

Why fitness impacts of different herbivores may combine nonadditively, and why it matters to the ecology and evolution of plant-herbivore communities

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Abstract

Background and aims – The manner by which the effects of multiple antagonists combine is a fundamental issue in ecology. This issue has been especially important in plant-herbivore evolutionary ecology—particularly predicting whether the combined fitness impacts of multiple herbivores on a shared host plant can be inferred by simply adding the individual impacts that each herbivore has when feeding alone. Despite accumulating empirical data, relatively little theoretical progress has been made in explaining why impacts of herbivore damage often combine nonadditively, as well as predicting the conditions that lead to a greater-than-additive (synergistic) or to a less-than-additive (subadditive) pattern.

Material and methods – Based on considerations of limiting resources and source-sink relationships, I proposed and tested two hypotheses: 1) The fitness impacts of two species of herbivores that affect the same resource (i.e. feed on the same tissue in a similar fashion) will combine in a synergistic pattern (if that resource is not limiting reproduction when plants do not experience herbivory), and 2) The fitness impacts of two herbivores that affect different resources (i.e. feed on different tissues) will combine in a subadditive pattern. I performed a field experiment in which horsenettle (*Solanum carolinense*) was exposed to a factorial combination of four levels of leaf herbivory and five levels of simulated floral herbivory.

Key results – The results were consistent with both hypotheses: 1) The combined fitness impact of flower damage that was simulated as being caused by two florivorous species feeding on the same plants was greater than the sum of the same total amount of damage when the two species were simulated as feeding individually; and 2) The combined fitness impact of the leaf and floral damage was less than the sum of the same total amount of damage when the two species fed individually.

Conclusions – The main ecoevolutionary implication of these results is that subadditive impacts of leaf- and flower-feeding herbivores could weaken selection for resistance in horsenettle (or any plant species that hosts multiple herbivores), and thus subadditive impacts may contribute to the maintenance of diverse herbivore communities sharing a species of host plant.

Keywords

coevolution, ecoevolutionary dynamics, *Epitrix*, florivory, herbivore impact, Limiting Resource Model (LRM), multiple herbivores, nonadditivity, *Solanum carolinense*, tolerance of herbivory

INTRODUCTION

Living organisms regularly interact—either directly or indirectly, mutualistically or antagonistically—with

individuals of many other species that share their environment. While the potential intricacy of interactions between species makes community ecology a fascinating discipline, the sheer number of potential interactions

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also makes predicting the ecological and evolutionary dynamics of communities particularly challenging. Studying the interactions of two species at a time is a good start, but the inferences might not hold up when the influence of another species in the community is also considered. In general, it is helpful to know when individual effects of pairwise interactions can simply be added together to understand the whole community, and when individual effects are likely to combine in a nonadditive fashion.

The issue of the whole picture not equalling the sum of the parts has been particularly prominent in the study of the ecological and evolutionary effects of herbivores on their host plants (Strauss 1991; Morris et al. 2007; Irwin and Brody 2011; Stephens et al. 2013; terHorst et al. 2018; Mesa et al. 2019). While much insight has been gained through a focus on pairwise interactions between a species of herbivore and its host plant, most plants are simultaneously (or sequentially) attacked by diverse communities of multiple species of herbivores that collectively may feed on all parts of a plant (e.g. Root and Cappuccino 1992; Lawton et al. 1993; Agrawal 2005; Hochwender et al. 2005; Wise 2007b). It took two decades after the publication of Ehrlich and Raven's (1964) seminal paper on plant-herbivore coevolution before theoreticians started to address seriously the possibility that novel outcomes might emerge from the consideration of more than one species of herbivore (Levin 1983; Fox 1988; Gould 1988; Levin et al. 1990). Recently, interest in the interactions involved in multiple-herbivore communities has peaked again within the burgeoning discipline of ecoevolutionary dynamics, with its focus on indirect ecological interactions and rapid evolutionary responses (Johnson and Stinchcombe 2007; Hendry 2017; terHorst et al. 2018).

The effects of the interactions between a plant and an herbivore can be altered by a second species of herbivore in two main ways. First, the presence of one species of herbivore (or its damage) may alter the amount of damage inflicted by a second species, either through direct or indirect competitive or facilitative interactions (Denno et al. 1995; Kaplan and Denno 2007; Wang et al. 2014; Callejas-Chavero et al. 2020). Second—even if the herbivores do not affect the amount of damage caused by each other—the impact that a certain amount of herbivore damage has on a host plant's fitness may depend on the amount of damage inflicted by a second species. In other words, the fitness impacts of given amounts of damage by different herbivores may not combine predictably as the sum of the individual impacts that would be suffered due to the same amounts of damage when each herbivore is feeding alone. The current study focuses on this second scenario, which I will refer to simply as nonadditive impact.

Multiple studies have compared separate and combined fitness impacts of two species of herbivores sharing a host plant (Hufbauer and Root 2002; Strauss and Irwin 2004; Morris et al. 2007; Kafle et al. 2017). Such studies

usually employ a factorial design in which one or more levels of damage by one species is crossed with one or more levels of damage by a second species. Whether the damage is real or simulated, the experimenter controls the amount of damage so that potential competitive or facilitative interactions between species are eliminated, and any nonadditivity of combined impact will thus not be obscured by ecological interactions that could affect amounts of damage. These studies show a range of results in a variety of systems, proving at least that nonadditivity of impact is likely a common phenomenon in plant-herbivore communities.

What has been largely lacking is a theoretical context to explain why herbivores' impacts might not combine independently. In particular, what conditions cause herbivores' impacts to combine in a greater-than-additive (i.e. synergistic) or less-than-additive (i.e. antagonistic, or hereafter, subadditive) fashion remains an unresolved question (Morris et al. 2007; Stephens et al. 2013; Mesa et al. 2019). I suggest that insight into the causes of nonadditive impact can be gained from casting the issue in terms of tolerance of herbivory, and in particular how resource limitations and other stressors can affect a plant's tolerance of different types of herbivory. Specifically, the relationship between the amount of herbivore damage and the fitness impact of that damage defines a plant's tolerance of that damage. As such, nonadditivity of impact occurs when damage by one herbivore alters the host plant's tolerance of the damage that is inflicted by another herbivore.

In that vein, the Limiting Resource Model (LRM) of plant tolerance—which was formulated to explain the varied effects that environmental stressors can have on plant tolerance of herbivory—can be applied to predict patterns of nonadditivity of impact by substituting the resource stress envisaged by the LRM with stress caused by damage of a second herbivore (Wise and Abrahamson 2005, 2007). Specifically, if the two herbivores primarily affect the same resource, and if that resource is the main limiting factor on plant fitness, then the impacts are likely to combine additively. If that resource is not limiting in the absence of herbivory, then the combined feeding is more likely to cause that resource to become limiting, and thus the impacts are likely to combine in a synergistic fashion. Finally, if the two herbivores mainly affect different resources, then the plant's tolerance of damage by either herbivore is expected to be greater (i.e. have a less-negative fitness-damage slope) when the other is also feeding. In other words, the impacts are likely to combine subadditively if the two herbivores mainly affect different resources.

For the sake of applying the LRM rationale, plant tissues and organs can be thought of in terms of the main resources that they acquire or the main functions that they perform (Wise and Abrahamson 2008). Most simply, these functions can be categorized as sources of carbon (e.g. leaves) or as sinks of carbon (e.g. flowers and fruits). While roots are sinks of carbon, they are sources for water

and nutrients. Stems are harder to classify, as they serve as a means of transport of multiple resources, in addition to structural roles. Nevertheless, consideration of whether different herbivores mainly affect sources or sinks may provide insight into whether the fitness impacts of the herbivores will combine in an additive, synergistic, or subadditive fashion on a shared host plant (Puentes and Ågren 2012; Wise and Rausher 2016).

For instance, consider a plant whose reproduction is limited by its ability to assimilate carbon. Its reproduction would thus be considered source limited, and, as a result, it may produce an excess of flowers (sinks) relative to the number of fruits it can fill. In this scenario, an herbivore species that feeds on the plant's flowers (i.e. a florivore) may have no impact on the plant's reproduction because it affects sinks, which are not a limiting "resource". If a second florivore also feeds on the plant, flowers are then more likely to become limiting. Therefore, the combined impact by the feeding of two florivores is likely to be greater than the sum of the impacts of each of the two florivores if they had been feeding in isolation. In other words, feeding by one species of herbivore causes the plant to be relatively less tolerant of feeding by another herbivore that feeds on the same plant organs.

Now consider a plant whose reproduction is essentially equally limited by sources and sinks of carbon. Any feeding on leaves (folivory) or on flowers will cause the plant to be respectively source or sink limited; thus either type of herbivory alone would be expected to have a negative impact on the plant's reproduction. However, if both herbivores feed on the same plant, their combined impact is likely to be less than the sum of their individual impacts when they fed alone. Consider that the leaf herbivory will cause the plant's reproduction to be source limited. In the presence of substantial leaf damage, floral herbivory will have minimal impact on reproduction until the flower damage becomes severe enough to cause the plants to become sink limited. Likewise, in the presence of substantial floral damage, the plant's reproduction will be sink limited, and leaf herbivory will have minimal impact until the loss of leaves becomes severe enough to cause the plant to become source limited. In essence, the presence of one type of herbivory causes the plant to become relatively more tolerant of feeding by a different type of herbivore.

The ecological relevance of how herbivore impacts combine is obvious, both from a basic and an applied perspective. For instance, information on nonadditivity could help inform policies on pest suppression in agricultural crops (Barber et al. 2012; Gagic et al. 2016) and strategies for biological control of weeds by herbivorous insects (Wooley et al. 2011; Musedeli et al. 2019). More contentious, however, is the issue of whether nonadditivity of herbivore impact is relevant to the evolution of host plants. As efforts were being made to apply the hypotheses of plant-herbivore coevolution to communities involving multiple species, Gould (1988) was among the first to identify the potential for nonadditive

impacts to play a significant role. Soon thereafter, in an effort to clarify terminology and provide a heuristic to guide future research on multiple-species communities, Rausher and colleagues specified definitions and criteria for pairwise and diffuse coevolution (Hougen-Eitzman and Rausher 1994; Iwao and Rausher 1997; Inouye and Stinchcombe 2001; Stinchcombe and Rausher 2001). Specifically, if a third species altered any part of the coevolutionary dynamics of two other species, then any coevolution among the three would be considered to be diffuse. Nonadditivity of combined impact was specified as one of the main causes of diffuse coevolution, in addition to ecological interactions among herbivores that alter amounts of damage and genetic correlations in plants' resistances to different herbivores.

In the next decade, the role of nonadditivity of impact in diffuse coevolution (or simply diffuse evolution) came under scrutiny. In a series of reviews, Strauss and colleagues argued for broadening the purview of coevolution beyond resistance to herbivory and advocated for a trait-centred approach (Strauss and Irwin 2004; Strauss et al. 2005; Haloin and Strauss 2008). The emphasis was placed on analysing selection gradients acting on specific traits, and the utility of factorial studies in which experimenters control the amount of damage was questioned. The criteria for diffuse (co)evolution were revised, with a trend toward minimizing the potential role of nonadditivity of herbivore impact to the point that nonadditivity of impact—in the sense envisaged by Gould (1988) and Rausher (Hougen-Eitzman and Rausher 1994; Iwao and Rausher 1997)—has been largely absent in recent treatments of ecoevolutionary dynamics in plant-herbivore communities.

In this paper, I argue that deemphasizing the potential role of nonadditivity of impact in the context of evolution of plant resistance in multiple-herbivore communities may be unwise. My argument consists of three definitions and a proposition. First, when an herbivore is allowed to feed freely in a host-plant population, the relative damage level caused by an herbivore on a plant defines the plant's "operational" resistance to that herbivore (operational resistance is considered a composite trait that incorporates the cumulative effects of more specific traits, such as levels of secondary chemicals, and it is generally quantified as the complement or the inverse of the amount of damage). Second, natural selection can be quantified by a selection differential (or gradient), which is the slope of the regression of relative fitness on a trait (or traits), such as operational resistance to an herbivore (or herbivores). Third, nonadditivity of impact can be defined as occurring when the damage caused by one herbivore alters the relationship between the level of damage by caused by a different herbivore and the host plant's fitness. Because nonadditivity of impact affects the slope of the damage-fitness relationship, nonadditivity exerts an influence on the strength of selection for operational resistance, and thus nonadditivity imparts diffuseness into the process of coevolution between plants and their herbivores.

This nonadditivity may not always be detected in the field due to the presence of other factors, such as ecological interactions among herbivores or allocation costs of resistance traits that also affect selection gradients for resistance. The potential for these other factors to obscure the presence of nonadditivity of combined impact is a major reason why factorial experiments in which the researchers control the amounts of damage can play a role in revealing a more complete picture of the coevolutionary dynamics of multiple-herbivore communities.

Here, I report on the results of a field experiment on the herbaceous weed *Solanum carolinense* L. (horsenettle) in which individual plants were exposed to a range of levels of leaf damage and flower damage in a factorial design. The impact of this damage on horsenettle's seed production was analysed to test two main hypotheses: 1) The fitness impacts of two species of herbivores that affect the same resource or tissue (in this case flowers) will combine in a synergistic pattern (if that resource is not limiting reproduction when plants do not experience herbivory); and 2) The fitness impacts of different types of herbivory (leaf and flower damage) will combine in a subadditive pattern. I also explored how these nonadditive patterns may be expected to affect the strength of selection for resistance imposed by the herbivores on the host plant, and I argue that these results have important implications for horsenettle's evolution of resistance against its multiple-herbivore community.

MATERIAL AND METHODS

Natural history

Horsenettle (*Solanum carolinense* L., Solanaceae) is a common herbaceous weed in its native range of the southeastern United States, as well as in parts of Europe, Asia, South America, and Australia (Bassett and Munro 1986; Imura 2003; Follak and Strauss 2010). Horsenettle spreads vegetatively via perennial lateral roots in disturbed areas, such as pastures and old agricultural fields (Sylvester 1946; Albert 1960; Ilnicki et al. 1962). It reproduces sexually through the production of flowers that mature acropetally on racemes that bear an average of 7–8 flowers each (Wise et al. 2008). Large ramets (i.e. stems) may produce more than a dozen racemes, while small ramets may produce none (Wise et al. 2008). The flowering period for horsenettle in the southeastern U.S. is roughly June through August, while a typical ramet remains in flower for about one month (Wise et al. 2008). Most of the flowers are perfect (i.e. cosexual or hermaphroditic), but a small proportion are male, and this proportion varies among genets (Elle 1998; Wise and Cummins 2007; Wise and Hébert 2010). Horsenettle is obligately out-crossing, and pollen is transferred mainly by buzz-pollinating insects such as bumblebees (Richman et al. 1995; Quesada-Aguilar et al. 2008). Fertilized ovaries may mature into round yellow berries with a diameter

commonly between 1 and 2 cm (Wise and Sacchi 1996; Wise and Cummins 2007). Many fertilized ovaries abort, apparently due to a lack of resources available to fill the fruits (Solomon 1985; Steven et al. 1999; Wise 2018).

The most-abundant leaf-feeding specialists (folivores) of horsenettle in the geographic area of this study include the eggplant flea beetle (*Epitrix fuscata* Crotch, 1873), the false potato beetle (*Leptinotarsa juncta* (Germar, 1824)), the eggplant tortoise beetle (*Gratiana pallidula* (Boheman, 1854)), the eggplant leafminer (*Tildenia inconspicua* Murtfeldt, 1883), and the eggplant lace bug (*Gargaphia solani* Heidemann, 1914) (Wise 2007b). In the field in which the current study was performed, flea beetles were the most damaging of the folivores (Wise 2010; Wise and Rausher 2013). Adult flea beetles feed on leaves from plant emergence to senescence, producing a characteristic shot-gun pattern of small holes in nearly every leaf (Wise and Weinberg 2002).

Flowers of horsenettle are also susceptible to high levels of herbivore damage. In a large study including 929 horsenettle plants in the same field site as the current study, plants lost a mean \pm 1 standard deviation of $51 \pm 20\%$ of their flowers to florivory (Wise and Rausher 2013). Ten percent of the plants lost more than three-quarters of their flowers, while 10% lost fewer than one-quarter of their flowers. The majority of flower damage was caused by two beetle species: the potato bud weevil (*Anthonomus nigrinus* Boheman, 1843) destroyed a mean of $31 \pm 18\%$ of the flower buds per plant, and the false potato beetle destroyed a mean of $12 \pm 16\%$ of the flowers per plant (Wise and Rausher 2013). Both of these herbivores are present through the entire flowering period of horsenettle (Tuttle 1956; Wise 2007b; Wise et al. 2008).

Source and propagation of plants

This study involved ten horsenettle genets (genetic individuals) that were originally collected as roots in the spring of 1997 from an oldfield population at Blandy Experimental Farm (39°03'43"N, 78°03'49"W) in Boyce, Virginia, USA. These ten genets were part of a larger research program, and more details on propagation methods can be found elsewhere (Wise 2007a).

The field-collected roots were planted in 18.9-liter (5-gallon) pots filled with commercial growing medium (Wesco Growing Media III, Wetsel Seed Company, Harrisonburg, VA, USA). These pots were placed on wooden pallets in full sunlight in a semi-protected propagation area at Blandy Farm, where the plants were allowed to grow through senescence in autumn. The roots were placed in cold storage over winter, and cuttings of new root growth were used to perpetuate the genets through 2002 using similar procedures. The two main goals of these propagation procedures were to generate numerous clonal replicates of the genets for a series of experiments and to purge the plants of potential maternal or carryover effects due to variation in the microhabitats of the original source fields.

From 1 to 4 May 2000, I removed from refrigeration the horsetettle roots that were grown in 1999 to begin the experiment that is the focus of this paper. I created equal-sized root cuttings by dipping a root into a 100-mL graduated cylinder containing 98 mL of water and cutting the root at the point at which it displaced exactly 2 mL of water. I planted 30 root cuttings for each of 10 genets separately in 3.8-L (1-gallon) plastic pots in Wesco Growing Media III. The pots were then placed onto wooden pallets outdoors in a semi-protected propagation area, where I watered them and monitored plant growth for 5–6 weeks. Once a ramet emerged, I attached a Fibe-Air™ plant sleeve (Kleen Test Products, Brown Deer, WI, USA) to its pot to prevent herbivory. If more than one ramet emerged, I clipped all but the largest down to the soil surface. On 13 Jun. 2000, I selected 20 healthy ramets from each of the 10 genets to participate in the experiment. Some ramets had initiated racemes, but no flowers had opened yet.

Experimental design

The experiment employed a three-way factorial design, with a folivory treatment (four levels) crossed with a florivory treatment (five levels), crossed with plant genet (10 genets). Each of the 20 damage combinations was assigned randomly to one ramet from each genet. Each of the 200 ramets was then randomly assigned to a position where it would be transplanted into an oldfield at Blandy Farm (39°04'00"N, 78°03'40"W) within an existing horsetettle population. The transplanting positions consisted of seven rows, two meters apart, with two meters between positions within each row.

The 200 ramets were transplanted into the field site on 15–17 Jun. 2000. I blended the transplants into the field setting by covering the growing medium with field soil and leaf litter. A plastic plant label was placed in the soil in front of each transplanted ramet, and a flag was staked near each location with the flag colour indicating the folivory treatment.

Experimental protocol

Damage treatments

The folivory-treatment levels were achieved using an insecticide-spraying regime to create a range of naturally imposed leaf herbivory. Specifically, I sprayed ramets with carbaryl in the form of concentrated SEVIN® (Bayer CropScience LP, Research Triangle Park, NC, USA) at 1.5 tablespoons of SEVIN® per gallon of water at four different frequencies throughout the growing season. Judgments regarding when to spray were determined subjectively by visual inspections of the plants. Between 17 June and 7 September, the ramets in the four folivory-treatment groups were sprayed 11, four, two, or zero times. The highest frequency was intended to keep folivory as low as possible, so plants were sprayed after heavy rains or when folivores started to reappear. The fourth group was

never sprayed; therefore, the damage levels of this group represented ambient damage levels in the field—rather than unnaturally high levels.

Although the insecticide affected all species of leaf-feeding insects, this study focused on flea beetles, which were by far the most common folivore in this horsetettle population. Moreover, in a separate study in the same field, flea beetles imposed far greater natural selection for resistance than any other horsetettle-feeding folivore (Wise and Rausher 2013). Damage by flea beetles was quantified non-destructively on each ramet in mid-August, soon after flowering had ceased in the experimental population, using a transparent plastic grid. A 92.7-mm² square on the grid was centred along the length of the right half of each leaf of sufficient size, with its left edge abutting the leaf's midvein (or its right edge, if tissue was lost to other herbivores on the right half of the leaf). This square was subdivided into 25 identical squares. The number of these squares that covered tissue with (and without) damage by flea beetles was counted for each leaf on each ramet. The sum of the number of squares covering flea-beetle-damaged tissue for all leaves on a ramet was divided by the total number of squares covered by the grid (damaged plus undamaged) to obtain a total flea-beetle-damage index (hereafter, "FB index") for each ramet. A mean of 12 leaves (standard deviation of 6 leaves) was included per ramet. The FB index provides a relative damage metric that is assumed to be monotonically related to the actual area of leaf tissue consumed by the beetles.

When a raceme started to develop, it was marked with a dot of coloured paint for future identification and covered with a small mesh bag (attached with a twist tie) to prevent natural florivory. The florivory treatments involved manually clipping the pedicels of maturing flower buds to generate a natural range of flower damage: 0%, 20%, 40%, 60%, or 80%, spread evenly within and among racemes (Fig. 1). Each ramet was checked every three days to apply the florivory treatments, which started on 18 June and continued until early August.

For the analyses, the simulated florivory was treated as though there were two species of florivores (F1 and F2), each of which destroyed 0%, 20%, or 40% of the flowers. Specifically, half of the ramets in the 20%-florivory group were randomly assigned to represent 0% damage by F1 plus 20% damage by F2, while the other half were assigned as 20% F1 + 0% F2. Similarly, one-third of the ramets in the 40% group were randomly assigned as 0% F1 + 40% F2, one-third were assigned as 40% F1 + 0% F2, and one-third were assigned as 20% F1 + 20% F2. Half of the ramets in the 60% group were randomly assigned as 40% F1 + 20% F2, and half were assigned as 20% F1 and 40% F2. Finally, all of the ramets in the 80% group were assigned as 40% F1 + 40% F2. There was no distinction in the manner in which the manual damage was physically applied to represent the two different simulated herbivores. The two main florivores are both beetles that dispatch of the flower buds rather quickly (Wise 2007b).

Pollination

The mesh bags that were placed over the racemes to prevent folivory also prevented pollinators from accessing flowers. Therefore, flowers had to be manually pollinated in order for fruit-set to occur. Pollen was procured by gathering fresh flowers each morning from multiple horsenettle ramets at Blandy Farm. I used a battery-powered tomato pollinator to buzz the anthers of each collected flower, releasing its pollen into a glass vial. I introduced pollen onto stigmas of open flowers in the field experiment using a camel-hair paintbrush. Each ramet was pollinated at three-day intervals, which was frequent enough that no open flower would go unpollinated—except for the staminate (male) flowers, which cannot set fruit. The last perfect flower in the experiment was pollinated on 11 August. The pollination of every flower was considered appropriate because a previous study in a similar field at Blandy Farm showed that horsenettle's fruit production was not pollen limited (Wise and Cummins 2002).

Seed production

Seed production was used as the fitness proxy for ramets in the statistical analyses. Rather than attempting to count all the seeds, I measured diameters of fruits to the nearest mm, as fruit size has been found to explain 90% of the variation in seed number in horsenettle fruits (Wise and Cummins 2007) with the following prediction formula:

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

where d is the mode of at least three diameter measurements (in cm) around the middle of the fruit. Fruit ripening occurred in a staggered fashion across racemes, with all fruits on a single raceme ripening simultaneously. I collected fruits as soon as they ripened

to take diameter measurements before fruits could be removed by frugivores—mainly meadow voles, *Microtus pennsylvanicus* (Ord, 1815). Once a ramet had completely senesced, I collected all of its remaining fruits. After hard freezes on 8–9 October killed the rest of the ramets, I collected and measured any fruits that remained. No attempt was made to estimate the fitness of ramets through the paternal route—that is, siring offspring via pollen. Because the majority of horsenettle's flowers produce both ovules and pollen, it is likely that maternal and paternal fitness are positively correlated. Even if this is not the case (cf. Elle and Meagher 2000), it seems unlikely that siring success would completely counteract any pattern of nonadditivity found for seed production.

Data analysis

Adjustments to sample size

It became apparent after the first insecticide spraying that ramets of one of the 10 horsenettle genets were poisoned by SEVIN[®]. While all of the sprayed ramets of this genet were eventually killed by repeated sprayings, the unsprayed ramets remained healthy throughout the experiment. This genet was omitted from the analyses, reducing the total sample size to 180 ramets. Other than a reduction of folivory, none of the ramets of the remaining nine genets appeared to be affected (negatively or positively) by the insecticide. Among these nine genets, 54 ramets produced fewer than five flowers. Because the simulated-florivory treatments could not be applied precisely for ramets with so few flowers, these 54 ramets were omitted as well, leaving a total of 126 ramets for the statistical analyses. Although these adjustments left an unbalanced design, there were ample replicates for each treatment combination to test the hypotheses.

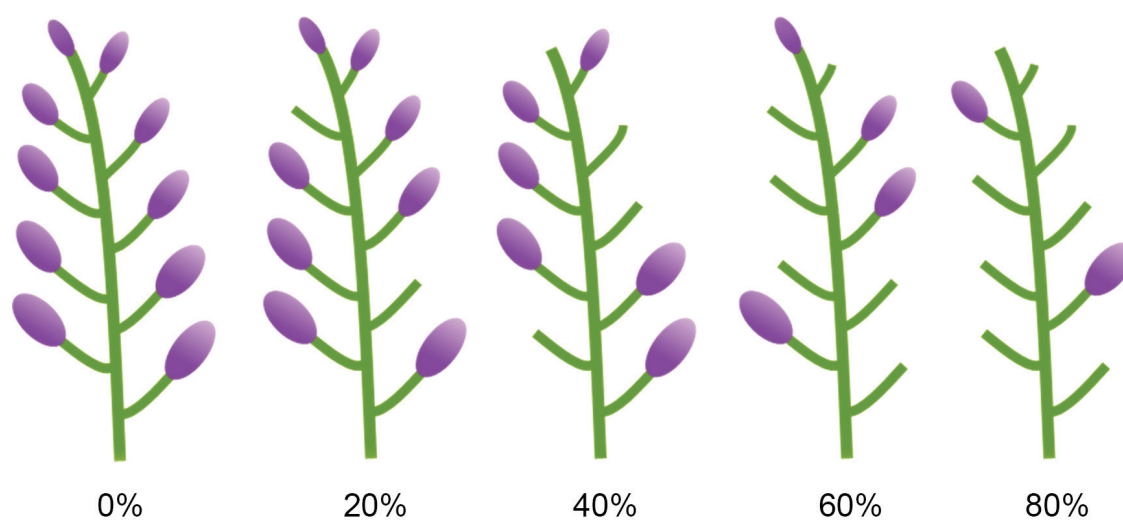


Figure 1. Five levels of simulated florivory in the field experiment. Flower buds (shown in purple) mature from the base to the tip of a raceme. The budless pedicels show which buds were clipped from the racemes to achieve the targeted levels of the florivory treatment.

Hypothesis 1: The impacts of two species of herbivores that affect the same resource will combine synergistically

To assess the additivity of impacts on horsenettle's seed production by the two simulated species of florivores, I performed an analysis of covariance (ANCOVA). Damage levels by each of the two florivores (i.e. the proportions of buds actually cut per ramet) were included as continuous explanatory variables. The FB Index was included as a covariate, and plant genet was included as a random-effects factor to account for the likelihood that genets would differ in seed production irrespective of damage treatments. A significant interaction term between the two simulated florivores would indicate that the impacts of the two species combined in a nonadditive fashion, and a negative value of the coefficient for the interaction term would indicate that the nonadditivity was of the synergistic type (i.e. the mean fitness of plants under combined-herbivore attack was less than would be predicted from simply subtracting the sum of the individual impacts of the same amounts of damage by the two herbivores when feeding alone from the mean fitness of undamaged plants).

Seed number was natural-log transformed for the ANCOVA to achieve homoscedasticity of residuals. Moreover, when herbivory is measured on a proportional scale, comparisons of its impacts (i.e. plant tolerances of herbivory) are more intuitively interpreted when fitness is on a logarithmic scale (Wise and Carr 2008). Because some plants produced zero seeds, a nonzero value had to be added prior to the log transformation. I added a value of 100 to the seed number of each plant before log transforming the data, which had the added benefit of reducing the skew of the distribution of the residuals. The ANCOVAs in this study were run using the REML procedure of JMP IN 4.0.4 (SAS Institute, Cary, NC, USA).

Hypothesis 2: Impacts of different types of herbivory will combine subadditively

To assess the separate and combined impact of florivory and folivory on horsenettle's seed production, I performed an ANCOVA similar to the one described above in which folivory and total florivory (sum of F1 and F2 damage) were treated as continuous explanatory variables. The FB indices were used as the folivory values (rather than insecticide-frequency categories). An interaction term between florivory and folivory was included in the ANCOVA to indicate whether the impacts of these two types of herbivory combined in a nonadditive fashion. A positive value of the coefficient for this interaction term would indicate that the nonadditivity was of the subadditive type (i.e. the mean plant fitness under combined-herbivore attack was greater than would be predicted by simply subtracting the sum of the individual impacts from the mean fitness of undamaged plants).

Influence of nonadditivity of impacts on selection for resistance

The ANCOVAs described above allowed for the detection of nonadditivity of impact. To obtain a more precise picture of how the impact of damage by one species varied over a range of damage levels by another species, I ran a series of regression analyses of plant relative fitness on damage levels by one herbivore at discrete levels of damage by the other herbivore. Specifically, I ran three separate linear regressions of relative fitness on the proportion of flowers damaged by simulated Florivore 1—that is, one regression for each set of ramets at the three damage levels caused by simulated Florivore 2. Within each Florivore 2 treatment level, I calculated relative fitness for each ramet by dividing the number of seeds it produced by the mean number of seeds produced by all the ramets within that Florivore 2 treatment level (cf. terHorst et al. 2015).

I then ran four separate linear regressions of relative fitness on the proportion of flowers cut—one regression for each set of ramets in the four folivory treatments. Within each folivory treatment, I calculated relative fitness for each ramet by dividing the number of seeds it produced by the mean number of seeds produced by all the ramets within that folivory treatment. Finally, I ran five separate linear regressions of plant relative fitness on the FB index—one regression for each set of ramets in the five florivory treatments. For these five regressions, relative fitness was calculated within each florivory treatment by dividing the number of seeds a ramet produced by the mean number of seeds produced by all of the ramets in that florivory treatment.

These regression analyses are analogous to phenotypic selection analyses, with the regression coefficients of the damage variables representing phenotypic selection differentials for resistance to that damage (Lande and Arnold 1983). However, instead of the damage levels being determined by resistance characters, they were determined by the randomized treatments imposed by the experimenter. Therefore, they cannot be interpreted as representing selection acting on resistance traits. Instead, the coefficients indicate the magnitude of the effects of damage itself on plant fitness, isolated from other factors that might be associated with resistance traits (such as allocation costs or ecological costs). A comparison of the magnitudes of these regression coefficients at different levels of damage by a second herbivore thus provides a clean picture of how the fitness impact of damage by one herbivore depends on the amount of damage caused by the other herbivore. That is, they detect and isolate the contribution of nonadditivity of fitness impact to selection for resistance, even though they cannot determine the total magnitude of selection on resistance traits.

RESULTS

Efficacy of herbivory treatments

The insecticide regime was effective at creating a range of folivory levels. With ambient levels of folivory (no spraying), the mean flea-beetle-damage index was 0.74 (Fig. 2A). Spraying twice, four times, and 11 times over the experiment decreased the FB index to means of 0.67, 0.44, and 0.17, respectively (standard errors of these means are shown in Fig. 2).

The simulated-florivory treatments were generally within 1–2% of their targeted means (Fig. 2B). The exception is that the mean percentage of buds lost in the 0% treatment was actually 4%. This discrepancy was due to the fact that a few flowers were accidentally damaged or were pollinated too late to set fruit. Such flowers were treated as “cut”. For the other treatment levels, any accidentally “cut” flowers were compensated for by cutting fewer than the targeted number of remaining flower buds. Such an accommodation was not possible in the 0% treatment group because no buds would have been targeted for cutting in that group.

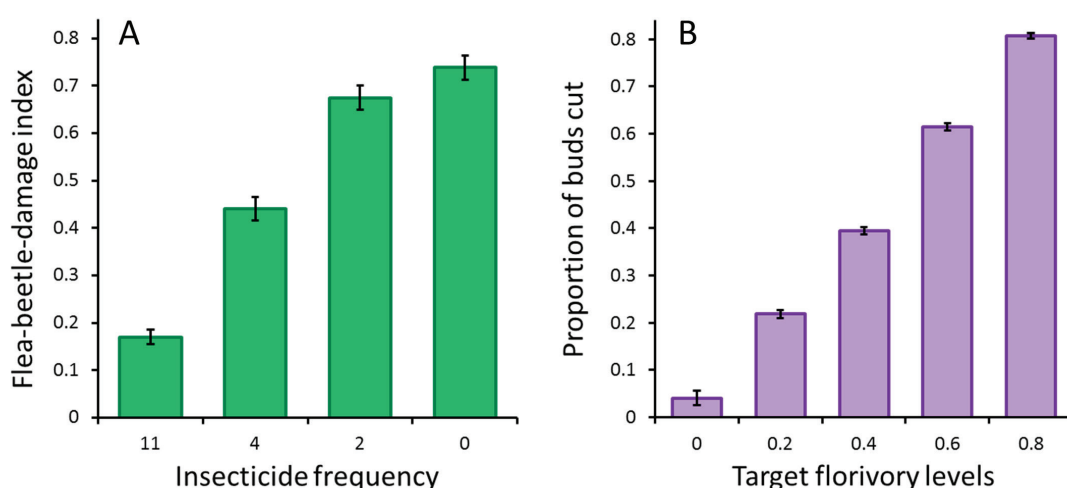


Figure 2. Efficacy of the herbivory treatments. Columns and bars represent means \pm one standard error. **A.** The folivory treatments are shown as insecticide frequency, which represents the number of times during the experiment that ramets were sprayed with SEVIN[®]. **B.** The targeted florivory treatments are shown as proportion of flower buds intended to be cut per ramet.

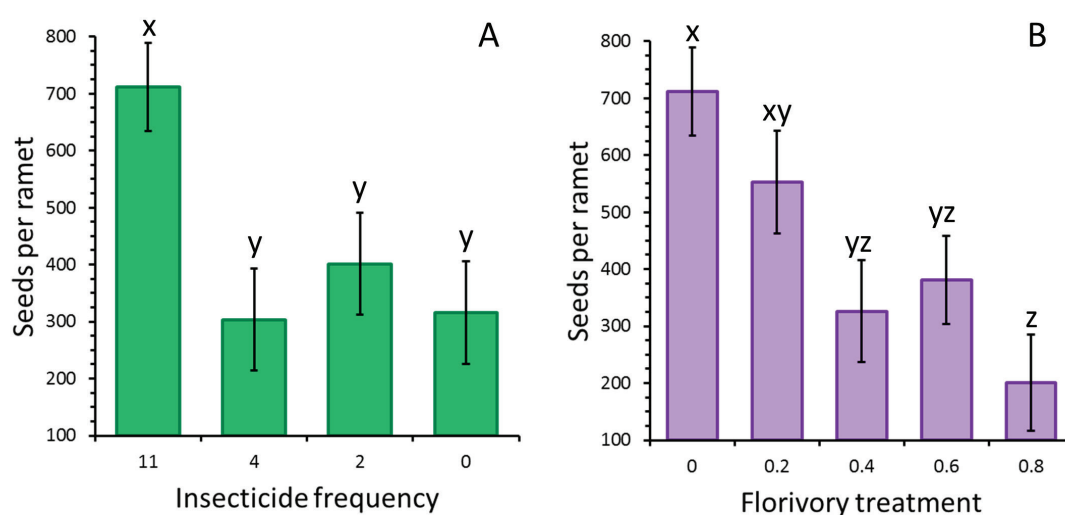


Figure 3. Effect of the damage treatments on horsenettle's seed production. **A.** Folivory: number of times insecticide was sprayed. **B.** Florivory: target proportion of flower buds cut. Columns and bars represent least-squares-means \pm one standard error calculated from an ANCOVA of seed number on plant genet, insecticide spraying frequency, target total florivory, and the interaction between the insecticide frequency and florivory treatments. Genet was treated as random and the damage treatments were considered as ordinal values. Within panels, bars that share a lower-case letter are not statistically significantly different from each other at $p < 0.05$ as determined by Tukey HSD tests.

Table 1. Summary of ANCOVA results for effects of folivory (flea-beetle-damage index) and simulated florivory (% flower buds cut) on seed production of horsenettle in the field experiment. Seed numbers were transformed as the natural log(seeds + 100). * There is a different coefficient for each genet.

Source of variation	d.f.	Parameter estimate	MS	F-ratio	p value
Plant genet	8	various*	3.38621	9.6702	< 0.0001
% Buds cut	1	-1.280	16.27093	46.4658	< 0.0001
FB Index	1	-0.276	0.60739	1.7346	0.19
% Buds cut × FB Index	1	1.470	1.49615	4.2726	0.041
Error	114		0.35017		

Table 2. Summary of ANCOVA results for effects of simulated flower damage (florivory) by two species on seed production of horsenettle in the field experiment. Seed numbers were transformed as the natural log(seeds + 100). Folivory (flea-beetle-damage index) was included as a covariate. * There is a different coefficient for each genet.

Source of variation	d.f.	Parameter estimate	MS	F-ratio	p value
Plant genet	8	various*	1.26075	9.0415	< 0.0001
FB index	1	-0.329	0.86560	6.2076	0.014
Florivore 1	1	-0.683	1.37587	9.8670	0.0021
Florivore 2	1	-1.008	3.01543	21.6252	< 0.0001
Florivore 1 × Florivore 2	1	-4.260	1.57214	11.2746	0.0011
Error	113		0.13944		

Impacts of herbivory on plant fitness

Folivory had a largely negative effect on seed production (Fig. 3A), with the most-frequently sprayed plants producing a least-squares mean \pm SEM of 711 ± 77 seeds, compared to 316 ± 90 seeds for plants experiencing ambient levels of folivory. However, the effect of folivory was noticeably nonlinear, with a substantial decrease in seed production once the FB index exceeded 0.17, and then a levelling off of impact up to the highest FB index of 0.74 (i.e. at ambient damage level). The main effect of folivory (FB index) was not statistically significant when the interaction between the FB index and florivory was included in the ANCOVA model ($F_{1,114} = 1.7346$, $p = 0.19$, Table 1); however, when that interaction term was not included in the model, the main effect of folivory was found to be statistically significant ($F_{1,113} = 6.2076$, $p = 0.014$, Table 2). In contrast, simulated florivory caused a statistically significant reduction in seed production even when this interaction term was included in the model ($F_{1,114} = 46.4658$, $p < 0.0001$, Table 1). The impacts of flower damage on seed production also showed evidence of nonlinearity (Fig. 3B). Specifically, a negative impact of simulated florivory was not evident until the plants lost at least 40% of their flowers. Plants that lost 80% of their flowers produced an average of 41% as many seeds as plants in the 0%-florivory group. The statistical significances of the folivory and florivory main effects must be interpreted with caution, however, because the interaction between the main effects was significant ($F_{1,114} = 4.2726$, $p = 0.041$, Table 1).

Hypothesis 1: The impacts of two species of herbivores that affect the same resource will combine synergistically

When flower damage was considered to be caused by two different species of simulated florivores, damage by either florivore alone caused significant reductions in log-transformed seed production (Table 2). However, the reduction when both were modelled to feed together differed significantly from what would be predicted by adding together their individual impacts (Florivore 1-by-Florivore 2 interaction: $F_{1,113} = 11.2746$, $p = 0.0011$, Table 2). The same ANCOVA run on the untransformed seed numbers provided a very similar inference regarding the interaction ($F_{1,113} = 5.2287$, $p = 0.024$). The negative value of the coefficient of the interaction term (Table 2) indicates that the impacts combined synergistically, consistent with the prediction of Hypothesis 1. For example, at mean levels of florivory by both simulated species (i.e. 20% each), a plant would lose 59 more seeds than would be predicted by the additive model. Moreover, at maximum damage levels of both species (40% each), a plant would lose 238 more seeds than an additive model would predict.

Hypothesis 2: Impacts of different types of herbivory will combine subadditively

The effect on log-transformed seed production that was caused by flower damage depended on the amount of leaf damage the plants experienced, and vice versa. In

Table 3. Results of regressions of horsenettle's relative fitness on simulated floral herbivory by one species (Florivore 1) at three different flower-damage levels by a second species (Florivore 2).

Florivore 2 level	N	Slope	SE slope	t-ratio	p value
0.0	48	-0.003	0.557	0.00	> 0.99
0.2	33	-0.627	0.600	-1.05	0.30
0.4	45	-2.374	0.679	-3.50	0.0011

Table 4. Results of regressions of horsenettle's relative fitness on flower damage (proportion of buds cut) at four different folivory levels (mean FB index at each insecticide-application frequency).

Folivory level	N	Slope	SE slope	t-ratio	p value
0.17	37	-1.24	0.31	-3.97	0.0003
0.44	29	-0.56	0.42	-1.34	0.19
0.67	32	-1.02	0.38	-2.71	0.011
0.74	28	-0.79	0.48	-1.76	0.091

Table 5. Results of regressions of horsenettle's relative fitness on leaf damage (FB index) at five different florivory levels (target proportion of flower buds cut).

Florivory level	N	Slope	SE slope	t-ratio	p value
0.0	27	-0.86	0.36	-2.37	0.026
0.2	28	-0.70	0.39	-1.79	0.085
0.4	22	0.10	0.53	0.19	0.85
0.6	23	0.19	0.50	0.37	0.72
0.8	26	-0.22	0.69	-0.32	0.76

other words, the individual impacts of the two types of damage combined in a nonadditive fashion, as evidenced by the significant interaction between the proportion of flower buds cut and the FB index ($F_{1,114} = 4.2726$, $p = 0.041$, Table 1). Notably, the same ANCOVA run on the untransformed seed numbers provided a nearly identical inference regarding the interaction ($F_{1,114} = 5.6349$, $p = 0.019$). The positive value of the coefficient of the interaction term (Table 1) indicates that the impacts combined in a subadditive manner, consistent with the prediction of Hypothesis 2. For example, at mean levels of flea beetle folivory and flower damage, a plant would lose 107 fewer seeds than would be predicted from the sum of the individual impacts that the same levels of folivory and florivory would have had if the herbivores were feeding separately (i.e. from an additive-impact model). Moreover, at maximal levels of both types of herbivory, a plant would lose 347 fewer seeds than the additive model would predict.

Influence of nonadditivity of impacts on selection for resistance

Flower damage by one simulated species of florivore had a statistically significant negative impact on horsenettle's fitness only at the highest level of flower damage by the other simulated species of florivore (Fig. 4, Table 3). Specifically, the identical amount of damage by Florivore 1 had either negligible impact, slight but non-significant

impact, or highly significant impact on horsenettle's fitness on ramets that respectively had either 0%, 20%, or 40% of its flowers damaged by Florivore 2 (Table 3).

Simulated florivory had by far its most significant negative impact when leaf damage was at its lowest—

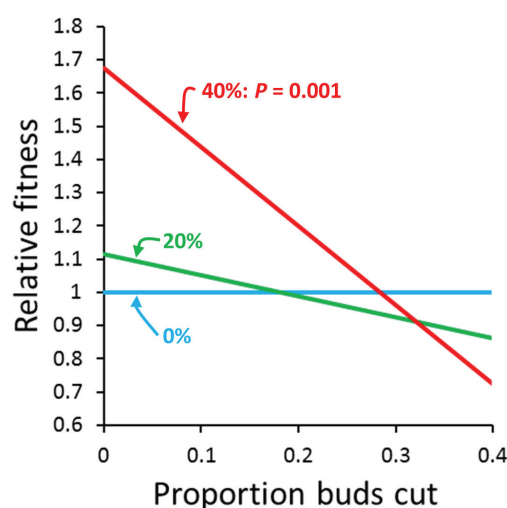


Figure 4. Results of regressions of horsenettle's relative fitness on flower damage by Florivore 1 at each of three levels of damage by Florivore 2. P values are shown only for regression slopes significantly different from 0 at an alpha of < 0.05. The numeric estimates of all slopes and associated p values are shown in Table 3.

that is, with the most frequent spraying of insecticide (Fig. 5A, Table 4). At higher levels of leaf damage, flower damage had less of an impact, and the impact was only marginally significant at the medium and highest levels of leaf damage (Table 4). Similarly, leaf damage had a statistically significant negative impact on horsenettle's fitness only when there was no flower damage (Fig. 5B, Table 5). Leaf damage had a slightly lower, but only marginally significant impact when 20% of the flowers were destroyed by simulated florivory. Once floral damage reached 40% or greater, leaf damage had essentially no impact on horsenettle's fitness (Table 5).

DISCUSSION

Hypothesis 1: The impacts of two species of herbivores that affect the same resource will combine synergistically

This result can be explained by the Limiting Resource Model (LRM) of plant tolerance with a consideration of source-sink dynamics (Geiger and Servaites 1991; Wise and Abrahamson 2005). Specifically, seed production in the absence of herbivory was apparently not sink-limited; that is, plants produced more flowers than could be matured into fruits, so flowers were not a limiting "resource". In particular, the plants could tolerate the loss of up to 20–40% of their flowers to simulated florivory without a significant reduction in seed production. The amount of damage caused by just one species of florivore was not severe enough to cause the seed production to become sink limited. However, the combined damage by two species caused the plants to become sink limited, thus crossing the threshold at which plants could no longer

tolerate the combined damage of two florivores without a concomitant reduction in seed production.

This pattern of synergistic impact of two herbivores feeding on the same resource is envisaged to result from a nonlinear relationship between damage level and fitness (Gould 1988). In other words, rather than each unit of damage causing the same decrement in plant fitness, the decrements depend on how much damage the plants have already suffered. Specifically, if plants can completely tolerate small amounts of damage, then the decrements caused by a unit of damage may be zero when there is little cumulative damage, but nonzero once cumulative damage levels pass the tolerance threshold. When viewed with this perspective, it becomes clear that the synergistic impact of two herbivores is a result of the same phenomenon that can cause a nonlinear relationship between the damage level of a single species of herbivore and its host-plant's fitness. Synergistic impact may be common in nature simply because two (or more) species of herbivores that feed on the same type of tissue tend to cause more damage than just one herbivore, and thus the combined feeding is more likely to overwhelm the plant's ability to tolerate the damage.

If the plants had already been sink-limited in the absence of florivory, then any amount of flower damage would have reduced seed production—that is, there would have been no tolerance threshold. If the damage-fitness relationship was linear, then each unit of herbivore damage would cause an equal fitness decrement, regardless of the cumulative amount of damage. As such, the combined impact of two species of florivores would be expected to be equal to the sum of the individual impacts of each herbivore species in isolation. Such an additive scenario of combined impact is likely to be quite common in nature when two or more species of herbivores feed

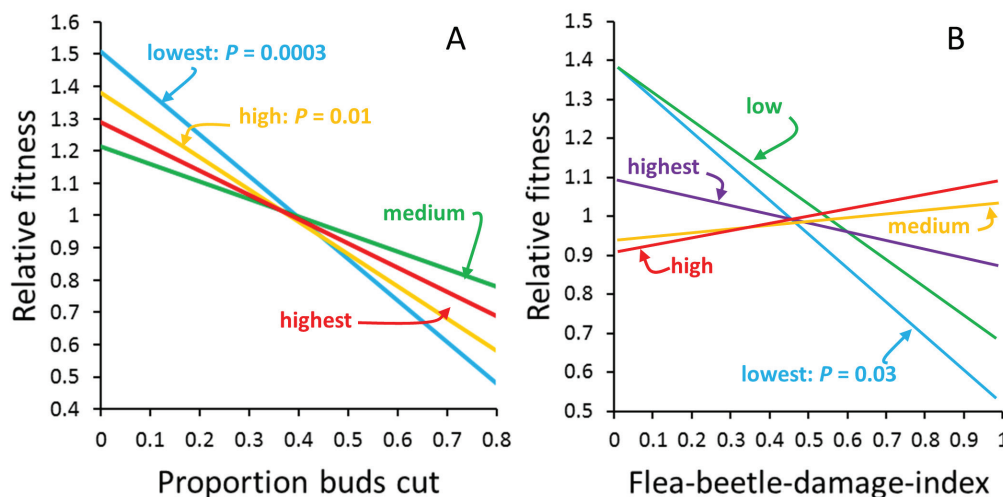


Figure 5. Results of regressions of horsenettle's relative fitness on (A) the proportion of buds cut at each of the four insecticide-treatment levels (lowest, medium, high, and highest represent 11, 4, 2, and 0 sprayings, respectively), and on (B) the flea-beetle-damage index at each of the five flower-damage treatment levels (lowest, low, medium, high, and highest represent targets of 0, 20%, 40%, 60%, and 80% of buds clipped, respectively). P values are shown only for regression slopes significantly different from 0 at an alpha of < 0.05 . The numeric estimates of all the slopes and the associated p values are shown in Tables 4–5.

on the same type of tissue and thus affect the acquisition of the same, limiting resource. The current study did not test this additive-impact hypothesis, however. Such a test would have required the plants to be sink (flower) limited in the absence of florivory. As detailed above, seed production in the experimental plants did not become sink limited until the damage level exceeded ~40% of the flowers.

The use of manually simulated damage in this experiment made the connection between nonlinearity, tolerance thresholds, and synergistic impact even clearer. That is, the damage could be envisioned as having been caused by just one hypothetical species of herbivore. The plants in this experiment tolerated damage ranging up to about 40% of flower buds with only a negligible loss of seed production; however, above that 40% threshold, seed production was reduced precipitously. Thus, there was an overall nonlinear relationship between florivory and plant fitness. However, a study of natural herbivory in this same field site revealed that the two most-damaging horsenettle florivores (the potato bud weevil, and the false potato beetle) destroy an average of only 31% and 12% of the flowers, respectively, while other florivores combined to destroy an average of 8% of the flowers (Wise and Rausher 2013). None of these florivores feeding alone would commonly cause the plants to cross the tolerance threshold. However, feeding by any one species in effect lowers the tolerance threshold for feeding by any of the other species of florivores, making synergistic impact increasingly likely in multiple-herbivore communities.

Although manually simulated herbivory has some advantages in experimental studies (Tiffin and Inouye 2000; Lehtilä 2003; Lehtilä and Boalt 2004), additional insight would be gained by studying actual herbivory. In this system, the two main florivorous species of horsenettle are beetles whose chewing destroys the flowers rapidly, and their damage tends to be spread across racemes. However, the potato bud weevils kill flowers in the bud stage by chewing through the pedicels after laying eggs in the buds, while false potato beetles consume flowers either in the bud stage or after a flower is open (Wise 2007b). In addition, meadow voles often destroy entire racemes by chewing their peduncles, and damage by larvae of the moth *Frumentia nundinella* (Zeller, 1873) (Gelechiidae) causes the maturation of parthenogenetic, seedless fruits (Wise 2007b). While the manner in which flower damage was simulated in this study may do a fair job at representing the general impact of the loss of sinks, a next step would be to investigate how subtleties in the damage patterns and manners of feeding of the different species might influence plant responses vis-à-vis the plant's tolerance thresholds.

Hypothesis 2: Impacts of different types of herbivory will combine subadditively

The results of this experiment were also consistent with the prediction of Hypothesis 2: the impacts of the two different

types of herbivore damage combined in a subadditive fashion to affect horsenettle's seed production. In other words, horsenettle expressed greater tolerance of each type of damage when damage by the other herbivore was relatively high than when damage by the other herbivore was low or absent. This pattern can also be explained by the LRM. Specifically, leaf feeding (folivory) likely decreased the supply of carbon available for seed production. With increasing levels of folivory, horsenettle's reproduction became relatively more source (leaf) limited and less sink (flower) limited. With more folivory, plants could tolerate a higher loss of flowers to herbivores because a greater proportion of the flowers would have aborted anyway due to a lack of photoassimilates needed to fill fruits.

Nonadditivity and diffuse (co)evolution

It is worth re-emphasizing that the regression slopes of relative fitness on damage in the analyses of this paper should not be interpreted as natural-selection differentials (or gradients) acting on resistance because the damage levels were not the result of any particular plant trait. Instead, the damage levels were assigned randomly by the experimenter. As such, the nonadditivity of impact found in this study cannot provide direct evidence of nonadditive selection acting on resistance, and thus it cannot provide evidence that diffuse (co)evolution is occurring in a natural horsenettle population. Nevertheless, the results of the ANCOVAs do show that the impact of damage by one herbivore can depend on the amount of damage caused by another herbivore (without influencing the amount of damage caused by the other herbivore). Moreover, the regression analyses clearly show that subadditive and synergistic impacts have opposite effects on the damage-fitness relationship—a relationship that is integral to the magnitude of a selection differential or gradient.

In a more natural setting, allocation and ecological costs of resistance traits may reduce the benefits of a reduction in damage and thus make it more difficult to detect the effects that nonadditivity of impact may have on selection for resistance. The advantage of a more controlled, factorial experiment is that it removes potentially obscuring factors from the picture and thus provides a more transparent view of how damage levels themselves affect plant fitness. This clearer view shows that nonadditivity may be a result of something as simple and fundamental as the balance of sources and sinks in plant reproduction. Such a fundamental balance would be expected to apply not only in a factorial experiment, but in nature as well. Thus, nonadditivity of combined fitness impacts of two herbivores on a shared host plant will contribute a diffuse component to selection for resistances to the herbivores, even if countervailing factors cause the overall gradients for selection on resistance to lose the signal of nonadditivity. In this sense, it is reasonable to conclude that nonadditivity of impact is a sufficient (though not necessary) criterion for diffuse (co)evolution in plants attacked by multiple species of herbivores.

Ecoevolutionary implications

The regression analyses suggest that nonadditivity of combined impact results in alterations of the strength of selection for resistance in shared host plants. In combination with other factors—such as amount of genetic variation for resistance traits, genetic correlations among resistance traits, and ecological interactions among herbivores—the manner in which fitness impacts combine will affect the expected evolutionary trajectory of resistance. Specifically, if the fitness impacts of multiple herbivores combine in a synergistic fashion on a shared host plant, the pace at which the plant population evolves resistance will accelerate (as long as the plant population possesses genetic variation for resistance mechanisms and the cost of resistance is not prohibitive). In contrast, if the fitness impacts combine in a subadditive manner, then the pace of evolution of resistance to at least some of the herbivores is likely to be slowed. Such evolutionary effects on resistance would then have feedback effects on the ecology and behaviour of the herbivores, which would affect the selective regime for resistance traits in the plant population, and so on.

Previous research on horsetnettle and its herbivores provides a basis for speculating on implications of the current results to the ecoevolutionary dynamics of this system. Consider, for instance, the horsetnettle-specialist moth *Frumenta nundinella*, which in a large field study destroyed an average of only ~3% of horsetnettle ramets' flowers (Wise and Rausher 2013). On its own, a minor florivore such as *F. nundinella* probably has negligible impact on horsetnettle's fitness, and thus there is likely to be no selective advantage for increased resistance to these florivores in horsetnettle populations located near the study area. However, if potato bud weevils are also present, then horsetnettle is likely to face much stronger selection for resistance against florivory in general. Thus, *F. nundinella* may be at a disadvantage in the coevolutionary arms race with horsetnettle due to selection for increased resistance to florivory brought about by damage caused by potato bud weevils. The survival and fecundity of individuals of *F. nundinella* may decrease, or their behaviour may change to focus on less-resistant plant species. Therefore, indirect ecological effects (namely, synergistically combining fitness impacts) would lead to evolutionary changes that cause further ecological effects in the plant-herbivore community.

Subadditively combining impacts are more likely to have the opposite effect, slowing the plant's evolution of resistance against members of its herbivore community. Consider that natural populations of horsetnettle are often heavily damaged by folivory, especially by flea beetles and potato beetles, but also by lace bugs, leaf-mining caterpillars, tortoise beetles, blister beetles, and others (Wise 2007b). Controlled experiments indicate that feeding by at least the first three species can cause significant impact on horsetnettle's reproduction (Wise and Sacchi 1996; Wise and Cummins 2006; Wise

and Mudrak 2021), and thus we might expect strong directional selection to increase resistance to these folivores. However, selection for resistance against leaf-feeding species was found to be relatively weak in a large field study on horsetnettle (Wise and Rausher 2013). In that field study, florivores destroyed an average of roughly half of the plants' flowers, and selection for resistance against the florivores was quite strong. The findings of the current factorial experiment suggest that the fitness effects of the leaf feeders in that field experiment was negligible because the flower feeders caused reproduction in the plants to be limited by the number of sinks (flowers) rather than the supply of sources (photosynthates) (Wise and Rausher 2016).

In the face of strong selection for increased resistance against florivory, some factors must be constraining an evolutionary response to that selection for damage levels to flowers to remain so high. Most simply, horsetnettle populations may possess a rather small amount of genetic variation for resistance against floral herbivory (Wise 2007a). If that is the case, before the plant population can begin to evolve resistance to leaf feeders, the plant population must wait for one of the following phenomena: for mutations that increase resistance to floral herbivory, for gene flow from populations with different alleles for florivory-resistance traits, or for a reduction in the florivore population due to natural enemies or an abiotic disturbance. Horsetnettle's evolution of resistance to potato bud weevils may also be constrained by internal (e.g. allocation) costs of resistance (Wise and Rausher 2016), negative genetic correlations with resistances to folivory by flea beetles and frugivory by meadow voles (Wise and Rausher 2013), and competitive interactions that likely result in the flowers that are saved from the weevils ending up being lost to feeding by potato beetles and meadow voles (Wise 2009).

With all of these potential genetic and ecological constraints, horsetnettle's evolution of resistance against potato bud weevils may be at a stalemate. Such a stalemate would allow other species of herbivores to maintain large populations on horsetnettle, unimpeded by selection for increased resistance. In this manner, subadditivity of combined-herbivore impact may play an important role in maintaining the persistence of the diverse multiple-herbivore community of horsetnettle.

CONCLUSION

Horsetnettle's abundance, economic importance, and weedy nature have made it a favourite model species for studying the evolutionary ecology of native plant-herbivore communities (Wise 2007b). Importantly, there is nothing about the biology of horsetnettle or its herbivores that would make the results of this experiment applicable only to this system. Like horsetnettle, most plant species are attacked by multiple species of herbivores. Therefore, subadditive impacts like those demonstrated in this study

may be a common stabilizing element in plant-herbivore communities in general. By extension, nonadditive fitness impacts might be an important phenomenon in understanding the ecoevolutionary dynamics in any coevolving community of multiple interacting species.

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