

HOST-PLANT-MEDIATED COMPETITION VIA INDUCED RESISTANCE: INTERACTIONS BETWEEN PEST HERBIVORES ON POTATOES

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Abstract. Plant-mediated competition among insect herbivores occurs when one species induces changes in plant chemistry, nutrition, or morphology that render plants resistant to attack by others. We explored plant-mediated interspecific interactions between the potato leafhopper (*Empoasca fabae*) and the Colorado potato beetle (*Leptinotarsa decemlineata*), two important pests on potatoes. Leafhoppers colonize fields in advance of beetles, and thus the possibility exists that previous feeding by leafhoppers induces changes in potato plants that have adverse consequences for beetles.

The consequences of leafhopper-induced resistance for beetle performance were studied in the greenhouse, field cages, and in large open-field plots. Potato plants were exposed to four densities of leafhoppers (none, low, moderate, and high), and visible feeding symptoms were measured as percentage leaf curling, chlorosis, and necrosis. The oviposition preference, performance, and survivorship of Colorado potato beetles were then measured on the four categories of induced plants in field-cage and greenhouse settings. In open field plots, survival on the four categories of induced plants was determined by placing cohorts of beetle adults onto plants and measuring the densities of resulting eggs, larvae, and emerging F₁ adults.

Leafhopper-induced symptoms on potato plants were density dependent, with the percentage of curled, chlorotic, and necrotic leaves increasing with leafhopper density. Previous feeding by leafhoppers adversely affected oviposition and larval performance of beetles. Fewer egg masses were deposited on plants that incurred high levels of leafhopper feeding. Similarly, larval development was delayed and emerging adult beetles weighed less when fed induced foliage from the high leafhopper-density treatment. Beetles survived less well in the field on plants experiencing moderate and high levels of leafhopper feeding as evidenced by lower densities of eggs, larvae, and emerging F₁ adults. Overall, leafhoppers and beetles competed through feeding-induced changes in plant quality. Notably, the asymmetric interaction took place at a large spatial scale in open field plots and had negative consequences that persisted to the next beetle generation. Ultimately, to establish an effective management strategy for crop pests such as leafhoppers, it is essential to consider the positive indirect effects of induced resistance along with the negative direct effects on crop yield.

Key words: biological control; colonization; economic thresholds; *Empoasca fabae*; feeding guild; induced resistance; *Leptinotarsa decemlineata*; pest management; plant-mediated interspecific competition.

INTRODUCTION

Herbivore-induced plant resistance and its consequences for other phytophagous insects are receiving considerable attention in both agricultural and natural systems (Damman 1993, Denno et al. 1995, Karban and Baldwin 1997, Agrawal et al. 1999, Thaler 1999a, Ohgushi 2005, Viswanathan et al. 2005, Denno and Kaplan, *in press*). Despite widespread interest in induced resistance for pest management, however, we simply do not know much about how one pest species might affect another via induced plant resistance. Because chemical elicitors of plant resistance (e.g., jasmonic acid) are currently being identified and may become important pest management tools (Thaler et al. 1996, Thaler 1999a), it becomes even more paramount to understand the

specific consequences of induced resistance for important agricultural pests.

Induced resistance occurs when herbivore feeding produces changes in host plants that diminish subsequent herbivore attack, changes that result from altered plant nutrition (Denno et al. 2000), morphology (Agrawal 1999), allelochemistry (Levin 1976, Faeth 1986, Rausher et al. 1993, Agrawal 1998, 1999), or some combination of these factors (Karbon and Baldwin 1997, Agrawal 1998, 1999). Notably, induced resistance can be local or systemic, may arise rapidly or with significant delay, and it may decay either quickly or quite slowly over several herbivore generations (Karbon and Baldwin 1997, Agrawal et al. 1999, Denno and Kaplan, *in press*). Thus, induced resistance allows for interspecific competition to occur indirectly between herbivore species that are spatially and/or temporally separated on the same host plant (Faeth 1986, 1992, Tomlin and Sears 1992b, Inbar et al. 1995, Denno et al.

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2000, Wise and Weinberg 2002, Denno and Kaplan, *in press*).

Induced resistance clearly has important implications in agriculture where it is essential to elucidate factors influencing the density of pest herbivores, both for establishing accurate economic injury levels (Dively et al. 1995), and ultimately for the development of biorational pest management programs that may involve chemical elicitors (Thaler 1999a, 2002). The prevalence of insecticide resistance and the concern over excessive use of pesticides in the environment demand a better understanding of pest dynamics in agricultural systems and the development of novel control approaches (Noronha et al. 2001, Matsumura 2004). One of the most notable cases of insecticide resistance has occurred with the Colorado potato beetle (Forgash 1985), a major pest of potatoes throughout North America (Ferro 1985, Forgash 1985, Hare 1990, Roush et al. 1990, Weber and Ferro 1994). Because potato is among the most important crops in the Northeast region of North America (USDA 1999a), and because it is susceptible to several species of insect pests that feed at different times through the growing season (USDA 2002), this system provides an ideal opportunity to explore the consequences of induced resistance and plant-mediated competition between insect herbivores sharing the same host plant. Leaf damage in potatoes and related plants in the Solanaceae results in the induction of a variety of nutritional and allelochemical compounds (Green and Ryan 1972, Tomlin and Sears 1992a, Hlywka et al. 1994, Thaler 1999a), all with known anti-herbivore effects (Karban and Baldwin 1997, Constabel 1999).

The two major pests of potatoes in the Northeast region of the U.S., the potato leafhopper (*Empoasca fabae*: Hemiptera: Cicadellidae) and the Colorado potato beetle (*Leptinotarsa decemlineata*: Coleoptera: Chrysomelidae), are temporally separated due to their differing overwintering habits, crop colonization phenologies, and current management practices (Dively et al. 1999a). In many fields, the potato leafhopper migrates into fields early in the season whereas the Colorado potato beetle colonizes later, after plants have been previously fed on by leafhoppers. Using a combination of greenhouse, field-cage, and large field-plot experiments, the possibility for delayed, plant-mediated competition between these two pests was examined. Specific objectives were to determine if previous feeding by potato leafhoppers had adverse consequences for Colorado potato beetles resulting in (1) reduced oviposition preference for induced foliage and (2) decreased performance (larval development time and adult body mass) and survival on induced foliage. Although there is a growing body of literature demonstrating that temporally and spatially separated herbivores can compete via induced resistance (Karban and Baldwin 1997, Agrawal et al. 1999, Viswanathan et al. 2005, Denno and Kaplan, *in press*), few have done so at a large spatial scale in an agricultural system. Such an

approach should ultimately allow pest managers to establish more accurate economic injury levels for agricultural pests that take into account both the direct impacts of an herbivore on crop yield as well as the possible indirect benefits that accrue from diminished populations of other pest species that feed later in the season. However, the interactive effects between pest species are usually not considered in developing thresholds or in making management decisions for most cropping systems (Welter 1996). Toward filling this void, this study aims to enhance our understanding of induced resistance as it influences interactions between two important pest herbivores.

METHODS

Study system

Potato is the most important agricultural crop in the Northeast region (e.g., Maryland) of the U.S. where >66 328 ha are grown annually with a gross value of U.S. \$295 million (USDA 1999b). The Colorado potato beetle has been a significant pest of potatoes throughout the Northeast region (Dively et al. 1995, USDA 2002). However, current management practices, which include the use of systemic insecticides at planting, have resulted in increases of a second pest, the potato leafhopper (Dively et al. 1999a).

In North America, potato leafhopper (*E. fabae*) attacks a wide range of crops and wild plants including several agriculturally important crops such as potato, alfalfa, beans, and soybeans (USDA 2002). Potato leafhopper cannot overwinter in the Northeast region and it migrates up from the south annually, colonizing potatoes in early June in Maryland and remaining in fields until harvest (Dively 1986, Lamp et al. 1994). Both adults and nymphs of the potato leafhopper feed on the vascular tissue of the leaves and stems of plants (Walgenbach and Wyman 1985, Ferro 1986, Lamp et al. 1994). With piercing-sucking mouthparts, leafhoppers tap into vascular plant tissue and inject saliva while feeding that causes abnormal cell growth and blocks phloem transport. Within two weeks, plants begin to show signs of "hopper burn," which is characterized by leaf curling, chlorosis, and ultimately necrosis of the leaves (Walgenbach and Wyman 1985, Ferro 1986, Lamp et al. 1994). Feeding by leafhoppers is also known to alter the amino-acid composition of potato foliage (Tomlin and Sears 1992b).

The Colorado potato beetle (*L. decemlineata*) is a major pest of potatoes in North America where it can cause severe defoliation and yield loss (Ferro 1985, Weber and Ferro 1994). Eggs are deposited on the undersides of foliage and both larvae and adults are voracious defoliators (USDA 2002). Larvae pass through four larval instars and a prepupal stage before pupating in the soil (Jacques 1988). Colorado potato beetles have two full generations and a partial third per growing season in Maryland, overwintering locally as adults and becoming active in late April (USDA 2002).

Contemporary management practices involve the application of a systemic insecticide to the soil at the time potato seed is planted in early March, precluding the first generation of beetles from inflicting significant damage on potatoes (Dively et al. 1999a, USDA 2002). However, by the time the second generation of beetles emerges in late June–early July, potatoes are no longer protected by the systemic insecticide, and this generation effectively colonizes fields where it can inflict severe damage (Dively et al. 1999a). Where Colorado potato beetles once colonized fields along with potato leafhoppers, this new soil-treatment approach has resulted in the temporal separation of the two pests whereby potato leafhoppers migrate into fields and begin feeding well before colonization by Colorado potato beetle (Dively et al. 1999a). Thus, this system provides an ideal opportunity to test for plant-mediated competition between two economically important pests.

Study sites

Greenhouse facilities were located at the University of Maryland, College Park, Maryland. Field-cage experiments were conducted in potato fields on the University of Maryland Farm (Beltsville). Open field plots were located at the Wye Research and Education Center (Queenstown) and the Central Maryland Research and Education Center (Upper Marlboro), two research sites separated by 75 km.

Greenhouse and field-cage assessment of beetle preference and performance on leafhopper-induced foliage

Leafhopper herbivory treatments.—To assess effects of previous feeding by potato leafhoppers on the oviposition preference and performance of Colorado potato beetles, plants experiencing four levels of leafhopper herbivory (none, low, moderate, and high) were established in the summer of 2001 at the Beltsville farm. In mid-May, tuber seed pieces (*Solanum tuberosum*, Kennebec variety) were planted in individual pots in standard potting medium, and allowed to grow in the greenhouse until aboveground foliage was observed. In early June, potted plants (64 per cage) were transferred to frame field cages (3.66 m² by 1.83 m in height) covered with saran screening (1.3 mm mesh). Plants were allowed to grow in the field cages for one month before leafhoppers were introduced in early July. To establish the four leafhopper herbivory treatments, field-collected leafhoppers (nymphs and adults) were released into cages at one of four preestablished densities (0, 3, 10, and 20 leafhoppers per plant), densities that span the range that occurs naturally in the potato fields (Dively et al. 1999b). Two cages for each treatment were established. Cages were monitored and leafhoppers were replaced to maintain desired densities for each treatment level. Leafhoppers were allowed to feed for two weeks, at which time visual signs of feeding damage were observed.

To confirm that leafhopper density treatments resulted in differences in visible feeding damage, all plants were removed from cages following leafhopper exposure and 20 plants from each of the four density treatments were scored for feeding damage by quantifying leaf curling (percentage) and leaf necrosis (percentage) (Dively et al. 1999b). To verify the persistence of the four leafhopper-density treatments, the number of potato leafhopper nymphs per plant was also censused on 20 plants per treatment at the end of the two-week exposure period. The effects of the four leafhopper treatments on leaf curling, leaf necrosis, and the number of remaining leafhopper nymphs were assessed using ANOVA followed by LSD means comparisons (SAS Institute 2001). Plants from the four leafhopper herbivory treatments were then used to test the effects of previous feeding on the oviposition preference and performance of Colorado potato beetles.

Oviposition preference of beetles for leafhopper-induced foliage.—To determine the effect of previous feeding by leafhoppers on the oviposition preference of Colorado potato beetles in the field, five blocks of 16 plants (four from each leafhopper herbivory level) were transplanted into the ground in a Latin square design at the Beltsville Farm. Within each block, plants were separated by 0.9 m. Saran screen field cages were then erected over each block of 16 plants. Thus, there were five blocks with four treatments per block and four replicate plants per block of each leafhopper feeding treatment. Sixteen pairs of field-collected Colorado potato beetles were released into each cage (one pair per plant) on 25 July. Beetles were allowed to settle for several days after which time eggs were visually censused on 8 August (peak oviposition time) by counting the number of egg masses on each plant. Treatment means were compared using ANOVA followed by LSD means comparisons (SAS Institute 2001).

Beetle performance and survival on leafhopper-induced foliage.—To assess leafhopper herbivory effects on the larval development time (days), adult body mass (grams), and survival (proportion) of beetles, 20 plants from each of the four feeding treatment levels were moved into the greenhouse. Four plants from each treatment were assigned randomly to each of five greenhouse benches (16 plants per bench). One cohort of 15 one-day-old, first-instar beetle larvae was caged on each plant in an organdy mesh bag on 24 July. Every other day, all sleeve cages were opened to record the developmental stage (instar) for all larvae in each cohort. This procedure was followed until all larvae reached the prepupal stage, at which time prepupae were removed from cages, counted, and then placed in buckets of soil for pupation. After emergence, the mass of all adults was determined. Development time (days from first instar to second, third, and fourth instars), larval survival (proportion of larvae surviving from first instar to prepupal stage), and adult wet mass (grams) was determined for each cohort, and treatment means

were compared using ANOVA followed by LSD means comparisons (SAS Institute 2001). Data were log-transformed as needed to meet assumptions of normality and homogeneity of variances.

Open-field assessment of beetle preference and performance on leafhopper-induced foliage

Leafhopper herbivory treatments.—To determine the effects of previous feeding by potato leafhoppers on the density of the life stages of Colorado potato beetles (eggs, larvae, and emerging adults), plants experiencing four levels of leafhopper herbivory (none, low, moderate, and high) were created in the summer of 2002 at both the Wye and Central Maryland research farms. At both locations a single field was planted with seed pieces of potatoes (var. 'Atlantic') in early April and subsequently divided into twelve plots. Each plot was six rows \times 10 m with 2 km separating plots. Potato plants were managed with standard commercial agronomic inputs including frequent overhead irrigation and applications of fertilizer as needed throughout the growing season.

Plots were randomly assigned one of four leafhopper herbivory treatments to each block (farm): none, low, moderate, or high (three plot replicates per treatment at each farm). The four levels of herbivory were achieved by manipulating natural populations of leafhoppers with selective insecticide applications of permethrin (FMC, Philadelphia, Pennsylvania, USA) to field plots. Since potato leafhoppers are the only major early-season herbivore inhabiting potato fields, sprays primarily manipulated leafhopper density and minimized non-target effects. No-herbivory plots were maintained with weekly applications of permethrin at a high rate (224 g/ha), whereas high-herbivory plots remained untreated and achieved ambient densities of leafhoppers. Low- and moderate-herbivory plots were treated with insecticide when plant symptoms indicative of low and moderate leafhopper density appeared. Plant symptoms used to indicate the four levels of herbivory and initiate spray regimes were based on the percentage of potato leaves exhibiting curling and yellowing, symptoms indicative of leafhopper feeding: 0% curling/yellowing (no herbivory), 10–30% (low), 40–60% (moderate), and 70–90% (high). Spray regimes were initiated when leafhoppers colonized plots in early June and ended in early July when potato beetles normally begin colonizing fields initially treated with soil insecticides at planting.

Once a week from early June through mid-July plots were sampled for leafhopper adults and nymphs to verify the efficacy of sprays in generating the four desired herbivory treatments. Adult density was estimated using sweep net sampling (10 sweeps per plot), whereas the density of nymphs was measured by visually searching the undersides of 10 random leaves per plot. Plants were also rated for evidence of leafhopper damage (percentages of leaf curling, yellowing, and necrosis) in all plots every 2 weeks. Two separate

observers independently estimated the percentage of leaves in each plot displaying damage symptoms (percentages of leaf curling, yellowing, and necrosis) and the average of these two observations was used as the plot mean (Dively et al. 1999b). Treatment effects on the density of leafhopper adults and nymphs (samples pooled from 5, 12, and 22 July) and symptoms of leafhopper herbivory (percentages of leaf curling, yellowing, and necrosis assessed on 22 July) were compared using ANOVA followed by a Tukey's means comparison test (HSD; SAS Institute 2001).

Beetle oviposition and survival on leafhopper-induced foliage.—To assess the impact of variation in early-season leafhopper herbivory on Colorado potato beetle survival, cohorts of field-collected adult beetles were placed into three replicate plots of each of the four herbivory treatments (none, low, moderate, and high) at each farm. Subsequently, differences in cohort survival across treatments were assessed by measuring the density of resulting eggs, larvae, and emerging F_1 adults over time. Field-collected beetles were used to establish each cohort of 20 adult beetles by placing one beetle on every other plant in the center two rows of each plot. Every other day from 8 July to 22 July (seven sample dates), plants in the center two rows were visually searched and all beetle life stages were counted and recorded. On 5 August, after larvae from cohorts had pupated in the soil and emerged as adults, we counted the number of emergence holes (estimate of F_1 adults) observed while walking through the center row (10 m) of each plot. This procedure provided an accurate estimate of cohort survival (emergence holes/10 m) through one complete generation. Treatment effects on the density of released adult beetles, eggs, early-instar larvae (first and second instars pooled), and late-instar larvae (third and fourth instars pooled) were compared using repeated-measures ANOVA with herbivory level and sampling date as main effects, and with farm as a blocking factor (SAS Institute 2001). Treatment effects on the number of emergence holes (F_1 adults) were assessed using ANOVA followed by a Tukey's means comparison test (HSD; SAS Institute 2001).

Several measures were taken to prevent contamination of field plots by local beetle populations. First, fields were planted at locations where potato was not grown in previous years, thus limiting the pool of overwintering adult colonists, which have notoriously poor dispersal capability because they disperse by walking rather than flight (Hare 1990). Furthermore, four buffer rows running along each side and 3 m at the ends of all rows were treated with the systemic insecticide imidacloprid (Bayer CropScience, Research Triangle Park, North Carolina, USA) at planting to form a treated outer perimeter of the fields. This management strategy provided an effective barrier, substantially reducing the number of immigrating adults (Dively et al. 1998). Last, all plants in each plot were visually searched prior to the

TABLE 1. Effect of intended experimental manipulations of leafhopper density on the resulting density of adults and nymphs and feeding-induced symptoms on potato plants in greenhouse and field experiments.

Intended leafhopper density	Resulting leafhopper density [†]		Percentage of leaves damaged		
	Adults	Nymphs	Curling	Yellowing	Necrosis
Greenhouse					
None	not assessed	0.30 ^a ± 0.10	2.75 ^a ± 0.37	not assessed	0.15 ^a ± 0.08
Low	not assessed	1.85 ^a ± 0.26	10.43 ^b ± 2.03	not assessed	0.50 ^a ± 0.11
Moderate	not assessed	15.20 ^b ± 1.03	79.50 ^c ± 2.11	not assessed	2.25 ^b ± 0.45
High	not assessed	29.70 ^c ± 1.79	94.75 ^d ± 1.56	not assessed	11.80 ^c ± 1.77
Field					
None	3.83 ^a ± 1.54	1.22 ^a ± 0.50	25.83 ^a ± 4.17	9.17 ^a ± 1.54	5.83 ^a ± 0.83
Low	11.16 ^a ± 4.08	2.16 ^a ± 0.86	41.67 ^a ± 5.43	15.83 ^a ± 3.52	10.42 ^a ± 2.62
Moderate	26.78 ^b ± 9.77	10.72 ^b ± 3.07	94.17 ^b ± 2.71	61.67 ^b ± 9.72	47.50 ^b ± 10.31
High	30.78 ^b ± 4.28	14.55 ^b ± 1.58	95.00 ^b ± 2.58	57.50 ^b ± 11.38	51.67 ^b ± 6.54

Note: Means (\pm SE) with different letters are significantly different ($P < 0.05$).

[†] Leafhopper density is the number per plant (nymphs in greenhouse), number per 10 sweeps (adults in field), and number per 10 leaves (nymphs in field).

establishment of cohorts and all ambient beetle eggs, larvae, and adults were removed.

RESULTS

Greenhouse and field-cage assessment of beetle preference and performance on leafhopper-induced foliage

Incidence of leafhopper herbivory and effects on potato plants.—There was a significant effect of treatment (0, 3, 10, and 20 potato leafhoppers per plant) on the number of leafhopper nymphs remaining at the end of the exposure period ($F_{3,76} = 172.10$, $P < 0.0001$; Table 1), verifying the persistence of the intended leafhopper density treatments. The leafhopper density treatments translated into significant herbivory effects on potato plants. In general, the percentage of curled ($F_{3,76} = 382.37$, $P < 0.0001$) and necrotic leaves ($F_{3,76} = 73.45$, $P < 0.0001$) increased with an increase in leafhopper density (Table 1).

Oviposition preference and performance of beetles on leafhopper-induced foliage.—Overall, previous feeding by potato leafhoppers resulted in potato foliage that was avoided by ovipositing female beetles, and feeding on such foliage had negative consequences for both the growth and survival of larvae. For example, when given a choice, female Colorado potato beetles deposited at least twice as many egg masses on plants that incurred no to moderate levels of leafhopper herbivory as they did on plants that were heavily fed upon by leafhoppers ($F_{3,76} = 4.10$, $P = 0.0094$; Fig. 1A).

Moreover, beetle larvae developing on plants that experienced a high level of leafhopper herbivory took longer to molt into second and third instars than larvae feeding on plants incurring no, low, or moderate levels of leafhopper feeding (second instars, $F_{3,76} = 4.71$, $P = 0.0045$, Fig. 1B; third instars, $F_{3,76} = 6.06$, $P = 0.0009$; Fig. 1C). Development time from first to fourth instar was not significantly protracted by feeding on leafhopper-induced plants, although there was a trend in this direction ($F_{3,72} = 1.82$, $P = 0.1503$; Fig. 1D).

Survivorship (first instar to prepupa) was significantly higher for beetle larvae developing on plants experiencing no, low, or moderate levels of leafhopper herbivory compared to larvae developing on plants subjected to a high level of previous leafhopper feeding ($F_{3,76} = 4.64$, $P = 0.0049$; Fig. 1E). Also, larvae developing on plants with low and high levels of leafhopper feeding damage molted into significantly lighter adults than larvae developing on plants experiencing no damage or moderate damage ($F_{3,68} = 5.29$, $P = 0.0024$; Fig. 1F).

Open-field assessment of beetle preference and performance on leafhopper-induced foliage

Incidence of leafhopper herbivory and effects on potato plants.—Spraying regimes were effective at establishing four levels of leafhopper density in open field plots (Table 1). For example, there were significant treatment effects on the density of leafhopper adults ($F_{3,20} = 11.34$, $P = 0.0002$) and nymphs ($F_{3,20} = 15.66$, $P < 0.0001$) that paralleled desired levels of leafhopper herbivory (no, low, moderate, and high). Moreover, the achieved levels of leafhopper density resulted in significant treatment effects on the symptoms of leafhopper feeding. Explicitly, the percentage of curled ($F_{3,20} = 49.21$, $P < 0.0001$), yellowed ($F_{3,20} = 12.55$, $P < 0.0001$), and necrotic potato leaves ($F_{3,20} = 17.55$, $P < 0.0001$) increased with an increase in leafhopper density (Table 1).

Beetle oviposition and survival on leafhopper-induced foliage.—In general, feeding on leafhopper-induced potato foliage had adverse effects on the oviposition and survival of beetles. Although there were no significant treatment effects on the density of adult beetles initially placed in the field ($F_{3,140} = 0.99$, $P = 0.399$; Fig. 2A), there were significant treatment effects on the number of egg masses that were deposited on plants ($F_{3,140} = 4.05$, $P = 0.009$; Fig. 2B). In general, beetles deposited nearly twice as many egg masses on plants that incurred no or low levels of leafhopper

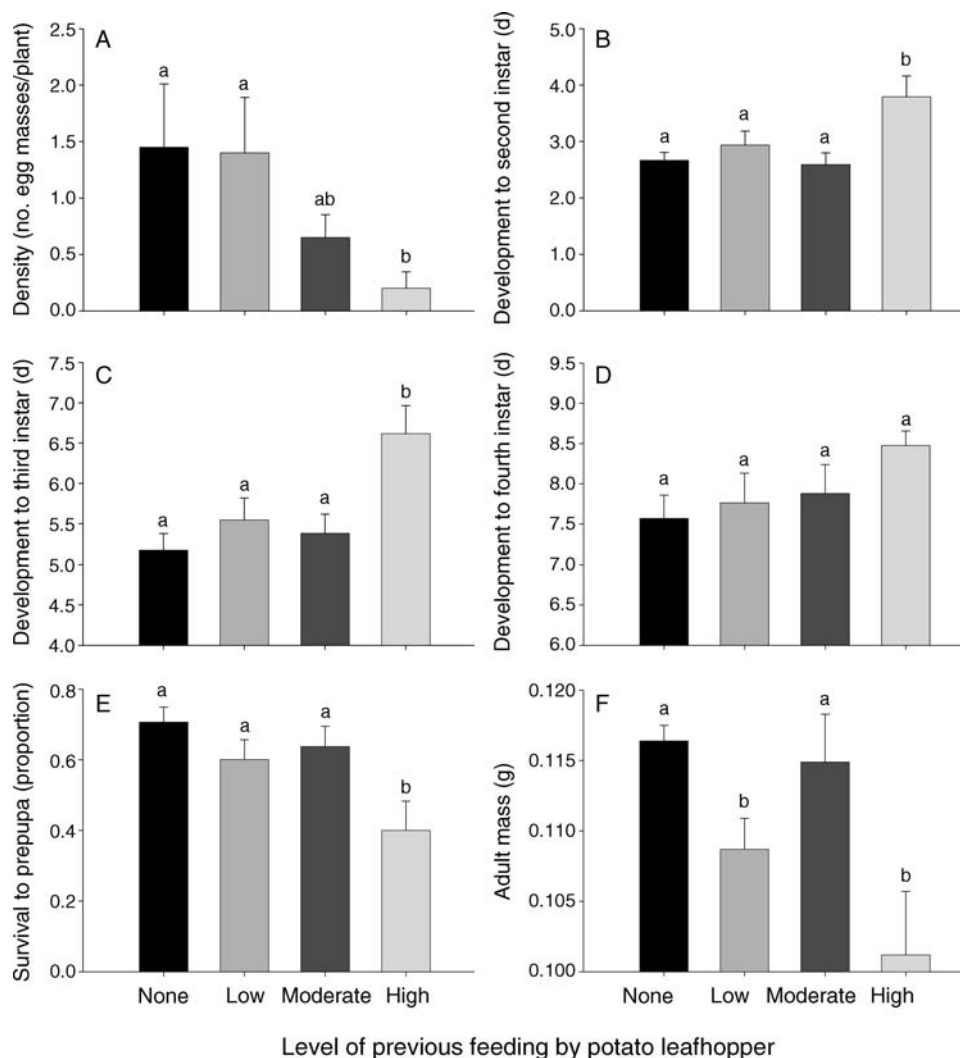


FIG. 1. (A) Number of Colorado potato beetle egg masses deposited on caged potato plants that had experienced one of four levels of previous feeding by potato leafhoppers: none, low, moderate, or high. (B–D) Development time, in days, (B) to second instar, (C) to third instar, and (D) to fourth instar. (E) Proportion of beetles surviving to prepupa and (F) adult body mass of beetles raised on potato foliage from the same four leafhopper-herbivory treatments. Means (\pm SE) with different letters are significantly different ($P < 0.05$).

herbivory than on plants that experienced a moderate or high incidence.

Moreover, there were significant effects of the leafhopper-feeding treatments on the density of early-instar ($F_{3,140} = 6.95$, $P = 0.0002$; Fig. 2C) and late-instar beetle larvae ($F_{3,140} = 3.72$, $P = 0.013$; Fig. 2D). Nearly three times as many early-instar larvae and almost four times as many fourth-instar larvae occurred on plants that incurred no or low levels of leafhopper feeding compared to the number of larvae on plants that sustained moderate and high levels of herbivory. That the difference in density of beetles on non-induced vs. heavily induced plants diverged from the egg to the late larval instar stage suggests that mortality occurred throughout beetle development. The larvae of Colorado potato beetles are extremely immobile, and it is unlikely

that differential emigration is a contributing factor to discrepancies in density across treatments. Notably, treatment effects on cohort survival carried over to the next generation such that the number of emergence holes left by F_1 adults was significantly higher in plots experiencing no leafhopper herbivory than in plots where potato plants incurred high levels of leafhopper feeding ($F_{3,20} = 3.90$, $P = 0.024$; Fig. 3). The fourfold difference in the density of F_1 beetles between no- and high-herbivory plots (Fig. 3) carried over from the density difference of late-instar larvae present in the same treatment (Fig. 2D) suggesting that treatment effects on pupal mortality were minimal. For this experiment, there were significant block effects (Wye vs. Upper Marlboro farms) and date effects (density across seven sample dates) on the density of all

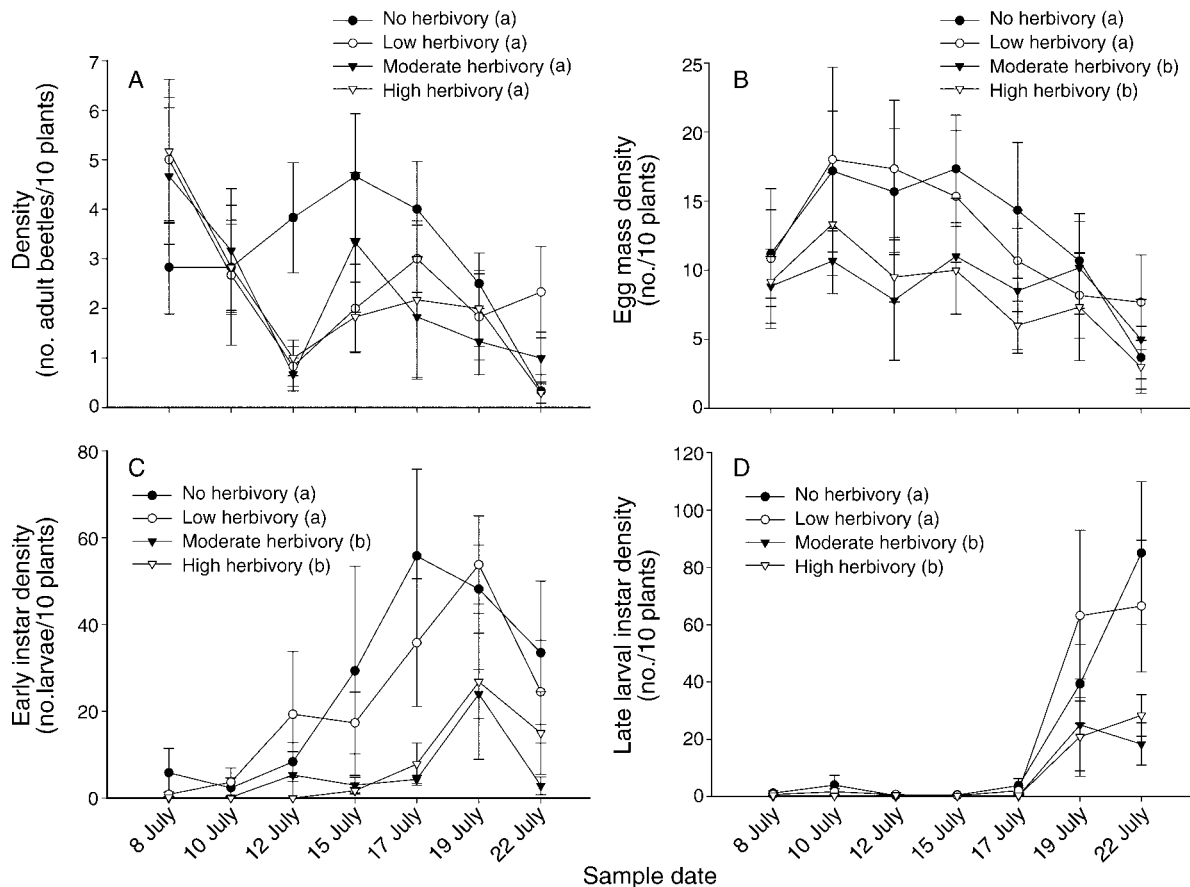


FIG. 2. (A) Density profiles (no. /10 plants) of adult Colorado potato beetles remaining after release (cohorts of 20 adult beetles released on 8 July) in open field plots of potatoes exposed to one of four levels of previous feeding (herbivory) by potato leafhoppers: none, low, moderate, or high. (B–D) Density profiles of the (B) eggs, (C) early-instar larvae, and (D) late-instar larvae of potato beetles in potato plots that experienced the same four leafhopper-herbivory treatments (eggs and larvae are the offspring of released beetle cohorts). Significant differences among treatment means (\pm SE) are indicated in the legend of each panel with different letters ($P < 0.05$).

immature developmental stages ($P < 0.05$). However, there were no significant treatment-by-date effects on the density of any immature stage ($P > 0.05$).

DISCUSSION

Data from greenhouse and field experiments show that previous feeding on potato plants by potato leafhoppers adversely affects the oviposition preference, larval performance, and survival of Colorado potato beetles. Specifically, (1) adult beetles deposited fewer eggs on leafhopper-induced foliage, (2) larvae developed slower and survived less well when raised on leafhopper-induced leaves, and (3) larvae molted into smaller adult beetles (Fig. 1). Moreover, the adverse effects of leafhopper-induced resistance on beetle survival were shown at a large spatial scale in open field plots (Fig. 2). Because leafhoppers and beetles occupied potato plants at different times, this represents a clear case of induced resistance, whereby one herbivore species affects another indirectly through feeding-induced changes in plant physiology or morphology (see Karban and Baldwin

1997, Agrawal et al. 1999, Denno and Kaplan, *in press*). Moreover, this is one of a growing number of studies demonstrating strong interspecific competitive effects between two distantly related taxa in different feeding guilds, a sap-feeder and a mandibulate herbivore (reviewed in Denno and Kaplan, *in press*). This particular interaction is asymmetric by default, because potato leafhoppers colonize potatoes in advance of Colorado potato beetles. Under different management practices beetles could precede leafhoppers in fields. However, previous research has shown that potato leafhoppers have greater effects on the amino-acid profile of potatoes than Colorado potato beetles suggesting that induced effects on plant nutrition are inherently asymmetric (Tomlin and Sears 1992a). For the most part, reciprocal effects are rarely examined in reports of plant-mediated interspecific competition, largely because researchers explore the effects of early-season feeders on late-season species (Damman 1993, Denno et al. 1995, Denno and Kaplan, *in press*).

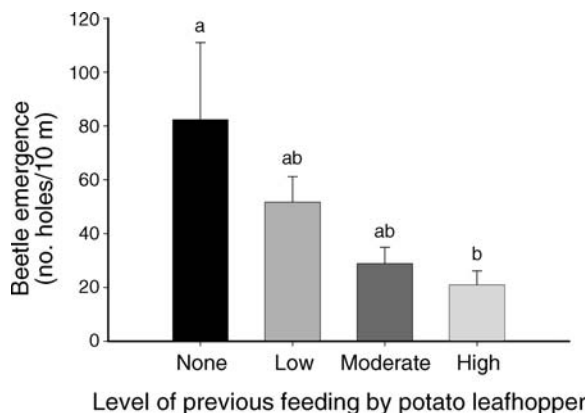


FIG. 3. Number of Colorado potato beetle emergence holes per 10 m of row space (index of F_1 adult density) in plots of potato plants that experienced one of four levels of previous feeding by potato leafhoppers. Means (\pm SE) with different letters are significantly different ($P < 0.05$).

Although the mechanism for induced resistance by potato leafhoppers remains an open question, several possibilities exist including altered plant nutrition, allelochemistry, morphology, or some combination of these responses. For example, natural resistance to herbivores in potatoes has been attributed to glycoalkaloids and proteinase inhibitors (Green and Ryan 1972, Tingey 1984, Sikinyi et al. 1997). Moreover, allelochemical-based resistance has been found in certain wild *Solanum* species that results in negative effects on the survival and fecundity of Colorado potato beetles (Pelletier et al. 1999). Notably, previous feeding by insect herbivores or applications of the elicitor methyl jasmonate result in increased concentrations of proteinase inhibitors and polyphenol oxidases (Green and Ryan 1972, Bolter and Jongsma 1995, Stout and Duffey 1996, Stout et al. 1998), glycoalkaloids (Hlywka et al. 1994), and several amino acids (Tomlin and Sears 1992b) in potato. Such compounds should be included as high-probability targets for investigating the mechanism underlying leafhopper-induced resistance in potato. In this study, symptoms of "hopper burn" such as leaf curling, chlorosis, and necrosis are indicative of an induced morphological-physiological change that may underlie the induced resistance to Colorado potato beetle. Any induced morphological change per se more likely influences oviposition preference by adult beetles than larval performance because there is no observational evidence that curled leaves deter larval feeding (Lynch 2005). Thus, induced-resistance effects on beetle performance in this system more likely have their basis in altered plant physiology than in altered architecture.

There are numerous examples in other systems in which plant-mediated competition between insect herbivores resulted from altered changes in plant nutrition (Inbar et al. 1995, Denno et al. 2000), allelochemistry (Stout and Duffey 1996, Stout et al. 1998, Inbar et al. 1999a, b, Bezemer et al. 2003, Van Zandt and Agrawal

2004), morphology (Agrawal 1998, 1999), or some combination of these mechanisms (Agrawal 1999). Together, these studies underscore the diversity of mechanisms that underlie plant-mediated competition between insect herbivores.

Overall, our research as well as that of others suggests that plant-mediated competition between insect herbivores is a widespread phenomenon (Damman 1993, Denno et al. 1995, Van Zandt and Agrawal 2004, Denno and Kaplan, *in press*), and certainly supports the view that seasonally displaced herbivores can compete via induced resistance. However, unlike other studies where induced resistance occurs at low herbivore densities (Karban and Baldwin 1997), the resistance factor imposed by potato leafhoppers on beetles appears to be density dependent (Figs. 1 and 2).

Importantly, the effects of induced resistance can extend to higher trophic levels such that slow-developing herbivores on induced plants experience a higher risk of attack from predators or parasitoids (Thaler 1999a, b, 2002, Kessler and Baldwin 2004, Denno and Kaplan, *in press*; I. Kaplan, G. P. Dively, and R. F. Denno, *unpublished manuscript*). For Colorado potato beetles, leafhopper-induced developmental delays are particularly evident early in development during the first and second instar stage (Fig. 1B and C). Notably, it is the younger instars of Colorado potato beetle that are particularly vulnerable to attack from predaceous stinkbugs, and leafhopper-induced developmental delays result in dramatic increases in mortality and thus the opportunity for enhanced biological control (I. Kaplan, G. P. Dively, and R. F. Denno, *unpublished manuscript*). Thus, the effects of induced resistance can cascade to other herbivores and their natural enemies, and thereby affect the structure and dynamics of the entire food web (Thaler 1999b, Van Zandt and Agrawal 2004, Ohgushi 2005).

Induced plant resistance clearly has important implications for the management of agricultural pests. Economic injury levels for pest management have been developed primarily for single pest species (Ferro 1986, Mahr et al. 1995), and interactive effects among pests are usually not considered (Welter 1996). However, potato growers in the mid-Atlantic area of the U.S. are faced with intra-seasonal decisions concerning the control of both potato leafhopper and Colorado potato beetle (Walgenbach et al. 1985, Dively et al. 1995, 1998). The current management approach assumes that the impacts of both pests are independent. Thus, control decisions regarding potato leafhoppers are based solely on current control costs and not on projected savings associated with the adverse induced resistance effects of leafhoppers on potato beetles. Current management practices for potato leafhoppers include the use of insecticides when densities reach a certain economic threshold, which has historically been rather conservative (Walgenbach et al. 1985, Dively et al. 1995). Based on our findings, a potato grower should be able to

accept a higher level of leafhopper injury, if losses due to Colorado potato beetle later in the summer can be prevented at a level sufficient to offset the direct costs of leafhopper damage. This is particularly likely given that significant adverse effects of induced resistance on beetles were seen in the field under just moderate levels of leafhopper herbivory (Fig. 2). Thus, the action threshold for leafhopper control should be higher when the delayed benefits of beetle reduction via induced plant resistance are considered (G. P. Dively, I. Kaplan, and R. F. Denno, *unpublished manuscript*). Importantly, higher action thresholds for potato leafhopper should also increase the chance of maximizing Colorado potato beetle mortality from natural enemies (I. Kaplan, G. P. Dively, and R. F. Denno, *unpublished manuscript*). Ultimately, to establish an effective management strategy for the multiple pests on potato, it will be essential to combine the positive indirect effects of induced resistance along with the negative direct effects on crop yield, a strategy which is not currently employed in potato (Dively et al. 1999b), or in other crops at large (Welter 1996).

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