

An experimental investigation of costs of tolerance against leaf and floral herbivory in the herbaceous weed horsenettle (*Solanum carolinense*, Solanaceae)

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Background and aims – A plant’s tolerance of herbivory depends on its ability to endure and compensate for damage so as to lessen the impact that herbivores have on the plant’s performance (e.g. its growth, reproduction, or fitness). While tolerance of herbivory is beneficial to plants, it is rarely complete, and individuals in plant populations tend to vary in their levels of tolerance. The goal of this study was to investigate potential costs associated with tolerance of leaf and floral herbivory in horsenettle (*Solanum carolinense*), a perennial herbaceous weed that is often subjected to high levels of damage from a diversity of herbivores.

Material and methods – We exposed 96 potted individuals across eight genets of horsenettle to factorial treatments of leaf herbivory by lace bugs and simulated floral herbivory by weevils. We quantified tolerance for each plant genet for both types of herbivory in terms of the impact of damage on the number of flowers opened, number of seeds produced, and root biomass (i.e. paternal, maternal, and vegetative tolerance, respectively).

Key results – Plant genets ranged widely in their ability to compensate for leaf and flower damage. While there was little evidence for tradeoffs in tolerance through the different routes, there was strong evidence of tradeoffs in genets’ abilities to tolerate herbivore damage to leaves and damage to flowers.

Conclusion – Tolerance is a useful defence strategy to cope with damage caused by herbivores, but its evolution may be constrained by concomitant costs and tradeoffs. The evolutionary role of the tradeoffs identified in this study are likely to be greater the more species of herbivores a plant hosts, and the more that herbivore levels vary both spatially and temporally.

Keywords – *Anthonomus nigrinus*; costs of tolerance; florivory; folivory; *Gargaphia solani*; horsenettle; *Solanum carolinense*; tolerance of herbivory; tradeoffs.

INTRODUCTION

In the face of a community of natural enemies that can collectively damage all parts of a plant, one of the most effective strategies for plants to cope with their enemies is simply to tolerate the attacks. In other words, instead of

minimizing the damage with resistance traits, plants may focus on traits that help them endure or compensate for the damage in a way that minimizes its impact on the plants’ fitness. This idea of plants tolerating herbivory has long been appreciated in the agricultural field (Painter 1958; Bardner & Fletcher 1974). However, among evolutionary

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ecologists, the widespread appreciation of tolerance as a defence strategy equal in importance to resistance did not occur until around the turn of the current century (Trumble et al. 1993; Rosenthal & Kotanen 1994; Fineblum & Rausher 1995; Karban & Baldwin 1997; Mauricio et al. 1997; Strauss & Agrawal 1999; Juenger & Lennartsson 2000; Stowe et al. 2000; Fornoni et al. 2003). While much about the ecology of herbivory tolerance has been learned in the past two decades, the reason that plant populations tend to maintain less-than-maximal levels of tolerance is still an open question (Stinchcombe & Rausher 2002; Leimu & Koricheva 2006; Dahlgren & Lehtilä 2015; Garrido et al. 2016; Carmago 2020; Núñez-Farfán & Valverde 2020). More precisely, it is not clear how often the evolution of tolerance is constrained by costs, and what sorts of costs are most important in natural plant populations (Agrawal 2011; Turley et al. 2013; König et al. 2014; Züst & Agrawal 2017; Garcia & Eubanks 2019; Avila-Sakar 2020).

The measurement of the costs of tolerance traits is rarely as simple as quantifying the metabolic investment into the production of a particular chemical. Instead, tolerance traits are more likely to involve opportunity costs that result from developmental, physiological, or growth constraints (Lehtilä 1999; Stowe et al. 2000; Tiffin 2000; Fornoni et al. 2003; Cipollini et al. 2014; Scholes et al. 2017; Avila-Sakar 2020). For instance, commitment to a structure or strategy to reserve resources for use in case of herbivore damage may keep a plant from reaching its reproductive potential if it is not damaged. Such opportunity costs may result in tradeoffs in abilities to compensate for damage through different routes (Strauss et al. 2003). For example, tolerance through the paternal route (e.g. pollen production) might come at a cost of less tolerance through the maternal route (e.g. seeds), and tolerance in terms of sexual reproduction might come at a cost of reduced tolerance through vegetative propagation. Furthermore, a plant's ability to tolerate one type of herbivore may come at a cost of reduced tolerance of a different type of herbivore (Tiffin 2000; Núñez-Farfán et al. 2007; Fornoni 2011). Because plant species are generally challenged by the attack of a community of multiple herbivores, such tradeoffs may be a powerful constraint on plants' ability to evolve increased tolerance. Even so, these types of tradeoffs have remained little investigated (Tiffin & Rausher 1999; Pilson 2000; Boalt & Lehtilä 2007; Manzaneda et al. 2010; Dahlgren & Lehtilä 2015; Pearse et al. 2017).

The goal of the current study was to investigate potential tradeoffs in a plant's tolerance of two different types of herbivory and through three reproductive routes. We performed a controlled experiment with potted individuals of the herbaceous weed *Solanum carolinense* (horsenettle). Specifically, 12 ramets of each of eight horsenettle genets were exposed to leaf herbivory and simulated flower-bud herbivory (i.e. folivory and florivory) in a factorial design. The folivory treatment involved leaf feeding by eggplant lace bugs (*Gargaphia solani*), while the florivory treatment involved manually imposed flower-bud clipping to simulate damage by potato bud weevils (*Anthonomus nigrinus*).

With the results of this experiment, we addressed four main questions: 1) How well does horsenettle tolerate damage to flower buds and to leaves (in terms of impact on

number of flowers opened, number of seeds produced, and root growth)? 2) How variable are the horsenettle genets in their tolerance of leaf and flower-bud damage through paternal, maternal, and vegetative routes? 3) Are there tradeoffs in horsenettle's ability to tolerate herbivory through paternal, maternal, and vegetative reproduction? and 4) Is horsenettle's ability to tolerate leaf herbivory negatively correlated with its ability to tolerate flower-bud damage?

MATERIAL AND METHODS

Natural history

Solanum carolinense L. (Solanaceae), or horsenettle, is a perennial herbaceous plant native to the southeastern United States (Bassett & Munro 1986). It has spread throughout most of North America, as well as parts of Europe and Asia (Imura 2003; NAPPO 2003), and it is considered a noxious weed of agricultural fields, pastures, and other disturbed habitats throughout much of its range (Gorrell et al. 1981; Frank 1990; NAPPO 2003). Its weedy nature is largely due to its ability to spread vegetatively through rapidly growing roots (Ilnicki et al. 1962; Nichols et al. 1991). Horsenettle reproduces sexually during a prolonged flowering period, from at least June through September in its native range (Wise & Cummins 2002). It has an andromonoecious breeding system, with a variable proportion of staminate (female-sterile) and perfect (hermaphroditic) flowers (Solomon 1985; Elle 1998; Steven et al. 1999; Wise & Hébert 2010). Racemes (inflorescences) are produced indeterminately, with large ramets producing more than 40 racemes (Michael J. Wise unpubl. res.). Individual racemes bear an average of 7–8 flowers (ranging from one to more than 20), and flowers mature acropetally (from the base to the tip of a raceme). Horsenettle is obligately outcrossing, relying on large-bodied bees to collect pollen to transfer to stigmas of perfect flowers on ramets of other horsenettle genets (genetic individuals) (Solomon 1986; Richman et al. 1995). Fertilized ovaries may mature into round, yellow-orange fruits (berries), usually ranging from 1 to 2 cm in diameter, though many fertilized ovaries abort before fruit expansion (Solomon 1985; Nichols et al. 1991; Wise & Sacchi 1996; Wise & Cummins 2006).

Horsenettle is subjected to a high level of damage by leaf-feeding herbivores (folivores) in its native range, with the eggplant lace bug (*Gargaphia solani* Heidemann, Tingidae) being among the most damaging species (Wise 2007b; Wise & Rausher 2013). Adult female lace bugs lay eggs in masses of an average of ~110 eggs (or many more in cases of communal egg-dumping) on the underside of leaves (Tallamy & Horton 1990; Loeb et al. 2000). Adults and nymphs feed by piercing horsenettle leaves and sucking out parenchyma tissue (Tallamy & Horton 1990; Loeb 2003). Their damage causes localized necrosis, leaf yellowing, and premature leaf abscission (Wise & Cummins 2006; Wise 2010). By far the majority of damage is caused by nymphs as they are led from leaf to leaf by their mother until they disperse from their natal plants soon after eclosion to adults (Wise 2018). These lace bugs have been reported to produce up to eight generations per year (Tallamy & Denno 1981, 1982).

Horsenettle is also subjected to high levels of damage by flower-feeding herbivores (florivores), with an average ramet losing roughly half of its flowers in a growing season (Wise 2007b; Wise & Rausher 2013). The most damaging florivore in the location of this study is the potato bud weevil (*Anthonomus nigrinus* Boheman, Curculionidae) (Wise 2007b). An adult female weevil lays a single egg (or rarely up to three eggs) in half-grown flower buds (Chittenden 1895; Tuttle 1956). She then fills the oviposition hole with a faecal plug and walks to the bud pedicel (Burke 1976). She chews through the pedicel until the bud drops, or at least until the damage causes the bud to senesce while still hanging on the raceme (Burke 1976). Weevil larvae feed inside the severed flower buds and emerge as adults within a few weeks (Chittenden 1895). Adult weevils are found throughout the entire flowering period of horsenettle (Wise 2007b).

Plant collection and propagation

Horsenettle roots from up to 30 horsenettle genets were collected in the spring of 1997 from a field population at Blandy Experimental Farm in Boyce, Virginia (39°03'43"N, 78°03'49"W). Details of the propagation procedures are available elsewhere (Wise 2007a, 2007b). Briefly, roots were placed in commercial growing medium (Wesco Growing Media III, Wetsel Seed Company, Harrisonburg, VA, USA) in 18.9 l (5 gal) plastic pots on wooden pallets in a semi-protected outdoor area in full sunlight each summer from 1997 to 2002. Roots were stored in refrigeration each winter, and new ramets were grown from cuttings of new root growth each spring. These procedures served to create genetically identical ramets from numerous horsenettle genets for use in experiments, as well as to purge the plants from potential carryover (non-genetic) effects that could be attributed to differences in microhabitat conditions within the plants' original source field.

In May of 2002, we removed horsenettle roots from refrigeration to propagate plants for experiments in the summer of 2002. For each genet, we cut roots into at least 30 equal-sized segments using water displacement in a 100 ml graduated cylinder. Specifically, a length of root was dipped into a graduated cylinder containing 98 ml of water, and the root was then cut at the point at which it displaced exactly 2 ml of water. The root segments were planted into 3.8 l (1 gal) round plastic pots in Wesco Growing Media III. The pots were kept in a greenhouse to monitor ramet emergence. If more than one ramet emerged in a pot, all but the first were clipped to the soil surface.

Experimental design

On 19 June 2002, we selected 12 ramets of each of eight genets for the treatments of the experiment described in this article. The chosen ramets were of intermediate size and were at least several days away from opening flowers. We randomized these ramets for three-way factorial experiment, with the three independent variables being genet, lace bug folivory, and simulated weevil florivory. The pots were then placed in randomized positions on greenhouse benches.

On 27 June, the plants were moved to randomized positions in rows on wooden pallets in a semi-protected

outdoor propagation area in full sunlight. Each pot was nested inside an empty 7.6 l (2 gal) pot to which a galvanized metal tomato cage had been attached. A fine mesh bag (custom-sewn from white "noseum" netting) was placed over each cage. Each bag was closed at the top with a plastic twist-tie and secured to the pot rim with string, which could be untied to allow experimenter access and then easily retied. The plants were watered as needed, which was daily for much of the duration of the experiment. The plants remained outdoors in their randomized positions through fruit ripening and plant senescence. On 11 November, the plants were moved back into the greenhouse for processing and final measurements.

Folivory (lace bug) treatment

Six of the 12 ramets of each genet were randomly assigned to host eggplant lace bugs. In late June, we collected broods of late-instar nymphs of eggplant lace bugs from local horsenettle populations by cutting off leaves on which groups of nymphs were feeding. The nymphs were transferred to leaves of potted horsenettle plants that were not part of the experiment, and the leaves with nymphs were covered with mesh plant sleeves. When the nymphs molted into adults, males and females were separated, and a single male-female pair was placed on the designated ramets in the experiment between 28 June and 2 July. Lace bugs were replaced as necessary, and all ramets had a single egg mass within about one week.

When the first brood of lace bugs began to eclose into adults, the mesh bags were left open at the top during the day to allow adults to disperse. Most of the lace bugs readily left the ramets through the top of the bag. To release stragglers from a ramet, the pot was moved away from the pallet, and the mesh bag was fully removed for a few hours. Except for the smallest ramets, generally one pair of lace bugs remained on the plants. We allowed a second brood of nymphs to develop on the ramets on which a female decided to oviposit. In late August, adults from the second generation began to emerge, and we allowed them to disperse as before. To make sure all plants were free of lace bugs, we removed all bags on 27 August and sprayed the plants with the insecticide carbaryl (1.5 tablespoons of concentrated SEVIN® per gallon of water; Bayer CropScience LP, Research Triangle Park, NC, USA). A continuous rain the next day rinsed away much of the insecticide residue. On 29 August, we reattached mesh bags to the pots, and the plants remained largely insect-free for the remainder of the experiment.

Florivory (simulated potato bud weevil) treatment

Four ramets of each genet were assigned to each of the following florivory levels: 0%, 33%, or 67% of buds cut. (Two of the ramets at each florivory level for each of the eight genets also experienced lace bug folivory, for a fully factorial design). Weevil florivory was simulated by cutting through the pedicel of a flower bud when the bud reached the size (about half-grown) at which weevils would be likely to oviposit in them. To achieve the targeted levels of florivory, each ramet was checked at least every three days. Racemes were labelled with dots of coloured paint at the base of the

peduncle so that we could keep track of the fate of each flower bud on each raceme. The buds mature from the base to the tip of a raceme over a period that can last several weeks, depending on the size of the racemes. On the ramets designated for 33% florivory, every third bud (starting with the second bud) was clipped with bonsai scissors. On the ramets designated for 67% florivory, every third bud was allowed to open (starting with the second). Thirteen of the 96 ramets produced fewer than six flowers. Because of the difficulty of matching target damage levels on ramets with so few flowers, these 13 ramets were omitted from the analyses. The actual proportions of buds cut on the remaining 83 ramets matched the targets very closely, with means and standard deviations of $32.9 \pm 2.5\%$ and $65.3 \pm 3.7\%$ (fig. 1). Bud clipping commenced on 20 June and finished on 30 July.

Pollination

Because horsenettle is obligately outcrossing and the mesh bags prevented access by pollinating insects, hand pollination of each flower was necessary to ensure fertilization. On the morning of each pollination day, we collected newly opened flowers from a mix of stock horsenettle plants from the greenhouse or from field populations to ensure that pollen from a variety of genotypes were included. Each flower was individually held upside down over a glass vial, and a

battery-powered “tomato” pollinator vibrated the anthers to release the pollen into the vial. We then applied pollen to receptive stigmas of open perfect flowers of the experimental plants using a camelhair brush. Flowers were pollinated on each ramet at least every three days from 23 June through 22 August, which was frequent enough to ensure that no open flowers went unpollinated.

Reproductive measurements

Fitness through the paternal route would ideally be estimated by the number of offspring sired by a plant, which would involve pollen production, successful transfer of pollen to receptive stigmas of plants of other genets, and a consideration of the quality of the pollen that was transferred (Elle & Meagher 2000). For simplicity, we assumed that a plant’s fitness through its paternal route is directly proportional to the number of flowers the plant opens. Previous studies on horsenettle have shown that the amount and quality of pollen, as well as the likelihood of pollinator visitation, does not differ between staminate and perfect flowers (Solomon 1985, 1986; Vallejo-Marin & Rausher 2007). Therefore, we simply used the sum of staminate and perfect flowers that successfully opened as the proxy for paternal fitness.

We used an estimate of the number of seeds produced as the proxy for fitness through the maternal route. Previous studies have shown that the diameter of a horsenettle fruit is a good predictor of the number of seeds it contains (Wise & Sacchi 1996; Wise & Cummins 2007). In mid-November, we removed each fruit, took at least three measurements of its diameter (d), and used the mode of the measurements to estimate the number of seeds each fruit contained, using a previously reported prediction equation:

$$\text{Seeds per fruit} = 70.1 - 23.0d + 2.18d^2 - 0.0415d^3$$

which has an r^2 of 0.90 (Wise & Cummins 2007). For increased precision, we dissected the smallest fruits (with a diameter of < 1 cm) to count the seeds, rather than using the prediction equation. The total number of seeds for all of the fruits a ramet produced was used as the estimate of that ramet’s maternal fitness.

For a perennial herbaceous weed such as horsenettle, vegetative reproduction can be an important component of an individual’s fitness. To quantify the potential for future vegetation reproduction, we measured root production for each ramet. In February, the roots from each pot were removed, thoroughly rinsed, and placed into paper bags for drying. The bags were placed into a drying oven set to 70°C , where they remained for one week, at which point the roots had attained a stable dry weight. The roots were then weighed to the nearest one-hundredth of a gram.

Tolerance estimates

Although conceptually each individual plant has a specific tolerance of any sort of stress (like herbivory), tolerance cannot, in practice, be measured on a single individual. Because individuals cannot be both exposed to and protected from the stress, tolerance is quantified by comparing performances of related individuals exposed to different

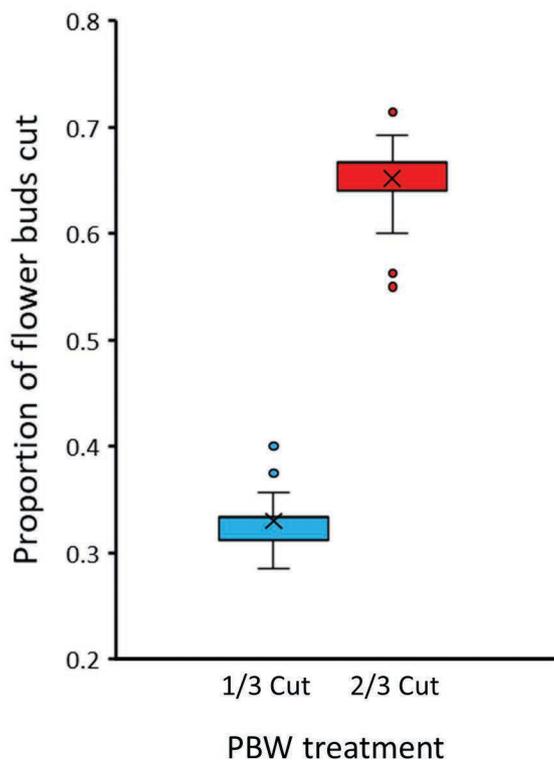


Figure 1 – Box and whisker plot of proportions of flower buds cut per ramet in the simulated potato bud weevil (PBW) florivory treatment. The boxes represent the interquartile range (IQR); the whiskers represent the range of data points that fell within $1.5 \times$ IQR above and below the upper and lower quartiles; the circles represent “outliers”; and the X’s represent the means.

Table 1 – Summary of results of the MANCOVA. The response variables comprised the number of flowers opened, the number of seeds produced, and the root biomass for each ramet. The simulated weevil-florivory and lace bug-folivory treatments were considered continuous covariates. $n = 83$ ramets across eight genets. Num. = numerator; Denom. = denominator.

Source of variation	Test	Value	<i>F</i> -ratio	Degrees of freedom		p value
				Num.	Denom.	
Genet	Wilks' lambda	0.1733	64.620	21	161.35	< 0.0001
Weevil florivory	<i>F</i> -test	12.597	235.137	3	56	< 0.0001
Lace bug folivory	<i>F</i> -test	0.5982	111.662	3	56	< 0.0001
Genet-x-weevils	Wilks' lambda	0.6753	11.259	21	161.35	0.33
Genet-x-lace bugs	Wilks' lambda	0.7345	0.8870	21	164.22	0.51
Weevils-x-lace bugs	<i>F</i> -test	0.0522	0.9744	3	56	0.41

levels of a stress. A plant's tolerance of herbivore damage is often quantified as the difference between mean performance (e.g. seed production) of damaged individuals and of undamaged individuals, or as a ratio of mean performance of damaged to undamaged individuals (Simms & Triplett 1994; Fineblum & Rausher 1995; Strauss & Agrawal 1999; Tiffin & Rausher 1999; Juenger & Bergelson 2000). A more flexible quantification involves a linear regression of plant performance on the amount of damage of a sample of related plants across a range of damage levels (Mauricio et al. 1997; Mauricio 2000; Simms 2000; Tiffin & Inouye 2000; Fornoni et al. 2003; Fornoni et al. 2004). The tolerance of a plant is then quantified as the slope of that linear regression, with a slope of zero indicating complete tolerance. The more negative the slope, the lower a plant's tolerance, while a positive slope indicates overcompensation for the stress.

For each of the eight horsenettle genets in our experiment, we calculated three estimates of tolerance of both lace bug damage and simulated weevil damage. The impact of damage on the number of flowers opened, the number of seeds produced, and the root biomass were used to quantify tolerance through paternal, maternal, and vegetative routes, respectively. For simulated weevil florivory, the predictor variable in the regressions of tolerance on damage was the actual proportion of ramets' buds that were clipped (rather than the target value for their treatment level). For lace bug folivory, the predictor variable was either 0 or 1, indicating the absence or presence of damage, respectively. The slopes for the lace bug regressions were thus equivalent to the difference in performance for plants in the two lace bug treatments for each genet (e.g. mean seeds produced by ramets with lace bugs minus mean seeds produced by ramets without lace bugs).

Statistical analyses

To assess the effect of leaf and flower-bud damage on reproductive parameters of horsenettle, we first performed a multivariate analysis of covariance (MANCOVA) for the three response variables (number of flowers opened, number of seeds produced, and root biomass) per ramet. The proportion of buds clipped and the lace bug treatment (0 or 1) were included as explanatory covariates. Plant genet was included as a predictor variable to account for genetic

variation in reproductive parameters unrelated to herbivore damage. Two-way interactions of genet-by-buds clipped and genet-by-lace bug treatment were also included to indicate whether the genets differed significantly in tolerance of herbivory. A two-way interaction term between buds clipped and the lace bug treatment was also included in the model. Following the MANCOVA, a univariate ANCOVA was performed for each of the three response variables individually. Because none of the two-way interaction terms were close to statistical significance in the MANCOVA, only the main factors were included in the univariate ANCOVAs. Plant genet was considered a random-effects factor for the ANCOVAs, and estimates were calculated using restricted maximum likelihood (REML). All statistical analyses were performed using JMP® v.4.0.4 (SAS Institute, Cary, NC, USA), which reports tests on random-effects factors using shrunken predictors, rather than traditional EMS estimates, in its REML procedure.

To investigate whether there were tradeoffs in different pathways of tolerance of a single type of herbivory, we calculated Pearson product-moment correlations among genet values of paternal, maternal, and vegetative tolerance for lace bug folivory and for simulated weevil florivory. To investigate the presence of tradeoffs between tolerances of different types of damage, we calculated Pearson product-moment correlations between the genets' tolerances of lace bug folivory and simulated weevil florivory for paternal, maternal, and vegetative tolerance.

The analyses in this study involved a total of 13 independent estimates of statistical significance, including one MANCOVA, three ANCOVAs, and three sets of three correlation coefficients. For simplicity, we consider the test-wise alpha rate of 0.05 as a cut-off for statistical significance in all tests, rather than making adjustments of alpha values for each set of tests, for the experiment as a whole, or across the entire research program of the experimenters. Instead, we caution that in a study with 13 independent estimates of statistical significance, the expectation is for 0.65 spuriously significant inferences due to random variation alone with a test-wise alpha of 0.05 (i.e. fewer than one type I error).

Table 2 – Summary of results of the ANCOVAs. The simulated weevil-florivory and lace bug-folivory treatments were considered continuous covariates, and plant genet was considered a random-effects factor. The analyses were run with JMP® v.4.0.4, in which “tests on random effects refer to shrunken predictors rather than traditional estimates” (SAS Institute, Cary, NC, USA). n = 83 ramets across eight genets for each response variable. The genet factor accounted for 37%, 42%, and 22% of the variation among ramets in flowers opened, seeds produced, and root biomass, respectively. d.f. = degrees of freedom; MS = mean square.

Source of variation	d.f.	MS	F-ratio	p value
A. Flowers opened				
Genet	7	69.319	58.005	< 0.0001
Weevil florivory	1	984.050	823.441	< 0.0001
Lace bug folivory	1	5.689	0.4760	0.49
Error	73	11.950		
B. Seeds produced				
Genet	7	236459	70.518	< 0.0001
Weevil florivory	1	1035251	308.737	< 0.0001
Lace bug folivory	1	238511	71.130	0.0094
Error	73	33532		
C. Root biomass				
Genet	7	64.153	30.211	0.0076
Weevil florivory	1	125.593	59.144	0.018
Lace bug folivory	1	828.983	390.380	< 0.0001
Error	73	21.235		

RESULTS

Both folivory by lace bugs and simulated weevil florivory had highly significant impacts on the reproductive performance of horsetettle (MANCOVA results, table 1). The plant genets were also highly divergent in their reproductive performance, but the interaction terms between genet and the herbivory treatments were not statistically significant (table 1).

The ANCOVAs provided a more specific picture of which reproductive parameters were affected by each type of damage (table 2). Specifically, averaged across simulated weevil-florivory treatments, lace bug folivory reduced mean seed production of ramets by 19% and root biomass by 36%, but only reduced the number of flowers opened by 5% (fig. 2). Simulated weevil florivory significantly reduced flower and seed production but increased root growth (fig. 2). Specifically, averaged across lace bug treatments, clipping one-third of the flower buds led to 30% and 8% decreases in the mean number of flowers opened and seeds matured, respectively. Clipping two-thirds (actually, 65%) of the flower buds led to respective decreases of 56% and 36% in flowers opened and seeds matured. Finally, ramets with one-third and two-thirds of their flower buds clipped increased their root biomass by a mean of 19% and 24%, respectively (fig. 2C).

Despite the non-significant interactions between plant genet and herbivory treatments in the MANCOVA (table 1), the genets did differ widely in their absolute responses to damage, and thus their ability to compensate for the damage. For instance, the loss of 2/3 of a ramet’s flower buds in the least-tolerant genets led to means of 71% reduction in flowers opened, 67% reduction in seeds, and 3% reduction in root biomass. In contrast, the loss of 2/3 of the flower buds in the most-tolerant genets led to a 24% reduction in flowers opened, 0% reduction in seeds, and a 64% increase in root biomass, on average. Similarly the impact of lace bug damage to the least-tolerant genets led to 21%, 29%, and 51% decreases in flowers opened, seeds produced, and root biomass, respectively. In contrast, lace bug damage to the most-tolerant genets led to a 75% increase in flowers opened, a 1% reduction in seeds, and a 5% reduction in root biomass.

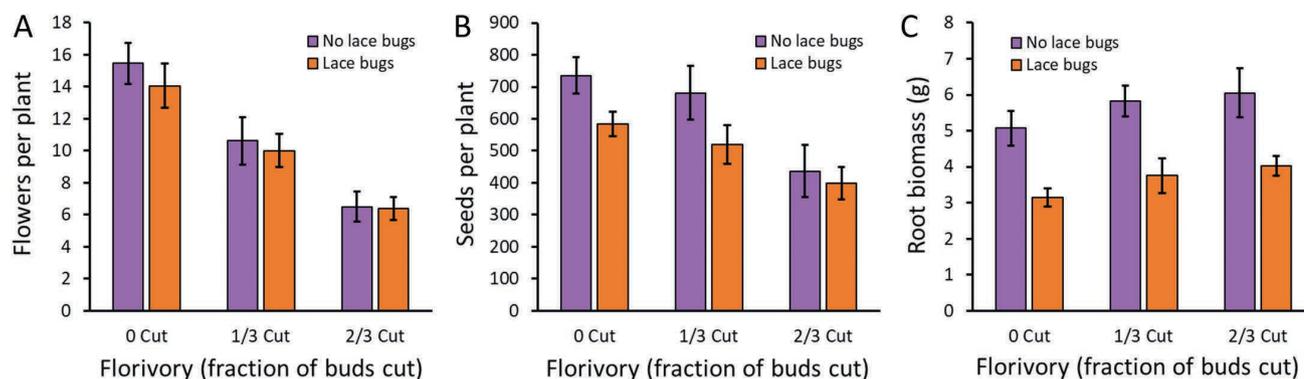


Figure 2 – Impact of simulated potato bud weevil florivory and lace bug folivory on paternal (A), maternal (B), and vegetative-reproductive (C) parameters of horsetettle. Columns and bars represent means ± one SEM. Orange = lace bugs absent; purple = lace bugs present.

The results of this experiment provided little evidence of tolerance costs in terms of tradeoffs in tolerance through different routes (fig. 3). On one hand, the genet correlations in tolerance through the paternal and maternal routes were highly positive for both types of herbivory (fig. 3A & D).

On the other hand, the genet correlations in tolerance through the sexual and vegetative-reproduction routes tended to be negatively correlated, particularly for tolerance of simulated weevil florivory in terms impacts on the number of flowers opened and on root biomass (fig. 3E). However,

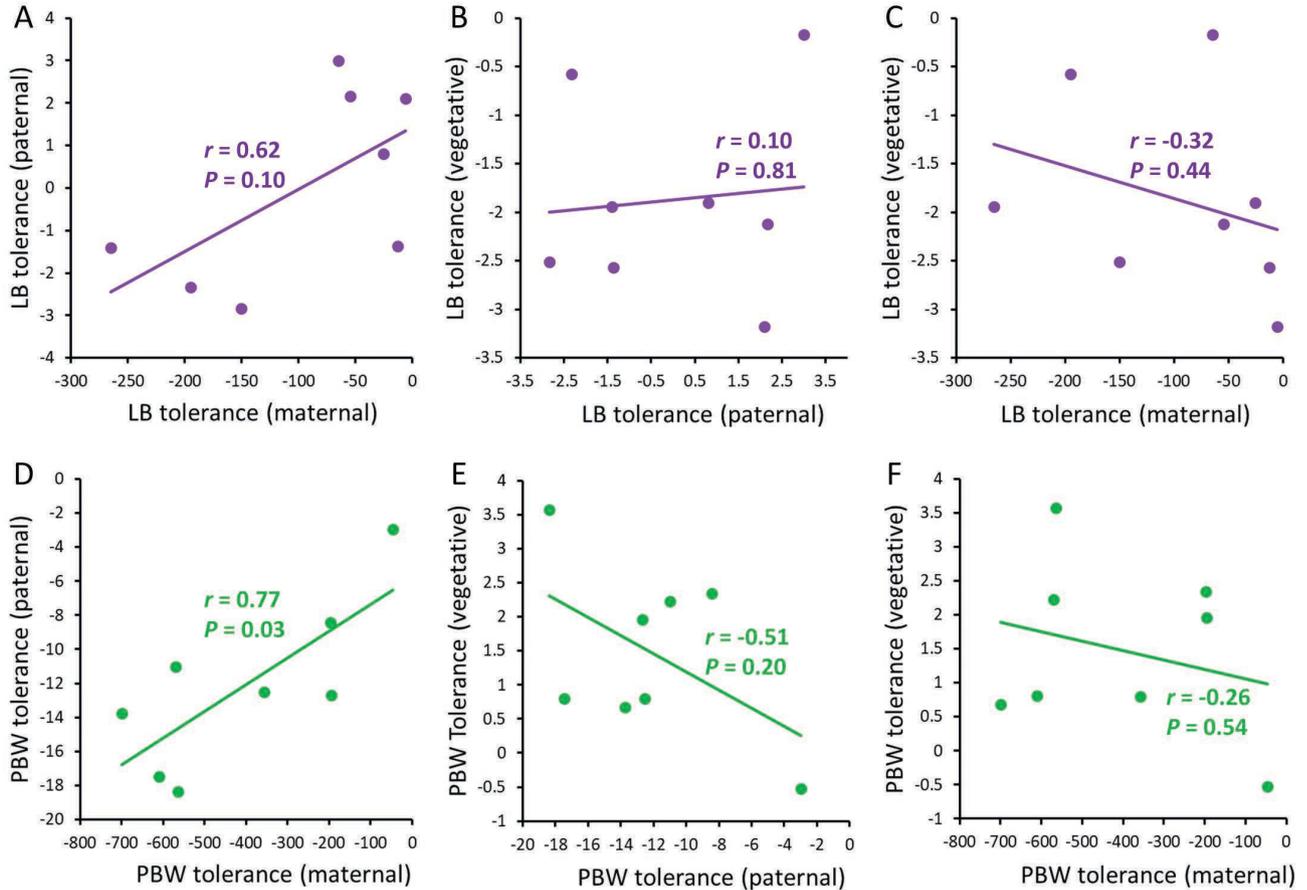


Figure 3 – Genet correlations in horsenettle’s tolerances of lace bug (LB) folivory (A–C) and simulated potato bud weevil (PBW) florivory (D–F). **A, D.** Paternal vs maternal tolerances. **B, E.** Paternal vs vegetative tolerances. **C, F.** Maternal vs vegetative tolerances.

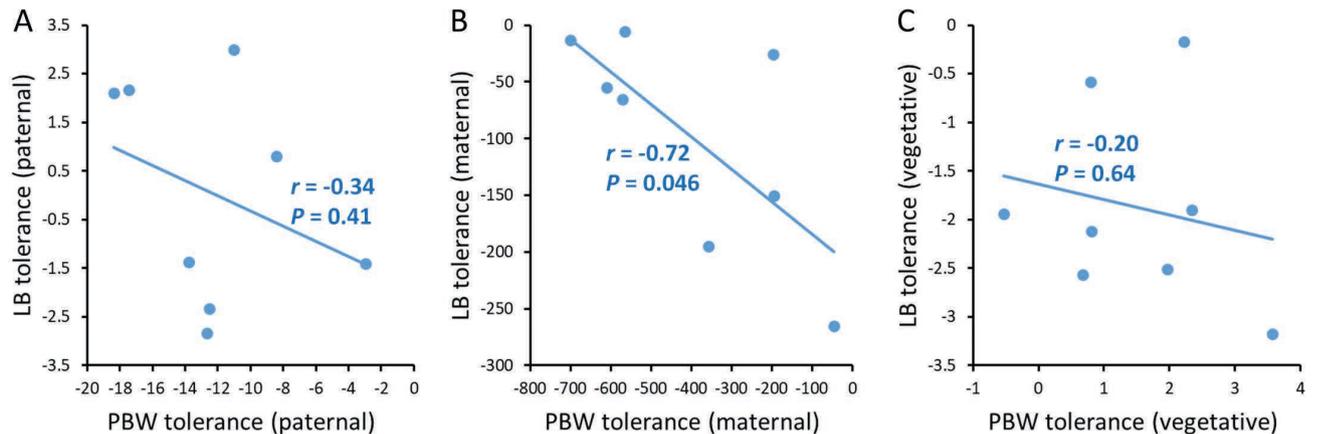


Figure 4 – Genet correlations between horsenettle’s tolerances of lace bug (LB) folivory and simulated potato bud weevil (PBW) florivory. **A.** Paternal tolerance (flowers opened). **B.** Maternal tolerance (seeds produced). **C.** Root biomass.

with only eight genets, none of these negative correlations were statistically significant (i.e. $p > 0.05$ for all correlation coefficients).

Finally, there was relatively strong evidence of costs in terms of tradeoffs between tolerances of the two types of damage (fig. 4). The genet correlations in tolerance of leaf and flower-bud damage for all three routes were negative, but the tradeoff was especially strong for tolerance in terms of seed production (fig. 4B).

DISCUSSION

Question 1: how well does horsenettle tolerate florivory and folivory?

Horsenettle in this experiment was fairly good at tolerating simulated weevil florivory. For instance, clipping of 65% of the ramets' flower buds led to a reduction of 56% in the mean number of flowers opened and to only a 36% reduction in seed production. The greater maternal than paternal tolerance can be attributed to the fact that there are more ways to compensate for bud loss in the currency of seeds than in the currency of opened flowers (Lloyd 1980; Stephenson 1992; Gómez & Fuentes 2001). For instance, previous research has shown that plasticity in the proportion of bud primordia that are matured can help horsenettle compensate in terms of both flowers and seeds (Wise et al. 2008). However, horsenettle can also produce more seeds by increasing the ratio of perfect-to-staminate flowers, reducing the rate of fruit abortion, and making bigger fruits containing more seeds (Wise & Cummins 2006; Wise et al. 2008).

Horsenettle ramets also responded to simulated weevil florivory by allocating more biomass to the growth of perennial roots. This sort of overcompensation is not surprising because, with fewer flowers on a ramet, roots would not have to compete with as many fruits for a finite supply of photosynthates at the end of the growing season. Reallocation of resources to perennial tissues is potentially a mechanism for plants to compensate for the loss of sexual reproduction in one year by enabling more growth – and likely more reproduction – in the following year (Wise et al. 2006; Wise et al. 2008).

Folivory by eggplant lace bugs significantly reduced sexual reproduction in horsenettle, with ramets exposed to lace bugs producing 5% fewer flowers and 19% fewer seeds than ramets protected from lace bugs. The lower paternal than maternal tolerance can be explained by the timing at which the effects of the two kinds of damage are felt by the plants. Specifically, the rate at which leaves were damaged by lace bugs accelerated throughout the summer. Thus, most of the stress caused by leaf herbivory occurred after the plants had finished flowering. Plants instead experienced the stress of the loss of leaves when they were filling their fruits. This result is consistent with a previous study that showed lace bug damage led to greater rates of fruit abortion in horsenettle (Wise & Cummins 2006).

Lace bug folivory had a greater negative impact on perennial root growth than on sexual reproduction, with ramets exposed to lace bugs having 36% less root biomass

than ramets protected from lace bugs. This result is similar to results of previous studies that have found decreased root growth in horsenettle ramets exposed to lace bugs (Wise & Cummins 2006; Wise 2018). The abscission of leaves fed upon by lace bugs would mean that there would be less photosynthate to allocate to root expansion late in the growing season. Thus, unlike the case for flower loss, the negative impacts of leaf loss would be likely to extend to succeeding years, as plant genets that avoided lace bugs would have a greater resource supply to tap into as they start their spring growth (Wise et al. 2008). However, resource supply is not the only factor that determines the trans-generational effects of herbivory. For instance, recent research has shown that sexually produced offspring of horsenettle plants that were damaged by the tobacco hornworm can outperform offspring of undamaged plants in some parameters (e.g. seed germination and seed production), at least under greenhouse conditions (Nihranz et al. 2020). Thus, other tolerance mechanisms might mitigate the negative impact of lace bugs on root growth.

Question 2: how variable is tolerance of florivory and folivory among horsenettle genets?

The non-significant statistical interactions between plant genet and the herbivory treatments in the MANCOVA (table 1) would suggest that the evolution of greater tolerance in this horsenettle population might be constrained due to a lack of genetic variation for traits that confer tolerance. However, with only eight plant genets, there was little statistical power to detect genetic variation. Therefore, any conclusion about a lack of genetic variation for tolerance in horsenettle populations based on this study should be considered tentative. Indeed, most studies of natural populations of other species have shown evidence for genetic variation in tolerance (Mauricio et al. 1997; Tiffin & Rausher 1999; Fornoni & Núñez-Farfán 2000; Hochwender et al. 2000; Pilon 2000; Stinchcombe & Rausher 2002; Fornoni et al. 2003), though there are exceptions (Juenger & Bergelson 2000; Strauss et al. 2003; Hakes & Cronin 2011).

Given the large absolute range in tolerance among genets (e.g. figs 3–4), it seems likely that a larger sample of genets would find the variation for tolerance in this horsenettle population to be statistically significant. Moreover, the range of tolerances among genets for both florivory and folivory was sufficient for a robust detection of tradeoffs, regardless of the non-significant interaction terms in the MANCOVA.

Question 3: are there tradeoffs in horsenettle's ability to tolerate herbivory through paternal, maternal, and vegetative routes?

Within any natural plant population, there is likely to be a range of growth- and reproductive-allocation strategies. For instance, some individuals may devote more energy to sexual reproduction, while others may invest more heavily in vegetative growth (Wise & Cummins 2006). This variation in strategies may lead to tradeoffs in the plants' abilities to tolerate herbivory through different pathways. For example, in a study of wild radish (*Raphanus raphanistrum* L.), Strauss et al. (2003) found a non-significant positive correlation

($r = 0.32$, $p = 0.36$) between male and female tolerance of caterpillar folivory, as measured by the numbers of flowers and seeds produced, respectively. (We are aware of no other empirical study that has reported correlations in tolerance through different reproductive routes.)

The evidence for tradeoffs in tolerance through different routes in this study was mixed. On the one hand, horsenettle's tolerances through the paternal and maternal routes were highly positively correlated for both simulated weevil florivory ($r = 0.77$) and lace bug folivory ($r = 0.62$). Such a high correlation is not surprising because traits that allow compensation by increasing the number of flowers that open would also provide plants with more ovaries in which to produce more seeds. In an andromonoecious plant such as horsenettle, this relationship between paternal and maternal tolerance would be uncoupled to the extent that the compensatory flowers were staminate. However, previous studies have shown that horsenettle generally responds to flower-bud loss by increasing the proportion of flowers that are perfect, rather than staminate (Solomon 1988; Wise et al. 2008) – a pattern that has also been observed in other systems (Hendrix & Trapp 1981; Hendrix 1984; May & Spears 1988; Krupnick & Weis 1998), and that would tend to increase the correlation between paternal and maternal tolerance in andromonoecious plants.

On the other hand, this study provided some indication of a tradeoff between horsenettle's ability to tolerate herbivory through compensatory sexual reproduction and through allocation to future vegetative reproduction. For instance, the genets' tolerances in terms of seed production were moderately negatively correlated with their tolerances in terms of root biomass for both simulated weevil florivory ($r = -0.26$) and for lace bug folivory ($r = -0.32$). In addition, tolerance in terms of impact on flower production was negatively correlated with root biomass for simulated weevil florivory ($r = -0.51$), but not for lace bug folivory ($r = 0.10$). With only eight genets in this data set, none of these negative correlations was found to be statistically significant; nevertheless, the results are at least suggestive of the potential for tradeoffs in different pathways of tolerance.

Question 4: is horsenettle's ability to tolerate leaf herbivory negatively correlated with its ability to tolerate flower herbivory?

While this study provided limited evidence of tradeoffs in tolerance of a single type of herbivory through different reproductive pathways, the evidence for a tradeoff in tolerance of different types of herbivore damage was relatively strong. This tradeoff was most clear in terms of maternal tolerance (through seed production), where the correlation (r) between tolerance of simulated weevil florivory and lace bug folivory was -0.72 . In particular, the genet with the highest maternal tolerance of flower-bud damage was the least tolerant of leaf damage, and the genet that was most tolerant of leaf damage was the second-least tolerant of flower-bud damage. The genet correlations between simulated weevil florivory and lace bug folivory in terms of paternal ($r = -0.21$) and vegetative tolerance ($r = -0.20$) were not statistically significant, but their negative

direction adds to the evidence of tradeoffs in tolerance of different types of herbivore damage.

All previous studies that have measured tolerance of two different types of damage run counter to the results of the current study. Specifically, Tiffin & Rausher (1999) found a significant positive correlation ($r = 0.48$, $p < 0.02$) for tolerance of folivory and tolerance of apical-meristem damage in the common morning glory, *Ipomoea purpurea* (L.) Roth. In contrast, two studies on wild radish revealed an absence of significant correlations between tolerances of foliar and apical-meristem damage (Boalt & Lehtilä 2007; Dahlgren & Lehtilä 2015). Similarly, Pilson (2000) found that tolerance of leaf herbivory by flea beetles and tolerance of seedpod damage by weevils were not significantly correlated in *Brassica rapa* L. More recently, Manzaneda et al. (2010) found no evidence of tradeoffs in tolerances of leaf damage caused by a specialist lepidopteran caterpillar, a generalist lepidopteran caterpillar, and manual clipping in the brassicaceous plant *Boechera stricta* (Graham) Al-Shehbaz.

It is notable that most of the previous studies have focused on tolerance of damage to source organs (e.g. leaves), while this study on horsenettle investigated damage to both source and sink organs. It is possible that the more functionally different the type of tissue damaged, the less overlap there will be in compensation mechanisms, and thus the more likely it is that there will be a tradeoff in tolerances. Because most species of plants are indeed attacked by a diversity of herbivores that collectively feed on all types of plant tissues, it seems likely that the data currently available underestimate the role that tradeoffs in tolerance of different herbivores may play in constraining the evolution of tolerance in natural plant populations.

CONCLUSION

Tolerance can be a highly effective way for plants to cope with damage caused by natural enemies, whether in place of or in conjunction with resistance (Hakes & Cronin 2011; Carmona & Fornoni 2013; Turley et al. 2013; Wise & Abrahamson 2017). However, a variety of ecological and genetic factors may lead plant populations to be less than maximally tolerant of herbivory. Most basically, a lack of genetic variation for traits that confer tolerance can prevent an evolutionary response in the face of natural selection for increased tolerance. Moreover, costs that counteract the benefits of tolerance can constrain the evolution of tolerance even in the midst of genetic variation that could enable the evolution of greater tolerance. In this study, horsenettle may experience costs in terms of a tradeoff in tolerance through sexual and vegetative routes, which could act to maintain tolerance through different routes at intermediate levels within plant populations. Horsenettle exhibited a stronger tradeoff in tolerances of damage to leaves and damage to flower buds. Because horsenettle populations tend to be attacked by a diversity of herbivores, tradeoffs between tolerances to different types of damage may be a strong constraint on the overall evolution of horsenettle's defence against its herbivore community.

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