Plant genotypic diversity interacts with predation risk to influence an insect herbivore across its ontogeny

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Abstract. A growing number of studies have manipulated intraspecific plant diversity and found dramatic changes in the densities of associated insect herbivores and their predators. While these studies have been essential for quantifying the net ecological consequences of intraspecific plant diversity, they have been less effective at uncovering the ways in which plant diversity alters trophic interactions within arthropod communities. We manipulated intraspecific plant diversity and predation risk in the field in a factorial design to reveal how a mixture of plant genotypes changes the response of an herbivorous beetle (Leptinotarsa decemlineata) to a common stink bug predator (Podisus maculiventris). We repeated the manipulations twice across the ontogeny of the beetle to examine how the effects of diversity on the predator–prey interaction differ between larval and adult stages. We found that intraspecific plant diversity, mixtures of susceptible and resistant varieties of potato (Solanum tuberosum), reduced larval survival by 20% and adult oviposition by 34%, which surprisingly put survival and oviposition lower in the mixed-genotype plots than in the resistant monocultures. Moreover, we found that predation risk reduced larval survival 25% and 11% in resistant and susceptible monocultures, respectively, but had no effect in the mixture. This result indicated that our genotypic mixing treatment interacted nonadditively with predation risk such that plant diversity altered the predator–prey interaction by changing the responses of the beetles to their stink bug predators. In addition, even though predation risk reduced larval survival, it increased adult overwintering survival by 9%, independently of plant treatment, suggesting that these interactions change through ontogeny. A key implication of our study is that plant diversity influences arthropod communities not only by changing resource quality, as past studies have suggested, but also by changing interactions between species within the arthropod community.

Key words: intraspecific plant diversity; Leptinotarsa decemlineata; nonconsumptive effects; Podisus maculiventris; predation risk; Solanum tuberosum; trait-mediated indirect interactions; tritrophic interactions.

INTRODUCTION

Intraspecific plant genetic diversity has dramatic effects on insect herbivores and their predators (Crutsinger 2006, Cook-Patton et al. 2011, McArt and Thaler 2013). Surprisingly, the effects of intraspecific diversity can be as great as the effects of plant species diversity (Cook-Patton et al. 2011). In general, manipulative studies of intraspecific plant diversity have found that increasing diversity tends to lead to higher densities of natural enemies of herbivores, lower densities of herbivores, and lower levels of plant damage in both natural and agricultural ecosystems (Grettenberger and Tooker 2017). These results, however, have been variable and inconsistent, with some studies indicating negative effects of diversity on plant damage and other studies showing increased natural enemy density but no change in herbivore density (Underwood 2009, Reiss and Drinkwater 2018). The large variability in outcomes resulting from plant diversity experiments, and the lack of a consistent link between increased natural enemy density and herbivore suppression, suggest that our understanding of the effects of intraspecific plant diversity on higher trophic levels may be missing key mechanistic components (Moreira and Mooney 2014). Resolving how intraspecific plant diversity influences herbivores and their interactions with predators would advance our fundamental understanding of the consequences of a key component of plant biodiversity and, moreover, could reveal new ways to use crop genotypic diversity as an ecological diversification strategy for sustainable pest management in agroecosystems.

A key gap among past studies of the effects of intraspecific plant diversity on herbivores and their predators is that they have not manipulated predators in conjunction with plant genotypic diversity. Instead, past studies have manipulated only plant diversity and then observed the net effects of plant diversity on herbivores and predators, without separating the effects of plant diversity on predator recruitment from their effects on the interaction between herbivores and predators (Root 1973, Andow 1991, Abdala-Roberts and Mooney 2014, Moreira et al. 2016). To improve our understanding of the consequences of plant diversity, we need to uncover how plant diversity alters the interactions between herbivores and their predators, acknowledging the potential for the bottom-up effects of plant diversity to interact with the top-down effects of predation risk (Moreira and Mooney 2014, Moreira et al. 2016). Studies that manipulate predation risk simultaneously with plant diversity would allow a deeper understanding of the ecological pathways by which plant diversity influences higher trophic levels. Moreover, predation by natural enemies is a key ecosystem service
that contributes to sustainable pest control, and it is essential that we understand how intracrop diversity will affect herbivore–predator interactions before we can use it for pest control.

One key, understudied component of predator–prey interactions is the nonconsumptive effect of predation, the behavioral and physiological responses of prey to the perception of predation risk (Werner et al. 1983). In insect herbivores, these responses include detrimental physiological stress and a reduction in time spent feeding, both benefits to plant productivity, and can represent half of the total effect of predation that cascades down to plants (Preisser et al. 2005). Intraspecific plant diversity might influence the responses of insect herbivores to predators because variability among individual plants in a population can influence herbivore behavior (Schultz 1983) or physiology (Wetzel and Thaler 2016) in ways that changes their responses to predation risk and alters their vulnerability to predation. Despite the potential for plant diversity to alter prey responses to predation risk and despite the relevance of this knowledge for our ability to manage agroecosystems using plant diversity and natural enemies, no studies have examined the effects of intraspecific plant diversity on the nonconsumptive effects of predators on insect herbivores.

A second gap among past studies of the consequences of intraspecific plant diversity is that they have not separated the potentially differing effects of intraspecific plant diversity on insect performance and preference at different stages of the life cycle. Past studies have measured colonization of open plots by the arthropod community (Johnson et al. 2006) or effects on one component of insect fitness at one life stage (e.g., adult density [Grettenberger and Tooker 2016] or biomass and survival of larvae [Kotowska et al. 2010]). Inferring mechanisms, which are necessary if we are to develop reliable methods for using intraspecific plant diversity to manage pests, would be facilitated by studies that measure effects at each life stage, from effects on larval performance, which may be affected by diet diversity (Stockhoff 1993, Ruel and Ayres 1999, Wetzel et al. 2016), to adult patch selection and oviposition behavior, which may be affected by the associational effects that plant types can exert on neighbors in diverse plant neighborhoods (Hambäck et al. 2014, Underwood et al. 2014).

We examined the interactive effects of plant genotypic mixing and predation risk on the Colorado potato beetle (Leptinotarsa decemlineata) using two field experiments that tracked individuals across an entire generation, including adult overwintering, and measured feeding, survival, growth, and oviposition behavior. Overwintering mortality is a key selective force each generation, but very little work has examined how it varies with biotic interactions like plant diversity and predation risk. First, we examined growth and survival at all life stages from first instar to adult including overwintering success in caged monocultures or mixtures of one susceptible and one resistant variety of potato (Solanum tuberosum). The first experiment examined the interaction between plant variability and predation on beetles after oviposition and so confined larval beetle to plots. In the second experiment, we examined behavior of adult beetles, including oviposition and feeding choices, using an open-plot experiment with monocultures and mixtures of the same potato varieties. Plant treatments in both experiments were crossed with a predation risk treatment that included the presence or absence of a common predatory stink bug (Podisus maculiventris). Previous work has shown that when Colorado potato beetle larvae perceive a threat of predation by this stink bug they reduce their rate of consumption and grow more slowly, but this effect depends on plant defense (Kaplan et al. 2014). How genotypic variability in plant defense influences responses to predators, however, is unknown.

**Methods**

**Organisms**

The Colorado potato beetle (CPB), native to Mexico, is a global pest on potato and other Solanaceae. Individuals have four instars, puate in the soil near the larval hosts, emerge as adults, and feed and mate before returning to the soil to overwinter. In the spring after overwintering, adults emerge and search for host plants for oviposition, feeding, and mating (Hare 1990). Most beetles are univoltine at our study sites in Tompkins County, New York, USA (42.4440° N, 76.5019° W). Experimental beetles came from a lab colony kept on cv. Green Mountain and refreshed annually with locally collected wild individuals. P. maculiventris (stink bug hereafter) is a generalist predator native to much of North America that has been released around the world for biological control of insect pests. All stink bugs used in this study came from a lab colony that was refreshed each year with individuals caught in Tompkins County, New York.

In both experiments, we examined the effects of monocultures or mixtures of two potato varieties: Yukon Gold, which is susceptible to CPB attack, and King Harry (originally NY-131), which was bred specifically for increased trichome density and generally for increased resistance to insect pests. Indeed, it has been shown in field trials to support low densities of insect pests, including CPB, and receive less insect damage (Brown 2012). Other than increased trichome density and higher levels of trichome-associated secondary metabolites, it likely also has other modified resistance traits, though these are not well quantified.

**Growth and survival field experiment**

We examined beetle survival and growth using a field experiment from June 2016–June 2017 at the Homer C. Thompson Vegetable Research Farm at Cornell University. We planted 120 plots (86 × 86 cm) with four plants in one row in the center of each plot with 20-cm spacing within rows. The plots alternated among three plant treatments: a susceptible monoculture of four Yukon Gold plants, a resistant monoculture of four King Harry plants, and a susceptible-resistant mixture with two plants of each genotype, alternated within the plot. We then erected a cage (86 cm per side) made of a PVC frame with amber screen (1 mm opening; Lumite, Alto, Georgia, USA) on each plot to prevent insects from colonizing. Fourteen plots had low germination, leaving us with 38 susceptible monocultures, 35 resistant monocultures, and 33 susceptible-resistant mixtures. On 10 August 2016 when plants had reached approximately 50-cm tall, plots received either 4, 8, 16, 32, or 64...
first-instar beetles, with density treatments spread evenly among plant treatments and with beetles spread evenly among plants within a plot, allowing us to account for potential density dependence.

We examined the interaction between the genotypic mixture and predation risk by introducing two male and two female stink bugs to one-half of the cages in each treatment group. Before introducing each stink bug, we surgically removed the tip of its rostrum using a razor blade. This manipulation has been shown in previous studies to prevent them from killing their prey without reducing stink bug survival, plant feeding, or foraging, and, moreover, these surgically altered stink bugs still exhibit hunting behavior that elicits nonconsumptive effects in CPB (Griffin and Thaler 2006, Kaplan and Thaler 2010, Thaler et al. 2012). Four days after introducing stink bugs to the cages, we introduced one more male–female pair of stink bugs to each cage in the predation risk treatment to maintain predation risk despite natural stink bug mortality.

After the start of the experiment, we censused each plot on day 8 of larval life (approximately the third larval instar), in fall before adults returned to the soil to overwinter, and in spring immediately after adults emerged from the soil after overwintering. In the day 8 larval census, we recorded the number of beetles on each plant, the length (from top of head to tip of last abdominal segment) of two larvae per plant (randomly selected), and plant height. In the fall census, we counted and sexed all beetles (all were adults) and measured the body mass and pronotum width of up to 20 beetles per plot, randomly selecting them from the total. In the spring census, we visited cages every 3 d starting before the first emergence and continuing until after the final emergence (20 May–29 June 2017) and collected all beetles on each visit. Beetles were frozen and later sexed, weighed, and had their pronotum width measured. We use wet mass as a response variable because in other work we found it to be tightly correlated with dry mass ($F_{1,248} = 6938, P < 0.001, R^2 = 0.97; \text{Wetzel and Thaler 2018}$).

We estimated the effect of plant treatment (susceptible, resistant, or the mixture), predation risk (stink bug presence), and their interaction on beetle survival using binomial generalized linear mixed models and growth using linear mixed models. In the adult censuses, the growth response was $\log$(length). In the adult censuses, the growth response was the first component from a principal component analysis of $\log$ mass and $\log$ pronotum width, which were highly correlated ($r = 0.67$). Models accounted for correlations among beetles within a plot with a random effect, and larval length models had an additional nested random effect for plant within plot. We included beetle sex as a covariate in adult growth models. We looked for direct effects of the genotypic mixture on plant growth by comparing plant height between monoculture and mixture treatments during the larval census when plant damage was still relatively low. We fit these models using lme4 in R (Bates et al. 2015, R Core Team 2017). We tested hypotheses about the effects of plant treatment, predation risk, and their interaction by comparing nested models using likelihood ratio tests (Bolker et al. 2009). For each response variable, we examined the difference between the mean response in polyculture and the mean response across both monocultures by comparing a null model without an effect of plant treatment to a model with a factor for monoculture or polyculture, effectively grouping both monocultures together. We also examined differences between each monoculture and the polyculture by comparing the null model to a model with dummy variables representing each plant treatment (resistant monoculture, susceptible monoculture, and polyculture).

**Adult behavior field experiment**

We examined the effects of the plant mixture and predation risk on adult oviposition and feeding choices using a field experiment in June–July 2016 with 120 patches of potted potato plants of the susceptible variety, the resistant variety, or a mixture of both. Each patch was assigned to one of five plant treatments: two resistant plants, two susceptible plants, four resistant plants, four susceptible plants, or a mixture of two resistant plants and two susceptible plants (24 patches per plant treatment). Including monoculture patches of two sizes (two or four plants) allowed us to separate the effects of focal plant frequency from the effects of focal plant density (Underwood et al. 2014). Changes in plant frequency lead to associational effects, which are a true effect of patch diversity, whereas changes in plant density lead to resource concentration or dilution effects, which depend on the abundance of a focal plant type and are not necessarily related to plant diversity (Hambäck et al. 2014).

The entire experiment was replicated across two temporal blocks separated by 10 d. In each experimental block, we staked 60 patches of potted plants in a 3.5-m grid on a turf field in an agricultural landscape. Plants were grown in 10.2-cm diameter pots in a greenhouse at Cornell University and averaged 50 cm tall when moved to the field. One-half of the patches in each plant treatment were assigned to a predation risk treatment, in which each plant received two male–female pairs of stink bugs in two separate clip cages (2.5-cm outer diameter, 2.5-cm height). Plants in control patches received two empty clip cages. Past work indicates that the odor emitted by the stink bugs is enough alone to elicit nonconsumptive effects in larval CPB (Hermann and Thaler 2014).

After setting up each block of patches and adding predators to clip cages, we added one male and one female beetle to the top leaves of each plant in the experiment (384 beetles total in each block). Every 24 h for 4 d, we counted the number of beetles and egg clutches on each plant. Egg clutches were removed after counting so that oviposition sites were never limited. After the fourth census, we counted the number of leaves on each plant with or without beetle feeding damage. We watered plants by hand daily during the experiment.

We estimated the relationships between the response variables (oviposition rate, feeding damage, and beetle density) and the plant and predation risk treatments and their interaction using generalized linear mixed models. For oviposition rate, we summed the total number of clutches deposited on each plant across the four census days and used a negative binomial distribution to account for overdispersion (Ver Hoef and Boveng 2007). We used random intercepts for block and patch to represent the potential correlations in oviposition among patches within the same temporal block and among plants within a patch. For damage, we used a binomial distribution and included random intercepts for
temporal block, patch, and plant. For beetle density, we used a Poisson distribution and included random intercepts for temporal block, patch, plant, and sampling date. We fit negative binomial models using glmmADMB and the others using lme4 in R (Fournier et al. 2012, Bates et al. 2015, Skaug et al. 2016, R Core Team 2017). We followed hypothesis testing methods described in the previous section except we also included patch size in models as an independent variable to separate the effects of neighborhood diversity from the effects of plant density (Hambäck et al. 2014).

### Results

#### Growth and survival field experiment

**Larval census.**—In the larval census on day 8 of the experiment, survival in the absence of predation risk was similar between resistant monocultures (mean [95% CI]: 0.77 [0.67, 0.85]) and susceptible monocultures (0.78 [0.69, 0.85]; Fig. 1a). The mixture treatment, however, reduced larval survival by almost 20% relative to mean survival across the two monocultures (mixture, 0.60 [0.49, 0.71]; monocultures, 0.77 [0.71, 0.82]; \( \chi^2 = 7.0, \text{df} = 1, P = 0.008 \)). This result indicates there were nonadditive, interactive effects of genotypic mixing that pushed survival well below the expectation based solely on the monocultures. Within the mixture treatment, larvae were evenly distributed between susceptible and resistant plants, with 50.3% of larvae on the susceptible genotype on average, even though by day 8 larvae had had enough time and were mature enough to have moved across multiple plants. This result suggests either that survival did not differ between genotypes or that, if mortality differed between genotypes within the mixture, movement of larvae maintained even densities of larvae between genotypes.

The predation risk treatment revealed that beetle survival depended on an interaction between plant treatment and predation risk (Fig. 1a; \( \chi^2 = 14.8, \text{df} = 5, P = 0.01 \)). Predation risk reduced larval survival by about 25% in resistant monocultures (to 0.68 [0.56, 0.77]) and by only 11% in the susceptible monocultures (0.67 [0.55, 0.77]). In the genotypic mixture, however, predation risk had no effect on survival (0.68 [0.56, 0.77]), suggesting that larvae responded differently to predation risk in genotypic mixtures than in monocultures.

The average body length of beetles that survived to the larval census was 10.8% and 12.7% higher in the susceptible monocultures and susceptible-resistant mixture plots relative to the resistant monocultures (Fig. 1b; \( \chi^2 = 8.34, \text{df} = 2, P = 0.015 \)). Mean beetle length in the mixture treatment, however, did not differ significantly from the mean length across both susceptible and resistant monocultures (\( \chi^2 = 2.8, \text{df} = 1, P = 0.093 \)), indicating that genotypic mixing did not alter beetle size relative to the additive expectation based on the monocultures. Within the genotypic mixture plots, larvae that were found on susceptible plants during the larval census were 9.9% longer relative to larvae that were on resistant plants (\( \chi^2 = 13.06, \text{df} = 1, P < 0.001 \)), reinforcing the notion that beetle size on each plant genotype was not affected by the genotype of neighboring plants. Finally, larval length did not vary with predation risk either alone (\( \chi^2 = 0.52, \text{df} = 1, P = 0.47 \)) or in combination with the plant treatments (\( \chi^2 = 0.82, \text{df} = 1, P = 0.36 \)).

**Fall preoverwintering census.**—In the fall census, which occurred shortly after adults eclosed and emerged from the soil and before they returned to the soil for overwintering, differences among treatments in survival mirrored results for the larval census. In the absence of predation risk, survival to adulthood was similar between resistant (0.71 [0.61, 0.80]) and susceptible monoculture treatments (0.67 [0.56, 0.76]; Fig. 2a). In the genotypic mixture, survival was on average 11.9% lower relative to the mean of both monoculture treatments (mixture, 0.57 [0.45, 0.68]; monocultures, 0.69 [0.61, 0.75]). This effect, however, was smaller than the one in the larval census and was not significant (\( \chi^2 = 2.9, \text{df} = 1, P = 0.089 \)), suggesting that the negative effects of
the genotypic mixture on beetle survival decreased in importance through beetle ontogeny. The addition of predation risk again had effects that varied by plant treatment, although the magnitude of the interaction was smaller and only marginally significant \( \chi^2 = 10.9, \text{df} = 5, P = 0.054 \), suggesting that the negative effects of predation risk on beetle survival that occur early in life may carry through to adulthood.

The size of beetles that survived to adulthood (first principal component of mass and pronotum width, explaining 83.3% of variance) was 0.25 standard deviations greater in the susceptible monocultures \( 0.64 [0.44, 0.84] \) than in the resistant monocultures \( 0.33 [0.16, 0.73] \) \( \chi^2 = 6.2, \text{df} = 1, P = 0.013 \) (Fig. 2b). Beetle size in the mixture treatment \( 0.64 [0.44, 0.84] \) was <0.05 standard deviations from the mean size across both monoculture treatments, again indicating the mixture did not alter the size of surviving beetles relative to the additive expectation. Predation risk by the stink bug had no effect on adult size either alone \( \chi^2 < 0.001, \text{df} = 1, P = 0.99 \) or in combination with the plant treatments \( \chi^2 < 0.001, \text{df} = 1, P = 0.98 \).

**Spring emergence census**.—The effects of the predation risk and plant treatments on survival between fall and spring, overwintering survival, contrasted results earlier in ontogeny. Overwintering survival was surprisingly 9.1% (95% CI: 0.5, 18) higher in predation risk treatments relative to risk-free treatments \( \chi^2 = 4.2, \text{df} = 1, P = 0.041 \), independent of plant treatment, suggesting that predation risk had a persistent positive effect on adults that opposed the immediate negative effect on larval survival. The effect of the genotypic mixture on overwintering survival, unlike previously in ontogeny, was only additive, such that survival in the mixture was similar to the mean survival between the resistant and susceptible monocultures \( \chi^2 = 1.2, \text{df} = 1, P = 0.28 \). Survivial in the susceptible monocultures, however, was 13.5% (2.6, 24.2) higher than in the resistant monocultures, suggesting that plant identity had an important effect on overwintering survival.

Lifetime survival, from first instar to spring adult, mirrored results for the larval census (Fig. 3a), though less closely than did the fall census because of the contrasting effects of the predation risk and plant treatments on overwintering survival. In the absence of predation risk, survival was similar between resistant \( 0.42 [0.33, 0.51] \) and susceptible monoculture treatments \( 0.46 [0.38, 0.56] \). Survival in the mixture \( 0.40 [0.32, 0.50] \) was reduced by only 3.7% relative to the mean of both monoculture treatments \( 0.44 [0.38, 0.51] \), continuing the trend of a reduction in the effect of genotypic mixing on survival through ontogeny. Lifetime survival was not meaningfully influenced by the predation risk treatment \( \chi^2 = 0.002, \text{df} = 1, P = 0.97 \) or the interaction between predation risk and plant treatments \( \chi^2 = 8.39, \text{df} = 5, P = 0.14 \).

The size of beetles that survived overwintering (first principal component of mass and pronotum width, explaining 87.7% of variance) was again slightly greater (0.33 standard deviations) in the susceptible monocultures \( 0.80 [0.61, 0.98] \) relative to resistant monocultures \( 0.37 [0.16, 0.57] \) \( \chi^2 = 9.5, \text{df} = 1, P = 0.002 \) (Fig. 3b). Beetle size in the genotypic mixture treatment \( 0.67 [0.48, 0.86] \) was again <0.05 standard deviations from the mean size across both monoculture treatments \( \chi^2 = 0.31, \text{df} = 1, P = 0.58 \). Predation risk by the stink bug had no effect on the size of adults surviving to spring either alone \( \chi^2 = 0.24, \text{df} = 1, P = 0.62 \) or in combination with the plant treatments \( \chi^2 = 0.35, \text{df} = 1, P = 0.55 \).

**Effects of genotypic mixture on plant growth**.—Plants of the susceptible plant genotype were, on average, 5.9 cm (95% CI: 3.4, 8.5) taller than plants of the resistant genotype. The heights of each genotype, however, did not differ in monoculture plots vs genotypic mixtures \( \chi^2 = 0.08, \text{df} = 1, P = 0.78 \). This result indicates that the genotypic mixture did not influence the growth of each genotype, suggesting that any effects of the genotypic mixture on beetle performance and responses to predation risk were not indirectly mediated by changes in plant growth.

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**Fig. 2.** Beetle (a) survival and (b) size in the fall, preoverwintering adult census of the growth and survival field experiment. Cages had four resistant plants (R), four susceptible plants (S), or a mixture of two of each genotype (Mix). Large black points and lines show group means and 95% CIs. Each small point is the mean survival rate or adult size in one plot. Survival plots are split by predation risk treatment. Adult size is represented by the first axis of a principal component analysis of mass and pronotum width and explains 83.3% of the variance in those variables. Adult size is shown for females only. Points are jittered for visibility.
Effects of beetle density on beetle growth and survival.—Neither survival nor growth to larval, fall, or spring census varied with CPB density in the presence or absence of predation risk or on any of the plant treatments (Appendix S2), indicating that at the range of beetle densities we tested, beetle vital rates were density independent.

Adult behavior field experiment

Oviposition.—Within monoculture patches, we found oviposition depended on an interaction between plant genotype and number of plants per patch ($\chi^2 = 17.3$, df = 3, $P < 0.001$; Fig. 4a). Among resistant monocultures, increasing patch size from two plants to four plants reduced mean oviposition per plant by 41.2%, whereas the same increase in patch size among susceptible monocultures had a nearly opposite effect: a 31.3% increase in oviposition.

In the genotypic mixture patches, the null expectation was that oviposition per plant would be equal to the mean oviposition in the two-plant monocultures or in the four-plant monocultures, depending on how beetles responded to patch size. Instead, oviposition in the genotypic mixture treatment was lower than in any single monoculture treatment (Fig. 4a). Oviposition in the mixture patches was 39.7% lower than the mean across susceptible and resistant patches with two plants ($\chi^2 = 7.3$, df = 1, $P = 0.007$) and 34.3% lower than the mean in the four-plant monocultures ($\chi^2 = 6.0$, df = 1, $P = 0.015$). This result indicates that genotypic mixing had nonadditive, negative effects that suppressed CPB oviposition at the patch scale.

At the within-patch scale, we examined the associational effects of each genotype on the other by comparing oviposition on plants of one genotype in its two-plant monocultures to oviposition on the same genotype in the four-plant monocultures and in the genotypic mixtures. Oviposition on plants of the susceptible genotype was reduced by 22.1% if two resistant plants were added to the patch, whereas it
The presence of predation risk did not alter genotypic mixing.

**Fig. S2**), suggesting that adult beetle movement decisions plant after 24 h was 0.78 (95% CI

**t**reatment (**t** = 0.28, **df** = 1,  **P** = 0.28). Feeding damage in the mixture was 7.9% greater than the mean of damage across both two-plant monocultures (χ² = 4.6, **df** = 1,  **P** = 0.03), and not significantly different from the mean of damage across both four-plant monocultures (χ² = 1.9, **df** = 1,  **P** = 0.17). These results suggest that, in contrast to the results for oviposition, genotypic mixing had no effects, or modest positive effects, on feeding at the patch level across both genotypes in the mixture. Examining feeding on each genotype separately within the mixture indicated that feeding was independent of patch size and identify of neighboring plants for both the susceptible (χ² = 2.8, **df** = 2,  **P** = 0.25) and resistant genotypes (χ² = 2.9, **df** = 2,  **P** = 0.23), indicating that associational effects did not influence feeding damage.

**Beetle density.**—The mean number of beetles remaining per plant after 24 h was 0.78 (95% CI 0.66–0.92), indicating slightly more than half of the two beetles we placed per plant decided to emigrate. At the end of the 4-d experiment, a mean of 0.28 (95% CI 0.23–0.34) beetles remained per plant. Beetle emigration, however, did not differ by plant treatment (χ² = 4.66, **df** = 4,  **P** = 0.32) (Appendix S1: Fig. S2), suggesting that adult beetle movement decisions were not influenced by differences between genotypes or by genotypic mixing.

**Predation risk.**—The presence of predation risk did not alter beetle oviposition (χ² = 0.12, **df** = 1,  **P** = 0.73), damage (χ² = 0.0, **df** = 1,  **P** = 1.0), or density (χ² = 1.03, **df** = 1,  **P** = 0.31) independently or in an interaction with plant treatment for oviposition (χ² = 8.08, **df** = 5,  **P** = 0.15), damage (χ² = 4.3, **df** = 5,  **P** = 0.51), or density (χ² = 4.83, **df** = 5,  **P** = 0.44).

**Discussion**

Our results indicate that a genotypic mixture between susceptible and resistant potato varieties can reduce the survival of larval Colorado potato beetle and discourage oviposition by adults. Moreover, the genotypic mixture interacted with predation risk such that it altered the predator–prey interaction by changing the responses of herbivores to their predators. Importantly for the population-level consequences of genotypic mixtures and predation risk, these effects varied through ontogeny, having opposite effects at different life stages.

**Consequences of genotypic mixing**

We found that larval survival was similar between resistant and susceptible monocultures, but that surviving larvae in resistant monocultures were substantially smaller than those in susceptible monocultures. Based on these monoculture results, one would have predicted, as a null hypothesis, that in the genotypic mixture larval survival would be no different from survival in both monocultures, and that larval size would be equal to the mean of the sizes across both monocultures. This prediction came true for larval size but surprisingly not for larval survival, which was drastically lower in mixture than in either genotypic monoculture alone. Nonadditive effects of plant diversity are often explained by selection effects (Loreau 1998) or nonlinear averaging (Ruel and Ayres 1999, Wetzel et al. 2016), where one plant type in mixture has disproportionately large effects on herbivores, effectively pulling herbivore responses toward the mean response in a monoculture of that plant and leading that plant type to dominate the outcome of the plant–herbivore interaction. This process, however, is not sufficient to explain our results because CPB survival was even lower in the mixture than it was in either monoculture alone. Instead, our results indicate that growing these susceptible and resistant genotypes in mixture led to an interaction that reduced herbivore survival nonadditively.

The nonadditive reduction in larval survival in the genotypic mixture may have arisen owing to potential costs of diets with plant genotypic diversity. Evidence has accumulated that diverse diets, especially when chemically defended, tend to reduce consumer performance relative to performance on a single best diet item (Lefcheck et al. 2013, Wetzel and Thaler 2018), which is consistent with the results of this study. This could have occurred if the two potato genotypes had qualitatively different resistance traits, in which case mixing genotypes would have increased the number of mortality factors facing a larva that moved between plants or forced potentially costly reacclimation (Wetzel and Thaler 2016). Additional physiological work, however, is needed to uncover the mechanisms behind this result.

The result that the genotypic mixture influenced larval survival but not size or growth of surviving larvae could have arisen via at least two mechanisms. First, the mortality caused by the genotypic mixture may have occurred primarily early in larval life, potentially the first time larvae switched from one host genotype to the other, and then larvae that survived this period in the mixture were able to maintain normal growth. Second, beetles in treatments with lower survival may have grown normally at the expense of body condition (e.g., lipid reserves), which we did not measure. Regardless of the mechanism underlying this pattern, it suggests that, in genotypic mixtures, CPB individuals would be similar in size but have lower survival.
A major goal in the recent biodiversity literature has been to compare the magnitudes of the effects of plant intraspecific diversity and plant species diversity (Cook-Patton et al. 2011). A recent study that reared CPB on potato, tomato, or both species found that the species mixture alone had no effect on CPB performance (Wetzel and Thaler 2018), which contrasts with the strong effects of the genotypic mixture in the present study. In the same previous study, however, CPB performance was drastically reduced when they were forced to switch between host plant species, mimicking events like predation or competition that can make insects move between neighboring plants. These studies, taken together, suggest that for CPB species diversity appears to be influential only when larvae are forced to switch plants (e.g., because of predation risk, defoliation of current host plant, or environmental conditions), whereas genetic diversity affects larvae even when they are free to move among plants of their own volition. One explanation for this difference is that CPB might be better at exerting choice between potato and tomato, different plant species, and less capable of discerning between susceptible and resistance genotypes of the same species. In addition, the species mixture in the previous study primarily influenced larval growth and not survival, whereas the genotypic mixture in the present study primarily influenced larval survival and not growth. The key point here is that comparing these studies suggests that plant intraspecific diversity and plant species diversity seem to have qualitatively different ecological consequences.

**Interaction between genotypic mixture and predation risk**

A key, novel finding from our study was that the effects of plant genotypic mixing interacted subadditively with the effects of predation risk, such that the threat of predation reduced beetle survival in the monocultures but not in the mixture. Our factorial manipulation of plant genotypic diversity and predation risk allowed us to show, for the first time, that plant genotypic diversity directly alters the interaction between predator and prey by changing the responses of prey to predation risk. There were multiple behavioral and physiological reasons to expect intraspecific plant diversity to have important effects on herbivore responses to predators (Schultz 1983, Wetzel and Thaler 2016), but empirical data were lacking. This result indicates that intraspecific plant diversity can have pervasive effects on insect ecology, not just influencing the patch-level recruitment of different species but also directly altering species interactions within a patch.

We propose two potential, somewhat contrasting interpretations of this result. The first interpretation is that the effects of predation risk and plant mixtures are redundant such that either factor alone achieves the full potential reduction in herbivore survival. This could occur if CPB were affected by plant mixtures and predation risk in a similar way. For example, CPB may need to rest and reduce feeding upon encountering a new host genotype to allow physiological reacclimation; resting also happens to be a key CPB response to perceived predation risk (Bernays 1997, Hermann and Thaler 2014, Kaplan et al. 2014, Wetzel and Thaler 2016), providing a potential redundancy in responses to these factors. This pattern could also arise from stink bug behavioral responses to plants: stink bugs in mixtures could have avoided the resistant variety and foraged primarily on the susceptible variety (Thaler et al. 2015). This behavior would result in high and low predation risk on the susceptible and resistant varieties, respectively, in mixtures. Given that predation risk had very little effect on CPB survival in the susceptible monocultures, concentrating predation risk on the susceptible varieties within the mixture may have negated the effects of predation risk in the mixture.

The second interpretation of the lack of effect of predation risk on survival in genotypic mixtures is that it suggests that genotypic mixtures may inhibit the ability of CPB to mount antipredator defenses. Prey defenses often have large direct costs (Beckerman et al. 1997, Schmitz et al. 1997), and inhibiting them could have increased CPB survival (in our study with nonlethal, “sham” predators). In natural settings, however, an inhibition of defenses would likely lead to higher mortality due to predator consumption. Testing this hypothesis would require measuring prey vulnerability to lethal predators in monocultures and mixtures. If confirmed, it would indicate genotypic mixtures enhance lethal predation by suppressing herbivore defenses.

**Effects of genotypic mixture on adult behavior**

CPB oviposition in the adult behavior experiment was reduced in the genotypic mixture relative to the mean oviposition rate across both monocultures. This result reflected the reduced survival of larvae in the genotypic mixture in the growth and survival field experiment, suggesting that female oviposition behavior to some extent enhances larval survival. In contrast, adult movement was unaffected and feeding potentially slightly increased by the genotypic mixture, which suggests the opposite: females may not be acting entirely in the best interests of each individual offspring. Adults may have stayed in genotypic mixture patches to feed despite the negative effects of mixtures on oviposition and larval survival because moving among patches may be energetically costly or dangerous. Thus, lingering may have potentially increased adult lifetime fitness via increased egg production, even though eggs produced while feeding would be in patches with relatively low larval survival. Rather than leave mixture patches, females simply reduced their oviposition rates while in those patches. Moreover, mixture patches may have been adequate for adults because they may be physiologically less susceptible to the negative effects of genotypic mixing. A key consequence of the lack of adult movement away from genotypic mixtures and the adult use of both genotypes in mixture, is that in a mixed-genotype population, adults will use both plant types and larvae will have to cope with both plant types. This indicates it is meaningful to examine larval growth and survival in mixed-genotype populations. The differences we found across growth and survival, movement, oviposition, and feeding suggests that some of the debate about the consequences of plant diversity for insects (e.g., Crutsinger 2006, Utsumi et al. 2011, Castagneryrol et al. 2012, McArt and Thaler 2013, Abdala-Roberts et al. 2016, Bustos-Segura et al. 2017) may be due to differences in the relative importance of these processes in different systems and highlights the importance of a life cycle approach.
The reduced oviposition in genotypic mixtures occurred because the presence of the resistant genotype decreased oviposition on neighboring susceptible plants but the converse was not true: the susceptible genotype did not increase oviposition on neighboring resistant plants. Instead, oviposition on plants of the resistant genotype decreased with increasing patch size, regardless of the identity of neighboring plants. This asymmetry in how each genotype influenced oviposition on the other genotype led to the observed lower overall oviposition in the genotypic mixture. In the parlance of the associational effects literature, which examines how plant neighborhoods influence insect damage or density on focal plants, the susceptible genotype was protected from oviposition by associational resistance from the resistant genotype.

CPB surprisingly did not reduce oviposition in patches with predation risk in the adult behavior experiment even though other studies, which looked at responses of CPB to larger aggregations of stink bugs, have shown that adults are capable of responding to cues from stink bugs (Herman and Thaler 2018). One hypothesis for this result is that adults perceive leaving a patch as costly in terms of reduced feeding time, and they may value their own feeding over safety for their larvae. Females continuously produce eggs, so they may be able to increase lifetime fitness more by feeding as much as possible than by seeking oviposition sites with low predation risk. Moreover, CPB adults, unlike larvae, are not especially vulnerable to attack by stink bugs because they have hard exoskeletons, excrete chemical defenses, and are strong fliers (Hare 1990). Finally, adults may ignore predation risk because this stink bug is highly mobile such that current location may not be a reliable cue for future predation risk.

The ontogeny of genotypic mixtures and predation risk

A major result from our study is that the effects of plant genotypic diversity on herbivores and their interactions with predators vary considerably through ontogeny. Genotypic diversity had strong negative effects on larval survival, but this signal weakened through pupation and had disappeared by adult emergence postwinter. Likewise, predation risk reduced larval survival but, later in ontogeny, increased adult overwintering survival. Finally, adults responded behaviorally to genotypic mixing via oviposition but not feeding or movement, and ignored predation risk (at a density of two predators per plant). These results indicate genotypic diversity has real biological effects on CPB and their responses to predators but also that these effects occur primarily early in ontogeny and may decrease in importance through time. One explanation for this is that CPB susceptibility to predators and plant resistance traits decreases ontogenetically, as is the case for other insect herbivores. In addition, stochastic events later in ontogeny may obscure early effects. This does not mean, however, that long-term effects are erased; recent work indicates predation risk experienced by parents can influence offspring via maternal effects (Tigreros et al. 2017). Overall, our results indicate that intraspecific plant diversity may influence arthropod communities not just by changing resource quantity or quality, as past studies have suggested, but also by changing the interactions between insect herbivores and their predators in ways that vary with ontogeny.

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Literature Cited


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