

Genetic Architecture of Tolerance to Foliar Damage in a Salix Hybrid System

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Abstract The release of apical dominance following the browsing of stems can alter plant architecture and provide tolerance to herbivore damage. Meristem changes are not associated with foliar damage, so tolerance responses to foliar feeding may not be robustly expressed. In this study, tolerance to foliar damage was characterized for six genetic classes of willows in an interspecific hybrid system (pure *Salix eriocephala* plants, pure *S. sericea* plants, F₁ hybrids, F₂ hybrids, backcrosses to *S. sericea*, and backcrosses to *S. eriocephala*). This characterization allowed the genetic architecture of tolerance to be explored. *Plagioderia versicolora*, a small leaf beetle, was used to inflict foliar damage. Cuttings of genetically identical plants were matched to create a metric of biomass tolerance (defined here as the ratio of biomass for a damaged plant relative to an undamaged plant). Initial size differences between cuttings influenced the relative performance of damaged/undamaged pairs, so a ratio of the cuttings (damaged/undamaged) was calculated using initial wet weights. The ratio of cutting weight explained meaningful variance in biomass tolerance ($F_{1,68} = 71.4$; $P = 0.0001$; $r^2 = 0.51$). Residual variance in biomass tolerance (the variance remaining in biomass tolerance following removal of variance explained by the ratio of cutting weight) was used to reduce the effect of differences in initial plant size for damaged versus undamaged plants. Residual variance in biomass tolerance differed significantly among genetic classes ($F_{5,64} = 2.7$; $P = 0.03$). Using a Tukey *post-hoc* test, F₁ hybrids had significantly greater tolerance to foliar damage than backcrosses to *S. sericea*. Using line cross analysis, the model expressing the genetic architecture for tolerance in this hybrid system included additive genetic effects (*a*) + dominance–dominance epistasis (*dd*). For this willow system, tolerance to damage appears to be a fundamental response, whether damage is associated with browsing or foliar damage. Given the epistatic interactions observed in the current study, coupled together with the potential complexity of growth/storage traits associated with the mechanisms of tolerance, the trajectory for the evolution of tolerance challenges easy interpretations.

Keywords Compensation, Compensatory Response, Gene Interaction, Herbivory, Heterosis, Hybridization, Speciation

1. Introduction

Genetic variation in plant tolerance to damage often exists, suggesting that evolution of tolerance is a possible response to herbivores.[1,2,3,4,5] Although the majority of tolerance research has centered on herbaceous plants (see citations above), the greater apparency of woody perennials[6] should favor the evolution of increased plant tolerance to damage for woody species.[7] Moreover, woody plants accumulate and store resources over multiple years, favoring the use of stored resources to compensate for damage.

Studies have documented compensatory responses across a range of woody plant species.[8,9,10] However, type of herbivory may influence whether woody plants show complete compensation, overcompensation, or

undercompensation for damage.[11] For example, compensatory patterns may differ depending on whether damage is due to browsing of stems or restricted to foliar damage. Commonly, overcompensation to damage has been associated with a release of meristems after browsing.[2,12,13,14,15] Damage to apical meristems changes plant architecture by releasing apical dominance and increasing growth of axillary meristems, thereby increasing branching.[16] In contrast to apical browsing, foliar damage is not associated with the same change in meristem growth, so the compensatory response in such cases may not be as great for this damage type.

Species-specific effects for tolerance have also been documented for woody;[17,18] however, information is lacking on the genetic architecture of tolerance. Populations or species can differ from each other through additive, dominance, or epistatic genetic effects on a trait, and the relative contribution of these genetic differences (i.e., genetic architecture) can be estimated by comparing means among genetic classes (i.e., pure species and hybrids[19]).

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Additive genetic effects alone can explain a significant proportion of the evolutionary trajectory of a trait,[20] yet non-additive genetic effects are also commonly found to explain species/population differences. In such cases, non-additive genetic effects must be considered in models of population/species differentiation.[21,22,23,24,25]

Non-additivity can arise if selection acts to favor the co-evolution of groups of alleles at different loci, either independently of the environment (co-adapted gene complexes[26]) or in certain environments (local adaptation [27]). Such gene complexes are evident when hybridization leads to the disruption of these gene complexes (i.e., outbreeding depression). Parental genomes can interact unfavorably in hybrids, either through unfavorable epistatic interactions among loci or underdominance,[28] resulting in hybrid breakdown and selection against hybrids (e.g., F₂ hybrids of *Mimulus guttatus* and *M. nasutus*[29]). Alternatively, parental genomes can interact favorably in hybrids, perhaps through favorable epistatic interactions,[30] overdominance of alleles, or masking of deleterious alleles, resulting in hybrid vigor and selection for hybrids (e.g., F₂ hybrids across planting location for differing populations of *Chamaecrista fasciculata*[21]).

Salix sericea x *S. eriocephala* are eastern North American willow species that commonly form fertile hybrids. In this hybrid system, mammalian browsers (including white-tailed deer, rabbits, and meadow voles) can aggressively browse plants, removing up to 90% of shoot length.[31] Tolerance to browsing is high; F₂ hybrid plants express overcompensation with regard to biomass tolerance and complete compensation with regard to reproductive tolerance.[10] While tolerance to *browsing* can be important in this system, *defoliation* can also occur; beetle species can greatly impact plants, sometimes completely defoliating them (CGH, pers. obs.). Tolerance responses to folivory should be examined in this system to determine whether expression of plant tolerance can be generalized across browsing and foliar damage. In this current study, six genetic classes (two pure species, F₁ hybrids, F₂ hybrids, and certain backcross hybrids) were examined to characterize tolerance to foliar damage. The degree to which beetle damage reduced biomass in these genetic classes was quantified to determine whether genetic classes differed in plant tolerance to damage. In addition, the genetic architecture of tolerance was examined to clarify how tolerance differs genetically between the two pure species.

2. Methods

2.1. Study Site and Species

Salix sericea is a 0.5–4 m high shrub with lanceolate leaves and sericeous hairs on the lower leaf surface. This species commonly inhabit swamps from Canada through Northeastern USA and along the Appalachian range to Georgia. *S. eriocephala* reaches heights of 6 m and has

narrowly oblong leaves. *S. eriocephala* occurs from Canada through Virginia, and as far west as Missouri. *S. sericea* and *S. eriocephala* commonly co-occur in swamps and along streams in central New York. *S. sericea* and *S. eriocephala* are known to hybridize naturally.[32]

Plagioderia versicolora (Chrysomelidae) is a small leaf beetle with a metallic blue-black coloration.[33] Although these European beetles are not native to the US, they have occurred in North America for more than 100 years. *P. versicolora* inhabits much of New England and the Midwest. This multivoltine beetle feeds on *Salix* species (as well as *Populus* species) throughout all stages of its 23–25 day life cycle.

This study was conducted in 2004 and 2005 at the Sosnowski site; this site is a low-lying swamp about 3 km from Milford, Otsego County, NY, where previous studies on field and common garden plants have been conducted. [34,35,36,37] *S. sericea* and *S. eriocephala*, and their interspecific hybrids occur naturally at the site, along with two other willow species, *S. discolor* and *S. bebbiana*.

2.2. Experimental Design

Genetically pure plants (determined with RAPD markers[38]) were crossed in April of 1998 to create full-sib families of *S. eriocephala* (designated as P_E) and *S. sericea* (designated as P_S). Full-sib families of F₁ hybrids were created by crossing a female *S. sericea* plant with a male *S. eriocephala* plant. The reciprocal cross does not produce viable seeds due to a pre-mating incompatibility trait in female *S. eriocephala* plants.[39] Full-sib families of F₂ hybrids were created by crossing unrelated F₁ hybrids. Backcross full-sib hybrid families were created by crossing F₁ hybrid females with either male *S. sericea* plants (designated as BC_S) or male *S. eriocephala* plants (designated as BC_E). Males of pure species were used to create backcrosses because females of one pure species have the pre-mating incompatibility trait.

In March/April of 2004, multiple cuttings were taken from individual plants to create genetically identical clones, weighed to the nearest 10 mg, and grown in the Vassar College greenhouse.[10] For each of the six genetic classes, a minimum of 4 independent, full-sib families were used to avoid anomalous results associated with having only a single genotype. In June, individuals were transported to the field site, and genetically identical ramets were paired for size. Plants were paired because tolerance cannot be quantified as a property of an individual.[11] Instead, characterizing tolerance requires evaluating fitness of individuals across of range of damage levels and requires at least two plants (a damaged and an undamaged plant). Such was the case in this experiment; each pair of willows involved identical clones that included an undamaged control plant and an experimental plant that received damage.

Each individual was planted in a 3.7 liter pot with a soil mixture (4:1:1 topsoil, peat, vermiculite), given 26 g of slow release fertilizer (10:10:10 NPK), and maintained in a pot for

the remainder of the experiment. Plants were grown in a fenced common garden on gravel that was removed from established field willows to reduce colonization by non-experimental herbivores. Plants were randomly arranged in their pairs, and all plants were irrigated with drip hosing. To prevent herbivore damage, control plants were sprayed with Sevin®, a carbaryl insecticide. Each experimental plant was left unsprayed to allow *P. versicolora* to feed on its leaves.

To inflict damage on experimental plants, *P. versicolora* eggs and larvae were incrementally added to the willows starting in early June and going through mid-July; numbers placed on plants ranged from 60 to 130 individuals, with a mean of 94 ± 2.6 (\pm SE). Additions of *P. versicolora* were made on plants where foliar damage appeared to be less than 20%; beetles were removed from plants where damage appeared to be greater than 40%. By mid-July, all insects were removed and further damage was prevented by using Sevin® applications.

To assign damage estimates accurately to damaged plants, every leaf of all damaged plants was scored for leaf damage; using visual representations of differing damage levels, every leaf on each plant was scored either 0%, 5%, 10%, 20% or another 10% increment up to 100%. For each plant, percent damage was calculated as the average damage across all leaves on a plant. Plants that experienced less than 15% damage or greater than 55% were excluded from the experiment in order to provide a narrower range for the damage treatment. To prevent additional foliar damage all plants were treated with Sevin® throughout the rest of the 2004 season and until plant harvest in late July of 2005.

The majority of plants produced catkins, but reproductive responses were not examined because catkin production was low (approximately 35 catkins/plant) and quite variable (0 to 108 catkins/plant). Still, differences in biomass tolerance (defined here as the ratio of biomass for a damaged plant relative to an undamaged plant) can explain meaningful variation in reproductive tolerance (the ratio of reproductive output for a damaged plant relative to an undamaged plant),[10] making biomass tolerance a useful estimate of reproductive tolerance. Leaves, stems, and roots of harvested plants were divided into separate bags, dried in an oven at 60-70°C for 48 hours, and weighed 24 hours after being removed from the oven.

2.3. Analyses

After eliminating paired sets of plants with non-correctable errors in their data, with missing information, or that were not within the range of 15 to 55%, 13 P_E pairs, 12 P_S pairs, 10 F₁ pairs, 13 F₂ pairs, 11 BC_E pairs, and 11 BC_S pairs remained (70 pairs total). For each pair, biomass tolerance was defined as the proportional change in woody biomass (i.e., biomass of the stems and roots of a damaged plant divided by the biomass of the stems and roots of the undamaged plant). Other studies have calculated tolerance as the *difference* in performance between a damaged and undamaged plant,[4] but using proportional changes allows

the study to be framed in a reaction norm/phenotypic plasticity context.[11] Moreover, choice of scale can influence results;[40] both the damage treatment and tolerance were proportional in this study.

Percent damage varied across the 70 damaged plants, so percent damage was evaluated as a potential covariate of biomass tolerance. Percent damage was not predictive of biomass tolerance ($F_{1,68} = 1.4$; $P = 0.24$), so this variable was not used. Initial size differences between cuttings may also have influenced the relative performance of a damaged and undamaged pair, so a ratio of the cuttings (damaged/undamaged) was evaluated using initial wet weights. The ratio of cutting weight explained meaningful variance in biomass tolerance ($F_{1,68} = 71.4$; $P = 0.0001$; $r^2 = 0.51$). Therefore, residual variance in biomass tolerance (the variance remaining in biomass tolerance following removal of variance explained by the ratio of cutting weight) was used to reduce the effect of differences in initial plant size for damaged versus undamaged plants.

Because relative root:shoot ratio (the root:shoot ratio of the damaged plant divided by that of the undamaged plant) was one trait found to predict tolerance in this willow system,[10] relative root:shoot ratio was calculated for plant pairs in this system. For genetically identical pairs where relative root:shoot ratio < 1, the damaged plant invests relatively more of its resources into shoot tissue and away from root tissue than the undamaged plant. This reallocation response was predicted to be associated with greater tolerance to damage than a response where the relative root:shoot ratio > 1 (e.g., where a damaged plant shifts fewer resources from roots to shoots compared to an undamaged plant). The ratio of cutting weight did not significantly influence relative root:shoot ratio ($F_{1,68} = 0.14$; $P = 0.71$), so raw data were used in the analyses for relative root:shoot ratio.

Instead of analyzing biomass tolerance directly, residual variance in biomass tolerance was examined using ANOVA to determine differences in biomass tolerance among the six genetic classes because the initial size of cuttings influenced the final biomass of plants.[41] ANOVA was also performed to evaluate differences among the six genetic classes for relative root:shoot ratio. Regression analysis was performed to evaluate whether relative root:shoot ratio influenced biomass tolerance. Tukey multiple comparison tests were used in all appropriate analyses.

Genetic architecture of tolerance—Line cross analyses were used to evaluate the additive and non-additive genetic differences in residual variance in biomass tolerance between *S. eriocephala* and *S. sericea* (using least square means[19]). *S. eriocephala* and *S. sericea* are assumed to be completely homozygous for alleles that control tolerance. These analyses test the extent to which additive, dominance, and epistatic interactions explain the deviation of mean tolerance of these species from a theoretical mean of a population with random segregation and assorting of all alleles (F_{∞} population).

We tested for significance of five genetic composite

effects [additive (a), dominance (d), additive-x-additive epistasis (aa), additive-x-dominance epistasis (ad), dominance-x-dominance epistasis (dd)] on residual variance in biomass tolerance. An additive composite effect (a) reveals a net difference between the two species in the additive effects of their genes. The dominance composite effect (d) reveals whether genes in one species are dominant on average over genes in the other species. Epistatic composite effects (aa , ad , dd) reveal net epistatic interactions between genes from the two species. There were 32 possible linear models for estimation of five genetic composite effects of the following form:

$$\begin{aligned} \bar{Y}_i = m + x_a [a] + x_d [d] + x_{aa} [aa] \\ + x_{ad} [ad] + x_{dd} [dd] + e_i, \end{aligned} \quad (1)$$

where \bar{Y}_i is the mean of the i th genetic class (species or hybrid), m is the overall mean, $[a]$, $[d]$, $[aa]$, $[ad]$, and $[dd]$ are the various genetic composite effects, x_a , x_d , x_{aa} , x_{ad} , and x_{dd} are the regression coefficients, and e_i is the residual error. The regression coefficients are based on the expected mean of F_∞ offspring as the intercept (versus F_2). [19,42] This allows for the mean in linear models to represent the mean after numerous generations of inbreeding. Composite effects were estimated using a S-Plus® program [43] that used a weighted least-squares model: [19]

$$\hat{a} = (\mathbf{M}^T \mathbf{V}^{-1} \mathbf{M})^{-1} \mathbf{M}^T \mathbf{V}^{-1} \mathbf{z} \quad (2)$$

$$\mathbf{C} = (\mathbf{M}^T \mathbf{V}^{-1} \mathbf{M})^{-1} \quad (3)$$

$$\mathbf{y} = \mathbf{M} \hat{a} \quad (4)$$

where \hat{a} is the vector of m , a , d , aa , ad , and dd , \mathbf{M} is the matrix of coefficients for these parameters from equations for predicted means (of each parental species and hybrid), \mathbf{M}^T is the transpose of \mathbf{M} , \mathbf{V} is the diagonal matrix of squared standard errors of means, \mathbf{z} is the vector of observed means, \mathbf{C} is the diagonal matrix of sampling errors of the elements in \hat{a} , and \mathbf{y} is the vector of predicted means. Thus, parameters (m , a , d , aa , ad , dd) were estimated from six means and standard errors (P_E , P_S , F_1 , F_2 , BC_E , BC_S).

Weighted residual sums of squares (RSS_w) were used to test goodness of fit of linear models:

$$RSS_w = \sum_{i=1}^k \frac{e_i^2}{SE_i^2} \quad (5)$$

where k is the number of genetic classes (6), e_i is the difference between the observed and predicted i th composite genetic effect ($i = a, d, aa, ad, \text{ or } dd$), and SE_i is the standard error of the i th estimated composite genetic effect. [19,44,45] RSS_w is χ^2 distributed if data are normally distributed (degrees of freedom = k - number of parameters in model); if significant, the model is inadequate to explain the observed means of the genetic classes.

Akaike's Information Criterion (AIC) was used to find the most parsimonious model, which is the best compromise

between the amount of variance explained and the number of parameters in the model. $AIC = RSS_w + 2K + \text{constant}$, where K is the number of parameters fitted into the model and the constant is equivalent for all models and dropped when comparing AIC scores among models. [45] The model with the lowest AIC score is the most parsimonious. We did not use sequential addition of parameters into the model (i.e., first additive effects are added, then dominance, and then epistasis) [46] because the ability to detect significant parameters that are added later in the model is affected by the order in which parameters are added. In addition, regression models that contain only parameters explaining most of the variance have parameter estimates that are more exact. [45]

A likelihood ratio test was used to test whether the removal of individual parameters significantly reduced the fit of the model. [19] The degree of reduced fit of the model is estimated as $\Lambda = RSS_w(\text{reduced model}) - RSS_w(\text{full model})$ and is χ^2 distributed with degrees of freedom equal to the number of parameters in the full model minus the number of parameters in the reduced model.

3. Results

For plants in the damage treatment, percent damage was constrained between 15-51%, with a mean of 30%. Damage did not differ significantly among genetic classes ($F_{5,64} = 1.8$; $P = 0.12$) (Table 1). Similarly, the ratio of cutting weight did not differ significantly among genetic classes ($F_{5,64} = 2.1$; $P = 0.08$) (Table 1).

Table 1. Trait Means (\pm SE) for Six Genetic Classes of Willow Plants. *Salix eriocephala* plants and their backcrosses are designated as P_E and BC_E ; F_1 hybrids and F_2 hybrid plants are designated as F_1 and F_2 ; *S. sericea* plants and their backcrosses are designated as P_S and BC_S . F-values and P-values represent statistical comparisons among genetic classes

Trait	P_E	BC_E	F_1	F_2	BC_S	P_S	F	P
Damage (%)	29 (2.5)	33 (2.8)	25 (1.9)	35 (2.9)	29 (3.2)	27 (2.7)	1.8	0.12
Ratio of cutting weight	1.2 (0.1)	1.2 (0.2)	1.0 (0.2)	0.9 (0.1)	0.8 (0.1)	1.1 (0.1)	2.1	0.08
Biomass tolerance	1.1 (0.1)	1.1 (0.1)	1.1 (0.1)	1.0 (0.1)	0.8 (0.1)	0.9 (0.1)	NA	NA
Residual variance in biomass tolerance	0.04 (0.06)	0.00 (0.05)	0.14 (0.05)	0.06 (0.05)	-0.13 (0.08)	-0.08 (0.04)	2.7	0.03

Biomass tolerance ranged from 0.4 to 1.9 across all plants, with a mean tolerance of 1.0 ± 0.04 (\pm SE), indicating complete compensation for damage. Residual variance in biomass tolerance also ranged greatly among genetic classes, with F_1 hybrids having the greatest expression of tolerance (0.14 ± 0.05) and BC_S plants having the least (-0.13 ± 0.08), for this measure. Residual variance in biomass tolerance differed significantly among genetic classes ($F_{5,64} = 2.7$; $P = 0.03$) (Table 1). Using a Tukey *post-hoc* test, F_1 hybrids had significantly greater tolerance to foliar damage than BC_S plants (Figure 1). In contrast, relative root:shoot ratio did not differ significantly among genetic classes ($F_{5,64} = 1.2$; $P = 0.30$), even though it ranged from 0.4 to 1.9, with a mean ratio of 1.00 ± 0.04 .

When residual variance in biomass tolerance was used to

examine genetic architecture of tolerance, the model including the mean, additive genetic effects (a) + dominance – dominance epistasis (dd) was most parsimonious in explaining differences between P_E and P_S [$\chi^2 = 3.65$; composite effects (\pm SE): $-0.03 (\pm 0.03) + 0.06 (\pm 0.03) + 0.16 (\pm 0.06)$ for mean, a , and dd , respectively]. However, dropping additive genetic effects did not significantly reduce the fit of the model to the data.

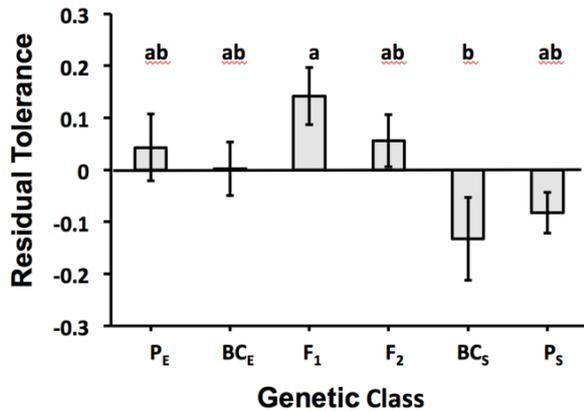


Figure 1. Bar graph representing mean residual variance in biomass tolerance (\pm SE) for six genetic classes of willow plants. *Salix eriocephala* plants and their backcrosses are designated as P_E and BC_E ; F_1 hybrids and F_2 hybrid plants are designated as F_1 and F_2 ; *S. sericea* plants and their backcrosses are designated as P_S and BC_S . Different lower case letters designate significant differences among genetic classes

4. Discussion

Overall, plants in this study showed full compensation to foliar damage, suggesting that tolerance traits provided the ability to compensate for damage, but not to overcompensate for damage. Biomass tolerance to foliar damage ranged greatly for willow plants, with several genetic classes presenting patterns of overcompensation (P_E , F_1 hybrids, and BC_E plants all showed biomass tolerance measures of 1.1), while F_2 hybrids showed complete compensation, and other genetic classes displayed patterns of undercompensation (P_S had a measure of 0.9, and BC_E plants had a measure of 0.8). In a prior study, Hochwender *et al.* also examined willow tolerance; [10] however, that study differed in three important ways: (1) only F_2 hybrids were examined for tolerance in the earlier study; (2) the previous study examined tolerance to *browsing* damage, while this current study examined tolerance to *foliar* damage, and (3) the former study used experimental plants that grew in the ground for two full years, while the present study grew plants in pots for just 1.5 years. The results from this current study are similar to those from the previous study in that both showed a high degree of tolerance (complete compensation and overcompensation). Nevertheless, in the earlier study, F_2 hybrids had a greater mean tolerance— 1.2 ± 0.05 (\pm SE)—than the F_2 hybrids here. While the differences in tolerance may be due to damage type, browsing versus foliar damage, the differences in the factors mentioned above may

also have played an important role. The expression of tolerance can be influenced by environmental factors that limit plant growth, [47] so a response of full compensation instead of overcompensation is not surprising for this current study. Overall, results suggest that plant tolerance to damage is a fundamental response to herbivore damage in this willow system, whether herbivore damage is associated with browsing or foliar damage. This outcome is also notable because it suggests that compensatory responses include traits other than those associated with meristem release following browsing.

Changes in relative root:shoot ratio did not provide a mechanism by which biomass tolerance was expressed. This finding differs from the earlier study. [10] In that study, relative root: shoot ratio did explain significant variation in tolerance for F_2 hybrids; plants responded by shifting more resources to shoot growth following damage compared to undamaged plants. Assuming that the differences between the two experiments were caused by damage type, our findings suggest that allocation responses are insensitive to foliar damage but sensitive to browsing damage. If true, the detection of relative root:shoot ratio as a mechanism in the browsing experiment (and the stronger tolerance response observed for browsing damage) may have been because browsing causes changes in apical meristems and plant architecture, but foliar damage does not appear to cause these changes. While relative root:shoot ratio did not provide a mechanism of tolerance for foliar damage, an alternative reason for this lack of response could be environmental conditions. Specifically, pot constraints in this current experiment may have limited the degree to which plants were initially able to invest in roots, so changes in allocation patterns from those initial root:shoot ratios were limited. Still, our study may have been constrained by sample size; in this study, only 70 pairs of plants were used (and those pairs included six genetic classes) whereas the previous study involved nearly twice as many pairs (and all pairs were from the same genetic class).

Hybridization has the potential to alter gene combinations with beneficial or detrimental effects on the phenotype. The hybrid fitness that results depends on a balance between the loss of favorable and unfavorable interactions within parental genomes. [48] Differences in tolerance to foliar damage were observed among the six genetic classes, but only F_1 hybrids and BC_S plants differed significantly (for residual variance in biomass tolerance). Even so, this outcome suggests that genetic variance in ability to tolerate foliar damage exists in this hybrid system. Moreover, line cross analyses supported the argument that non-additive genetic effects contributed to interspecific differences between means. For residual variance in biomass tolerance, F_1 hybrids had a significantly higher value than expected from a purely additive model of inheritance (0.14 ± 0.05 versus the predicted value of -0.02 ; one sample t-test using -0.05 as μ : $t_9 = 2.9$; $P = 0.02$). This outcome suggests favorable gene interactions—overdominance or masking of deleterious alleles. Indeed, non-additive genetic effects

(dominance x dominance) contributed to interspecific differences between means, suggesting heterosis for tolerance. In contrast, BC_S had the lowest mean residual variance in biomass tolerance of all six genetic classes; although this mean was not significantly different from the means of pure species, this result is noteworthy. In recombinant hybrids (like BC_S), epistatic interactions existing in pure species can become disrupted upon hybridization. If this disruption causes lower tolerance in recombinant hybrids than in the pure species, coadapted gene complexes may have been present in pure species. Such coadapted gene complexes can arise through selection that favors the coevolution of alleles at different loci.[26]

Two other studies that have examined this hybrid system shed light regarding the expression genetic architecture of tolerance. One study, which used line cross analysis to examine traits associated with plant growth, found that the expression of genetic architecture was contingent upon nutrient environment for root:shoot ratios (at least for female willow plants) (Czesak *unpublished data*). A second study found that the expression of non-additive genetic effects could alter the expression of shoot biomass and reproductive output among genetic classes.[23] In this second example, nonadditive genetic effects involving hybrid breakdown (and selection against hybrids) was expressed in most garden plots; however, in one plot, evidence supported non-additive genetic effects involving overdominance of alleles or masking of deleterious alleles, resulting in hybrid vigor. Given the potential complexity associated with the genetic architecture of growth/storage traits (i.e., potential mechanisms of tolerance), the epistatic interactions observed in the current study challenge easy interpretations regarding the trajectory for the evolution of tolerance. Models of selection often assume absent non-additivity, yet our ability to understand speciation based such models can be limited if non-additivity complicates responses to selection.[22] As the genetic composition of a population changes over time, non-additivity can change the additive effects of alleles, potentially changing which alleles are favored by selection[49] and thereby influencing the outcome of selection. Epistatic variance can also be converted to additive genetic variance, causing genetic variance - covariance matrices to change in response to selection.[50] Thus, the genetic factors responsible for past divergence of species may be difficult to resolve if non-additivity is ignored.

5. Conclusions

Our findings suggest that plant tolerance to damage is a fundamental response to herbivore damage in this system, whether herbivore damage is associated with browsing or foliar damage. Biomass tolerance to foliar damage ranged greatly, with several genetic classes presenting patterns of overcompensation. In addition, the results from line cross analysis suggest that both favorable gene interactions and

negative epistatic interactions influenced expression of tolerance. Given the epistatic interactions observed, as well as the potential complexity associated with growth/storage traits (i.e., potential mechanisms of tolerance), predicting the evolutionary trajectory for tolerance will be challenging.

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