peaks as high as 100% during the later part of the season (Hilbeck, 1994). Hilbeck indicated that *C. maculata*

was the most important predaceous species in the area. In laboratory experiments, Giroux et al. (1994) found no toxic effects of *Bt*- based insecticides to *C. maculata*.

The relationship between *L. decemlineata* egg density and egg consumption by *C. maculata* was determined in laboratory experiments by Hazzard & Ferro (1991). They found a Type II functional response to egg density. While these petri dish results are interesting, we feel that determining the impact of *C. maculata* on the rate of *L. decemlineata* adaptation to *Bt* toxins requires experiments conducted in more realistic environments. Here we report results of an investigation of *C. maculata*'s response to various *L. decemlineata* egg mass densities in arenas that mimicked different spatial scales of mixed plantings ranging from plant-to-plant mixtures to plot-to-plot mixtures of transgenic and susceptible plants. We used the predation data from these experiments to set parameters of a simulation model that predicts rates of pest adaptation to resistant host plants.

Materials and methods

Insects

L. decemlineata. Egg masses were obtained from a colony maintained at the Department of Entomology, North Carolina State University, and from a culture maintained at Philip Alampi Laboratory, Beneficial Insects Rearing, New Jersey Department of Agriculture. For experiments requiring up to 70 egg masses per day, we always used fresh egg masses. However, in preparing for large scale experiments, egg masses were sealed in petri dishes and frozen at -20 °C. Egg masses were thawed by leaving petri dishes at 4 °C overnight. Preliminary bioassays showed that thawed egg masses are readily accepted by *C. maculata* and do not deteriorate for over five days after thawing (data not shown).

C. maculata. Adults (15–25 days old) were obtained from USDA-APHIS-PPQ Mission Biological Control Laboratory, Mission, TX. Prior to experiments, insects were held overnight without food. Previous research has demonstrated that females are more responsive than males to food availability and will eat more (Hazzard & Ferro, 1991). Therefore, we used only female *C. maculata* when this was feasible. When only females were to be used in an experiment, we ensured accurate sexing by selecting only the females from mating pairs.

Experimental protocols

Small cage experiments

Metal, mesh-screened cages $42 \times 42 \times 42$ cm were used in all of the small-cage experiments. Two 3-week-old potted potato plants were placed in these cages with limited contact between the leaves of different plants. Eight female *C. maculata* were released in each cage. Plants were infested by pinning egg masses on top of single potato leaflets. All experiments were conducted in a greenhouse with controlled temperatures between 20 and 38 °C.

Presence-absence treatments. Four different egg mass densities were tested, each in a different cage: 1, 3, 10, and 20 egg masses per plant. Newly laid egg masses were pinned on only one of the two plants. Four *C. maculata* adult females were placed on each of the two plants. The number of *C. maculata* on each plant was recorded at 2, 4, 6, 20, 22, 24, 28, 30, 44, 46, 48, 52, 54, 68, 70, and 72 h after the initial placement. The number of eggs eaten was determined daily for three days. Egg masses were examined by removing them from the plants and inspecting them for predation under a dissecting stereomicroscope. Undamaged egg masses were replaced in the same spot from which they were taken. Attacked egg masses were replaced with new ones. Two replicates were set up for each egg mass density. The experiment was repeated four times.

Low versus high density treatments. The protocol was the same as in 'presence-absence treatments', except that one of each pair of plants always had a single egg mass instead of no egg masses (i.e., low density plants). The other plants had 1, 3, 10 or 20 egg masses and is referred to as the 'high density plant'. Egg masses on both plants were scored for percent of eggs eaten. The experiment was repeated three times.

Large cage experiments

Four benches, 265×120 cm, were constructed in a greenhouse in a rectangular arrangement such that each bench was 50 cm from neighboring benches on all sides. Forty, 3-week-old potted potato plants were placed on each bench (8 rows of 5 plants each). A large fabric net, supported by 4 m high poles, was placed over all four benches to prevent escape of the *C. maculata* adults. Two of the plots (on diagonally positioned benches) were infested with three egg masses per plant,

while the plants on the other two benches received an egg mass density of 0.1 per plant (i.e., 1 for every 10 plants). Forty *C. maculata* females were released in each plot (1 per plant). The number of *C. maculata* in each plot was counted twice per day and egg predation was scored daily. All egg masses in the low density plots were scored for predation, but in the high density plots, a total of 40 egg masses per plot (1 per plant) was examined. Attacked egg masses were not replaced in this experiment. Each of the three replicates of the experiment lasted for five days (frozen egg masses were used to prevent hatching). The placement of the high and low egg mass density treatments was alternated among the benches following each replicate.

Field experiments

Seed pieces of transgenic potatoes that expressed *Bt* toxins were provided by Monsanto Agriculture Company (St. Louis, MO). We chose to use a potato line that expressed *Bt*-toxin to prevent natural *L. decemlineata* infestations. Four plots were demarcated within a potato field of approximately 0.1 ha in a completely randomized block design. Plot size was 8.8×5.0 m. Each plot consisted of 12 rows of 17 plants each, but only 10 rows were used for the observations (the two border rows were excluded). Soil type was sandy loam. Preplant fertilizer (12–6–24) was applied at a rate of 600 lbs per acre and a post-planting top dressing of fertilizer (15–0–4) was applied at 200 lbs per acre. When potatoes were in the early bloom stage, plots were artificially infested with previously frozen Colorado potato beetle egg masses by pinning egg masses to the upper side of the potato leaves. Thereafter, approximately 5000 adults of *C. maculata* were released throughout the plots. The same egg mass densities used in the large cage experiments were chosen to assess *C. maculata* predation rates and movement in the field experiments, because these two densities (0.1 and 3 egg masses per plant) represent potential field situations in North Carolina (Kennedy, unpubl. data). Egg predation was scored every day using a stereomicroscope. All egg masses were counted in the low density treatments, but only 170 egg masses (1 per plant) were monitored in each high density plot. The number of *C. maculata* per plot was scored daily by examining 50 randomly chosen plants per plot. The experiment was repeated twice with each replicate lasting four days. The plots used for the high and low density treatments were switched between replicates of the experiment. The first replicate was initiated on June 7, 1994, and the second on June 16, 1994. During the time of these experiments, the plots were devoid of significant numbers of alternate *C. maculata* prey, such as aphids.

Statistical analyses

Small cage experiments

Presence-absence treatments. To evaluate the functional response of *C. maculata* to egg masses, data from the presence- absence experiments were fitted to the Holling disc equation (Holling, 1959) by using a nonlinear regression (SAS, 1989). A reciprocal linearization of the disk equation (Livdahl & Stiven, 1983) was used to obtain the initial estimates of a (area of discovery), and Th (handling time) as required in the SAS NLIN procedure. The percentage of beetles on the 'treatment' plant was used to investigate the effect of egg mass density on beetle preference. A Wilcoxon signed rank test was used to determine if, overall, plants with egg masses had more beetles on them than plants without egg masses. A repeated measures ANOVA was adopted to determine if there were differences among the 1, 3, 10 and 20 egg mass treatments regarding the extent of beetle preference (as measured by beetle position). The repeated measures design was also used to analyze effects of time during the course of an experiment and the effect of experimental block on the degree of beetle preference. Density treatments used in the experiment were analyzed using a random effects statistical model. Data were arcsin square root transformed before performing ANOVA.

Low versus high density treatments

Beetle preference for the high and low density plants was determined as described in 'presence-absence tests'. A repeated measures ANOVA was used to test whether the proportion of eggs attacked on the high density versus the low density plants was differentially affected by the four high egg mass densities. The ratio of percent predation on low versus high density plants was the dependent variable. Egg mass density and experimental block were considered random effects.

Large cage experiments and field experiments

The same general type of analyses used in the 'small cage' low versus high density tests, were used for the large cage and field tests. The most important difference in the analyses was that in the small cage tests the four densities of egg masses were considered random effects, whereas in the large cage and field tests the

two densities were chosen specifically to reflect expected field densities and were therefore considered fixed effects. Predator preference in the field was assessed by analyzing the percentage of beetles in the high and low density plots, over time.

Genetic model

A deterministic population genetic model described in Gould (1986) was used to examine how movement and predatory behavior of *C. maculata* might influence the rate of *L. decemlineata* adaptation to *Bt*-expressing potatoes that were planted as a mixture of plots, one-half of the plots containing 100% *Bt*-expressing plants and one-half containing 100% non-*Bt*-plants. The inheritance of resistance was assumed to be controlled by a partially recessive allele with an initial frequency of 0.02. In the absence of *C. maculata*, the fitness of *L. decemlineata* RR, RS, and SS genotypes on *Bt*-expressing plants was set at 1.00, 0.005, and 0.001, respectively. On non-*Bt* plants, all genotypes were assumed to have a fitness of 1.00 in the absence of *C. maculata*. The impact of *C. maculata* on the fitness of beetles in the *Bt* and non-*Bt* plots was determined based on results of four days of predation in the field experiments. A second simulation was run assuming that there was equal predation on beetle eggs in *Bt* and non-*Bt* plots.

Results

Small cage experiments

Presence-absence treatments. The relationship between *L. decemlineata* egg density and number of eggs eaten by *C. maculata* can be explained well by the Holling disc equation (Williams & Juliano, 1985):

$$Na/TP = aN/(1 + aThN),$$

where a = area of discovery, Na = number of prey attacked, T = total time, P = number of predators, and Th = handling time. Estimated parameters (mean $\pm$ confidence interval) were: $a = 0.47481 \pm 0.2772$, and $Th = 0.5232 \pm 0.1330$, with an $R^2 = 0.933$. An approximate curve is plotted in Figure 1. Mean daily consumption at different densities is shown in Table 1.

The percentage of female *C. maculata* on different plants over time is presented in Figure 2. It was clear that predator abundance was greater on plants with *L. decemlineata* egg masses relative to control plants (mean difference = 27.95 Wilcoxon signed rank test $S = 12939$; P < 0.0001). Different prey densities induced a significantly different *C. maculata* behavioral response as measured by location of the predators (ANOVA, F = 4.09; df $= 3, 9$; P = 0.043, see Table 2). There was also a significant effect of time (ANOVA, $F = 2.76$; df $= 15, 40$; P = 0.005).

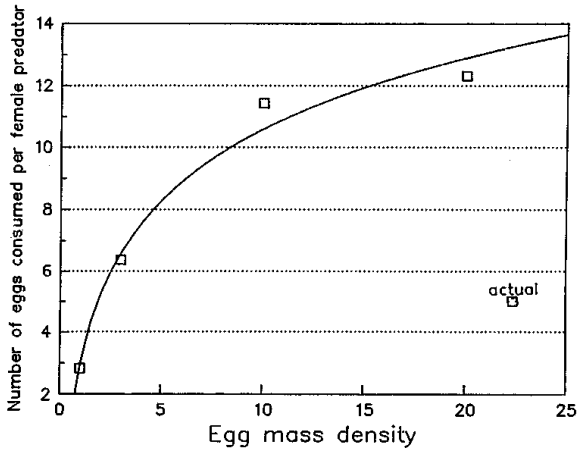

Figure 1. Relationship between daily egg consumption per *C. maculata* female and egg mass density. $R^2 = 0.933$ in the regression of experimental data against the data expected based on the Holling equation. The curve in this figure is a nonlinear fit to the data points.

Low versus high density treatments

Figure 3 presents the percentage of *C. maculata* on plants in the different treatments over time. Most of the predators were found on the plant with the higher egg mass density (mean difference = 18.42 Wilcoxon signed rank test $S = 17889$, P < 0.0001). There was a significant positive relationship between the difference in egg mass densities on the high and low density plants and the degree to which predators were aggregated on the high density plant (ANOVA, $F = 9.53$; df $= 3, 9$; P = 0.011, see Table 2). None of the other factors or interactions were significant. These results match those obtained in the presence-absence treatments, indicating that prey density does affect *C. maculata* position at a plant-to-plant spatial scale.

Analysis of variance of the ratio of the percent of the eggs eaten on high and low density plants indicated a significant relationship between the difference in egg mass densities on the high and low density plants and this ratio ($F = 7.83$; df $= 1, 6$; P = 0.031, linear contrast). However, this relationship was in the opposite direction of the effect of density on plant preference by adults. A higher proportion of eggs was preyed upon in the low density treatment than in the high

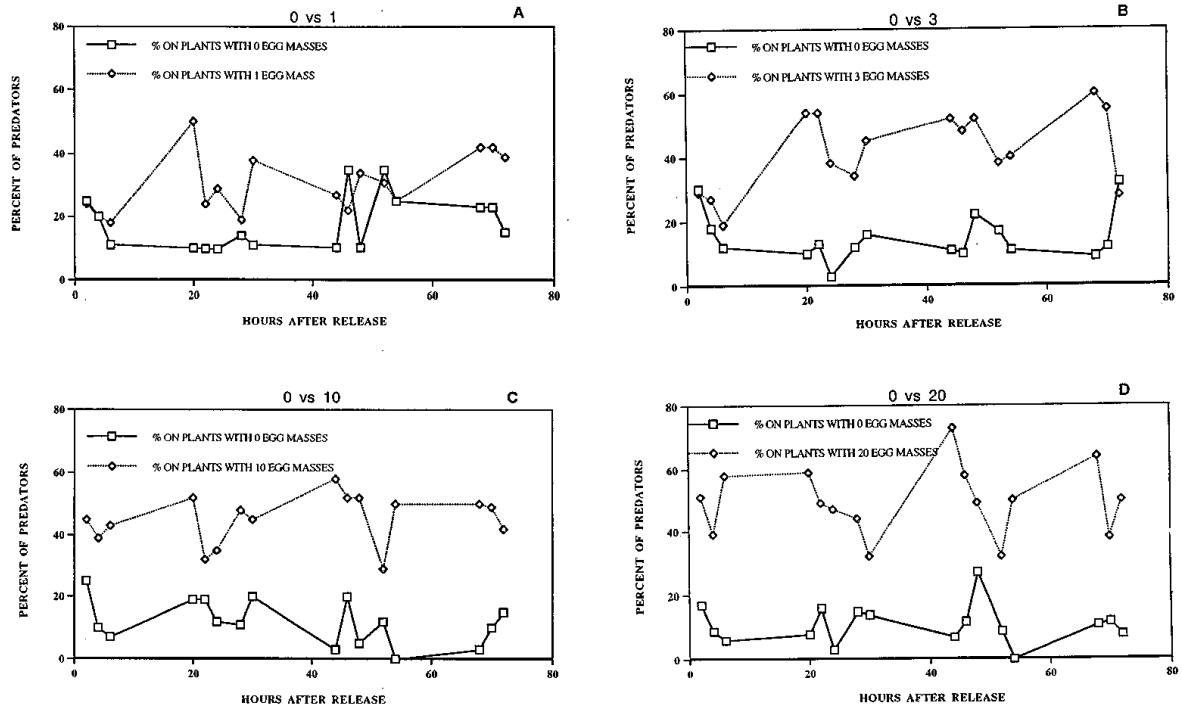

Figure 2. Predator position over time in small cage, presence-absence experiments. Solid lines indicate the mean percentage of *C. maculata* found on plants with egg masses. Dotted lines indicate the percentage of *C. maculata* found on plants without egg masses.

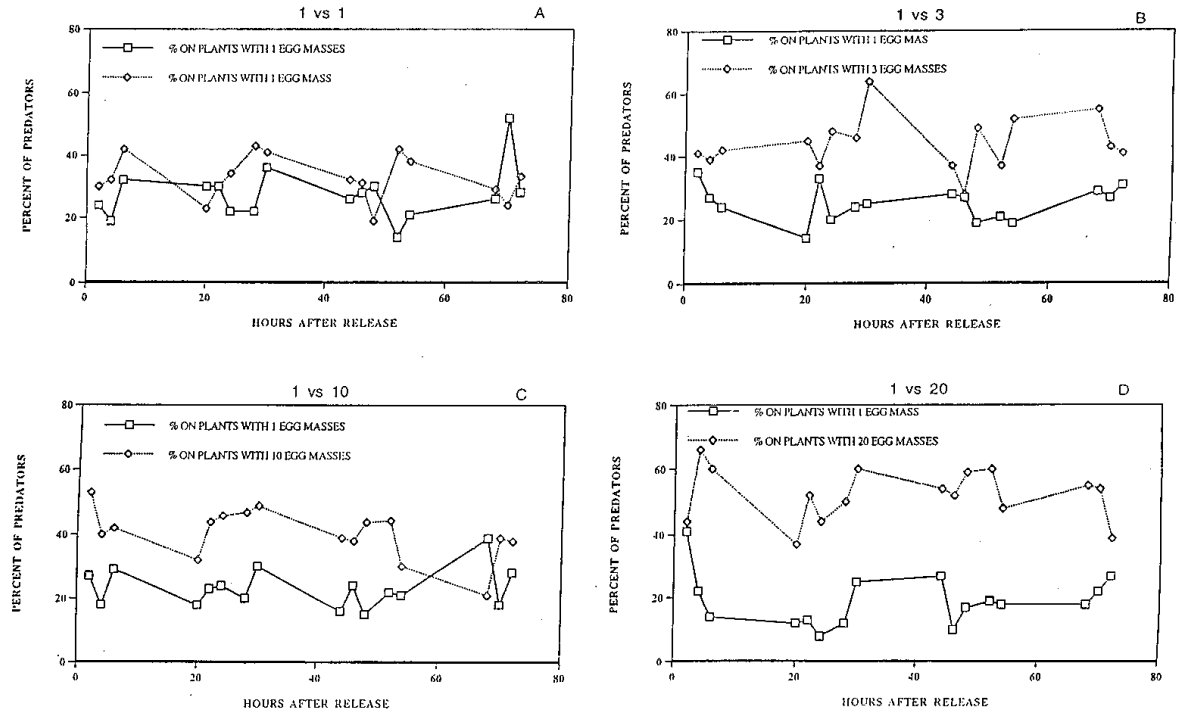

Figure 3. Predator position over time in small cage, low versus high density experiments. Solid lines indicate the percentage of *C. maculata* found on plants with varied egg mass densities; dotted lines indicate the percentage of *C. maculata* found on the plant treatment that always had one egg mass.

Table 1. Mean daily consumption (± standard errors) of *Leptinotarsa decemlineata* eggs per *Coleomegilla maculata* female at different prey densities. In the presence-absence test, one plant had no egg masses, while a second plant had either 1, 3, 10, or 20 egg masses. In the low versus high density experiment, one plant always had a single egg mass, while a second treatment plant had 1, 3, 10, or 20 egg masses

Experiment	Day	Number of egg masses			
		1	3	10	20
Presence-absence	1	2.98 (±0.82)	4.95 (±1.79)	10.49 (±2.38)	10.72 (±1.70)
	2	2.88 (±0.83)	6.72 (±1.77)	10.38 (±2.12)	11.56 (±1.56)
	3	2.63 (±0.83)	7.44 (±0.81)	13.52 (±1.88)	14.68 (±1.55)
Low versus high density	1	4.00 (±0.50)	7.77 (±0.72)	15.33 (±0.95)	18.60 (±0.93)
	2	4.15 (±0.59)	8.46 (±1.82)	12.69 (±1.39)	17.59 (±1.13)
	3	4.13 (±0.78)	8.02 (±1.04)	15.55 (±2.65)	20.07 (±1.75)

Table 2. Mean percentage of *Coleomegilla maculata* found on the higher density plants at different egg mass densities in the small cage experiment. The percentage on the control plant is equal to 100% minus the percent on the treatment plant because predators that were on neither plant were excluded from the analysis

Presence-absence test		Low versus high density test	
No. egg masses	Percent *C. maculata*	No. egg masses	Percent *C. maculata*
20	81.40	20	73.78
10	78.29	10	63.36
3	71.85	3	61.89
1	65.19	1	53.70

density treatments, and the significant linear contrast indicated that the higher the egg mass density on the 'high density plant', the bigger the difference in percent predation was on the high and low density plants (Table 3). This logically indicates an inverse density dependence in *C. maculata* predation on *L. decemlineata* eggs. The treatment × experiment interaction was not significant (ANOVA $F = 1.74$; df $= 6, 36$; P $= 0.139$). Mean daily egg consumption was generally higher in the low versus high density experiment compared to egg consumption in the presence-absence tests (Table 1). This indicates that the *C. maculata* used in the choice tests were more active than those used in the presence-absence tests.

Large cage experiments

Over the five day period of the experiment, an average of more than 40% of the *C. maculata* adult females released in the three replicates were detected in our daily samplings, but the mean number of individuals declined almost constantly over time (day 1 = 80 beetles, day 2 = 55 beetles, day 3 = 45 beetles, day 4 = 37 beetles, day 5 = 27.5 beetles). Predator location over time is shown in Figure 4A. More *C. maculata* were found in the high density plots than in the low density plots (ANOVA, $F = 107.53$; df, 1,26; P $= 0.0001$). The treatment × experiment interaction (ANOVA, $F = 35.69$; df, 2,26, P $= 0.0001$), and the treatment × experiment × time interaction (ANOVA, $F = 3.17$; df, 6,26; P $= 0.0181$) were also significant.

The percent predation per day was higher at low egg mass density than at high egg mass density (Table 4), but statistically, this difference was only marginally significant (ANOVA, $F = 3.71$; df, 1,28; P $= 0.068$).

Field experiments

The percentage of predators in the high and low density plots is presented in Figure 4B. To quantify the pattern of coccinellid aggregation in the field situation, it was important to consider the natural decrease of *C. maculata* numbers over time (Figure 5). We therefore estimated the average time *C. maculata* individuals remained in each plot using the method of Ives et al. (1993). This

Table 3. Daily percent egg consumption by *Coleomegilla maculata* when given a choice of one plant with low egg mass density and one plant with higher egg mass density in small cages

Egg mass densities	Mean consumption (%) (± standard error)		Ratio between percent predation on egg masses on high and low density plants**
	Low density	High density	
1 versus 20	33.62 (±4.73)	19.46 (±0.94)	1.90
1 versus 10	49.56 (±6.54)	28.02 (±2.21)	1.76
1 versus 3	49.46 (±6.61)	52.68 (±4.99)	1.12
1 versus 1*	65.43 (±5.61)	74.97 (±3.92)	0.94

* In the 1 versus 1 treatment, the 'high density plant' was arbitrarily assigned.
** Ratios were computed for each experimental replicate and then the mean was determined. Therefore, simply dividing the mean percent consumption of eggs on the low density plants by the mean percent consumption of the high density plants will not give the values in this column.

Table 4. Percentage (± S.E.) of eggs eaten per day in low (0.1 egg masses/plant) and high density (3 egg masses/plant) plots in tests conducted in a greenhouse and in the field

	Greenhouse experiments[a]		Field experiments[b]	
	Treatment		Treatment	
Day	Low density	High density	Low density	High density
1	22.70 (±6.17)	14.61 (±3.20)	30.54 (±5.57)	24.04 (±5.58)
2	5.59 (±2.27)	7.88 (±3.36)	31.71 (±4.22)	17.61 (±2.31)
3	10.26 (±5.32)	14.44 (±7.91)	50.17 (±6.45)	27.86 (±2.18)
4	19.97 (±8.98)	11.53 (±6.96)	44.81 (±13.73)	24.84 (±6.69)
5	26.07 (±12.42)	11.88 (±4.21)		

[a] Average of three experiments.
[b] Average of two experiments.

was determined by multiplying the fraction of predators remaining in each plot on a given day by the number of days since the release. The sum over all days was then calculated. Even after a single day, the number of *C. maculata* in the low density plots was substantially lower than those in the high density plots. Results obtained with this method are consistent with what we observed in cage experiments where *C. maculata* movement was more restricted (Figure 4A, B). Analysis of variance indicated that significantly more *C. maculata* were found in high density plots than in low density plots ($F = 48.71$, df $= 1, 16$, P $= 0.0001$). These results indicate that at the larger spatial scale predators aggregated in response to food availability. *C. maculata* individuals of both sexes were released in field plots. We did not assess whether males or females responded more strongly to egg mass density in the field experiments.

The scoring of eggs within egg masses for evidence of *C. maculata* feeding (Table 4) indicated that predation was significantly greater in low density plots than in high density plots (ANOVA, $F = 5.42$, df $= 1, 16$, P $= 0.031$). This result is similar to that of the greenhouse experiments, confirming the existence of inversely density-dependent predation.

At least some eggs in almost all egg masses were fed upon during the four-day field experiments. The average percent of egg masses found without even one egg attacked in the high density plots was 0.53%, and in the low density plots, all egg masses were attacked. After four days, 86.54% of the eggs in the low density plots were killed by predators, while only 66.28% were killed in the high density plots.

Predictions of the genetic model

By assessing the percentage of eggs in the high and low density field treatments that were not killed by the end of the experiment, we were able to arrive at an expectation for the difference in fitness caused by predation

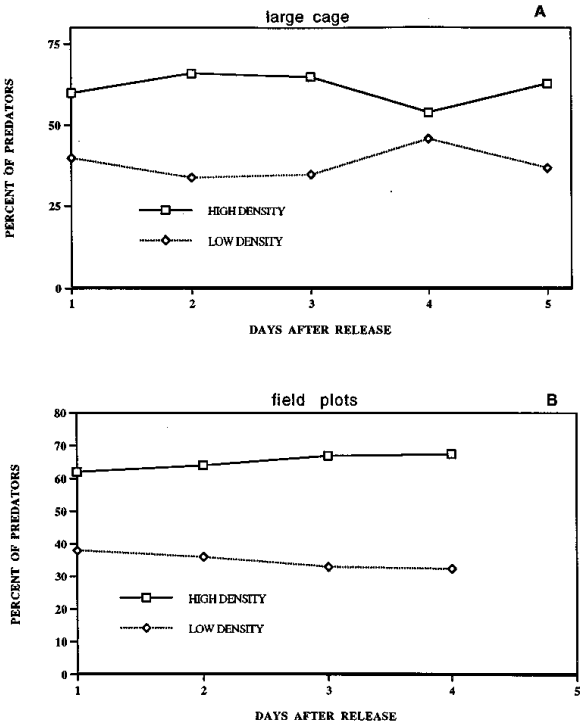

Figure 4. Predator position over time. A = large cage experiments; B = field experiments. In both A and B, one-half of the plots had three egg masses per plant, while in the other plots, there were 0.1 egg masses per plant.

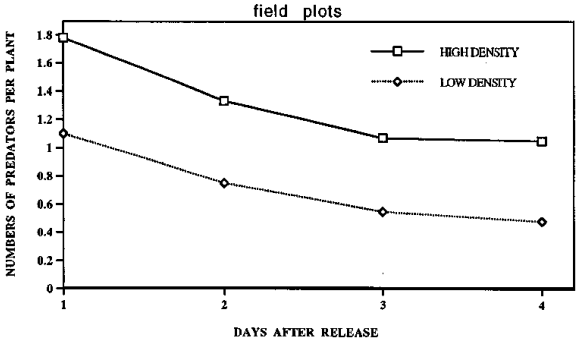

Figure 5. Natural decrease of *C. maculata* population over time in experimental fields. This decrease could have been due to dispersal and/or mortality of the beetles.

in *Bt* and non-*Bt* potato plots. In the low density plots (i.e., mimic of *Bt* potatoes), the overall egg survival in face of predation was 13.46% (i.e., 100%–86.54%). In the high density plots (i.e., mimic of non-*Bt* potatoes), 33.72% survived. We used these values to set genotype-specific fitnesses in hypothetical *Bt* and non-*Bt* fields. *L. decemlineata* with RR genotypes were assigned a fitness of 0.3372 in non-*Bt* fields and were

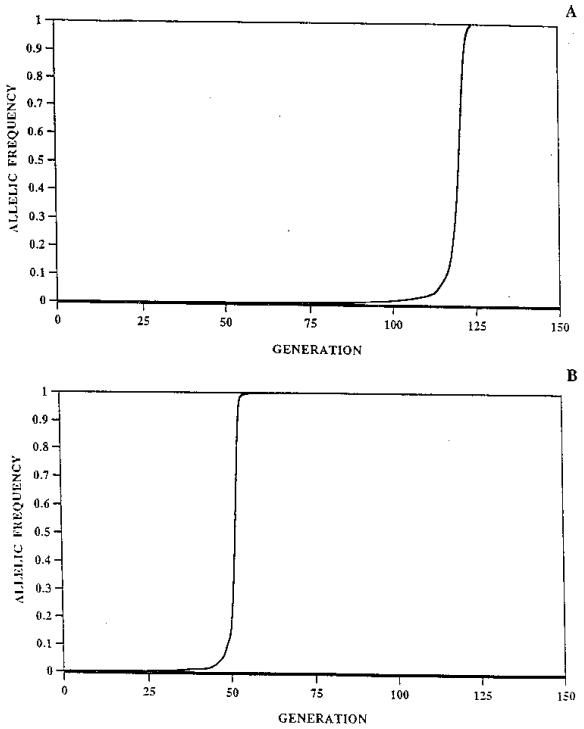

Figure 6. Results of single locus genetic model with fitness values set based on A) data from the field experiment (Table 4), or B) the assumption of equal predation in high *L. decemlineata* density, non-Bt plots, and in low density Bt plots.

assigned a fitness of 0.1346 in *Bt* fields because they were not affected by the *Bt*, but suffered mortality from predation. The RS and SS genotypes had a fitness of 0.3372 in the non-*Bt* plots, just like the RR genotypes. In *Bt* plots, however, the fitness of the RS genotypes were estimated to be $(0.005) \times (0.1346) = 0.000673$, and the fitness of the SS genotypes was estimated to be $(0.001) \times (0.1346) = 0.000135$ because of high toxin-induced mortality. Assuming that a beetle population lives in an area with one-half *Bt* plots and one-half non-*Bt* plots, the average relative fitness of the RR, RS, and SS genotypes would be 0.2359, 0.1689, and 0.1687, respectively. When these values were used to initialize the computer model, it took 118 generations for the resistance allele to reach a frequency of 0.5 (see Figure 6A). When the same genetic model was run under the assumption of equal mortality due to predation in *Bt* and non-*Bt* fields (50% in both), the model indicated that it would take only 52 generations for the resistance allele to reach a frequency of 0.5 (Figure 6B).

Discussion

Changes in feeding rate and in movement are fundamental responses of natural enemies to changes in prey density. These responses determine a natural enemy's effectiveness in controlling insect pest populations (e.g., Huffaker & Messenger, 1976; Hassel, 1978). To properly assess these characteristics in specific predators, experiments must be conducted over a range of spatial scales (Heads & Lawton, 1983; Freeman & Smith, 1990). Hodek (1973) pointed out different feeding responses of the same coccinellid species when tested in laboratory experiments and in a field situation. Ives et al. (1993) highlighted the importance of examining different spatial scales to appropriately examine *C. maculata* movement.

To collect adequate information on *C. maculata* interactions with *L. decemlineata* egg masses when the egg masses were the only source of food, we studied both feeding response and predator movement at different spatial scales (i.e., plant-to-plant and plot-to-plot prey density variation). Our results indicate that *C. maculata* distribution at a plant-to-plant spatial scale as well as in potato fields is driven by *L. decemlineata* egg mass density, but in spite of this aggregation, our experiments indicate that a significant number of beetles occur in areas where food density is low. The proportion of beetles in our high density treatments relative to our low density treatments did not reflect the difference in prey density in a one-to-one relationship (i.e., when the high prey density was $30\times$ the low prey density, the density of beetles in the high prey density treatments was $< 30\times$ higher than in the low prey density treatments). Therefore, an inversely density-dependent pattern of egg consumption was found. Our results have significant implications for biological control projects that use augmentation of *C. maculata*. While *C. maculata* will aggregate in high pest density areas, the extent of aggregation is not sufficient to cause spatially density-dependent predation (see Huffaker & Messenger, 1976 and Hassel, 1978).

Our results are also useful for understanding genetics of tritrophic interactions in a crop system including transgenic plants resistant to *L. decemlineata*. When our data were used in a genetic model to assess how *C. maculata* would affect the rate at which *L. decemlineata* adapted to *Bt* toxin in potatoes, the results indicated that the inverse density dependence would significantly slow down the rate of adaptation if *Bt* potatoes were mixed with non-*Bt* potatoes at a plot-to-plot spatial scale. This result makes sense at an intuitive level. In a mixed deployment of transgenic and non-transgenic potatoes in the field, a much lower density of egg masses on transgenic plants is expected. If predators prey in an inversely density-dependent fashion, the likelihood of a *Bt*-resistant *L. decemlineata* individual reaching adulthood on transgenic plants will be lowered, thus slowing the rate at which the resistance alleles increase in frequency. Our field experiment only examined a single set of relative densities of prey, and our simulation model made a number of assumptions about genetics of resistance. Therefore, the quantitative impact on resistance development predicted from our work should not be considered as a general prediction. The qualitative outcome that *C. Maculata* could slow the development of resistance in plot-to-plot mixtures is somewhat more robust.

We studied a simple system in which there was only one predator and one prey species. In most field situations, generalist predators such as *C. maculata* have a choice of more than one prey species. In the case of *C. maculata* in potatoes, aphids often serve as a source of prey. It will be important to know whether the results that we obtained in our one prey/one predator system would hold in a more species-rich field environment. Our experiments and our analyses are also limited because they did not examine how density-dependent spraying of broad and narrow spectrum pesticides based on economic thresholds would interact with the genetics of our tritrophic system. Further experiments are certainly needed before any final conclusions can be made about how natural enemies will affect the rate at which pests adapt to engineered plants. However, our results clearly point out that adaptation to plant defenses in agricultural and natural settings does not occur in isolation and can be significantly influenced by the ecological setting in which the target plant and herbivore occur.

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