



## Using hybrid systems to explore the evolution of tolerance to damage

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**Abstract.** Hybridization is common and important to the adaptive evolution of plants. Hybridization has resulted in the formation of new species and the introgression of traits between species. This paper discusses the advantages of using hybrid systems to explore the evolution of tolerance to herbivore damage (i.e., the ability to diminish the negative effects of damage on fitness). The major consequence of hybridization likely to make it influential for tolerance evolution is that hybridization generates broad variation in traits that can be selected for or against. In addition to generating greater variation in tolerance to damage and its putative traits (e.g., traits associated with allocation patterns and meristem production), hybridization can generate greater independence among tolerance traits and between tolerance and defense traits. Greater independence may provide a greater ability to discern mechanisms of tolerance, give a greater probability of detecting allocation costs of tolerance, and provide an effective means to evaluate tradeoffs between tolerance and defense. Interspecific hybrid systems can also be used to evaluate the importance of co-adaptation of tolerance traits. Moreover, recombinant hybrids can be used in selection studies focusing on tolerance to damage to discern whether parental combinations of tolerance traits are favored over novel combinations. Research in hybrid systems that investigate the selective importance of tolerance, the patterns of inheritance of tolerance traits, and the genetic architecture of plant species involved can be vital to our evaluation of the adaptive role of tolerance to damage.

**Key words:** allocation costs, chemical defense, epistasis, genetic architecture, hybridization, selection experiments, tolerance to damage, tradeoffs

### Introduction

Hybridization between species or subspecies is common in nature (Stebbins, 1959; Grant, 1981; Harrison, 1993; Rieseberg *et al.*, 1996; Arnold, 1997), and has often been used as a tool to explore the process of species formation and maintenance of species boundaries (Barton and Hewitt, 1985; Hewitt, 1988). Not surprisingly, studies examining hybridization and the maintenance of

species boundaries have focused on systems in which hybrid individuals are unfit relative to parental species (Barton and Hewitt, 1985; Hewitt, 1988). This has led to the general perception that hybridization is a maladaptive event (Arnold, 1997). However, recent evidence suggests that hybrid fitness is variable. Hybrids can have intermediate fitness to their parental species (Rieseberg and Wendel, 1993; Arnold and Hodges, 1995), have equal or higher fitness than parental species (Graham *et al.*, 1995; Emms and Arnold, 1997; Burke *et al.*, 1998), or have lower fitness (Millar, 1983; Heywood, 1986). In addition, the relative fitness of parental species and hybrid classes can depend upon environmental context (Wang *et al.*, 1997). Thus, instead of viewing hybridization as a “violation of the basic aspects of divergent evolution . . . it may be more constructive to view it as a creative and ongoing process in the evolutionary history of numerous groups of organisms” (Arnold, 1997). Shifting the paradigm of interspecific hybridization from a framework wherein hybridization is considered only to be a maladaptive event to one in which hybridization is also regarded as potentially adaptive can free evolutionary ecologists to use a powerful tool for examining trait evolution.

Natural hybridization can influence the evolution of parental species by altering genetic diversity of parental species through introgression (Keim *et al.*, 1989; Rieseberg and Wendel, 1993; Rieseberg, 1995; Arnold, 1997). Furthermore, hybridization can play an important role in adaptive evolution through the creation of new plant species (Grant, 1981; Stace, 1987; Arnold, 1997). The major consequence of hybridization likely to make it influential for plant evolution is that hybridization generates broad variation in traits and combinations of traits, thereby enhancing the potential for adaptation (Lewontin and Birch, 1966; Stebbins, 1973). Indeed, this variation is almost synonymous with hybridization (Grant and Grant, 1992; Rieseberg and Wendel, 1993; Arnold and Hodges, 1995; Rieseberg and Carney, 1998; Orians, 2000).

The variation generated through hybridization may be effectively utilized to study the adaptive responses of plants to herbivores. Because herbivores commonly decrease plant fitness, herbivores are potentially an important selective force affecting host plant traits (Marquis, 1992). Consequently, natural selection should increase the frequency of plant traits that reduce damage by herbivores. Of studies investigating plant-herbivore interactions, the majority of research has focused on specific traits that provide a defensive function against plant enemies (i.e., traits that decrease the amount of damage incurred by a plant) (Berenbaum and Zangerl, 1992; Herms and Mattson, 1992). However, plants also possess traits that provide tolerance to damage (i.e., traits that allow plants to diminish the negative impact that damage can have on fitness). In the agricultural literature, tolerance to damage has long been recognized to be as important as defense in contributing to plant protection (Painter, 1968; Smith, 1989). Recently, tolerance has also begun to receive

more attention among evolutionary ecologists (Simms and Triplett, 1994; Fineblum and Rausher, 1995; Mauricio *et al.*, 1997; Stowe, 1998; Tiffin and Rausher, 1999; Hochwender *et al.*, 2000; Stowe *et al.*, 2000).

Applying general evidence regarding hybridization, which has been gleaned from a variety of hybrid studies, as well as utilizing results from our current studies using a hybrid willow system, we address the potential advantages of using hybridization as a tool to explore tolerance to damage. We categorize the advantages into four topics and discuss each. Firstly, hybridization can potentially generate greater variation in tolerance and its putative traits. Secondly, hybridization may generate greater independence among tolerance traits, as well as between tolerance and defense traits. Greater independence would provide a greater ability to discern mechanisms of tolerance, give a greater probability of detecting allocation costs of tolerance, and provide an effective means to evaluate tradeoffs between tolerance and defense. Thirdly, interspecific hybrids offer insight into the patterns of inheritance for tolerance. Particularly, the importance of co-adaptation of tolerance traits can be evaluated. Finally, recombinant hybrids can be used in selection studies focusing on tolerance to damage. Such studies can be used to discern whether only certain gene combinations are favored by selection (e.g., whether parental combinations of tolerance traits are favored over novel combinations).

#### *Creating variation in tolerance*

One important prerequisite for a population to respond to natural selection is that genetic variation for a given trait must exist. Both directional and stabilizing selection, however, reduce genetic variation for any trait upon which selection is acting. Moreover, recent models examining tolerance to damage suggest that positive feedback between tolerance and plant enemies should result in fixation of tolerance traits (Roy and Kirchner, 2000; Tiffin, 2000), thereby eliminating all variation in tolerance. Thus, when studies explore a single lineage, results may provide only limited insight into the evolution and mechanisms of tolerance to damage, as well as the costs associated with tolerance. Comparative studies using several species can also be problematic because the evolutionary histories of different species will be distinctly unique in ways unknown to the researcher. An alternative approach that can overcome both of these problems is to use plants from a hybrid complex (Anderson, 1939; Lewontin and Birch, 1966). By generating recombinant hybrid individuals, researchers can potentially generate greater variation in tolerance to damage through segregation and recombination, while also maintaining a evolutionary history that is consistent across individuals.

Because interest in adaptive evolution in hybrid systems has just recently gained momentum (Rieseberg and Wendel, 1993; Arnold and Hodges, 1995;

Arnold, 1997; Rieseberg and Carney, 1998), as has interest in the evolution of tolerance to damage (Simms and Triplett, 1994; Fineblum and Rausher, 1995; Stowe, 1998; Hochwender *et al.*, 2000; Stowe *et al.*, 2000), no empirical evidence has documented how much variation in tolerance to damage exists for recombinant hybrids compared to parental species. In general, though, the distribution of a trait is expected to show greater variance for recombinant F<sub>2</sub> hybrids than for either parental species or for F<sub>1</sub> hybrids, whether the trait expressed involves one or several genes (Mather and Jinks, 1982). Experimental hybridization of isolated, intraspecific animal and plant populations concur with this expectation; increased variance is commonly found for traits in F<sub>2</sub> hybrid populations compared to F<sub>1</sub> and parental populations (Endler, 1977; Mather and Jinks, 1982; Burdon, 1990a, b; Edmands, 1999). For example, observed patterns for plant height using intraspecific hybrids of *Nicotiana rustica* support this expectation (Mather and Jinks, 1982). Furthermore, interspecific F<sub>2</sub> hybrids of *Nicotiana* and *Viola* expressed a much broader range in floral or foliar traits than either parental species (Brainerd, 1924; Anderson, 1939). Rieseberg and Ellstrand (1993) also found that morphological characters expressed in later generation hybrids exhibited great variation.

Plant physiological traits are particularly relevant when examining whether variation in tolerance is increased through hybridization, as allocation of carbohydrates, proteins (and therefore nitrogen), and phosphorous are all important physiological functions that can affect how plants tolerate damage (e.g., Barber *et al.*, 1996; Marquis *et al.*, 1997; Louahlia *et al.*, 1999). No studies exist that specifically test whether hybridization increases variation in these physiological traits. However, several lines of evidence suggest that hybridization may alter variation in plant physiology and therefore variation in tolerance. Firstly, interspecific hybrids of *Populus* demonstrated dramatic differences in how biomass was partitioned among leaves, branches and roots (Scarascia-Mugnozza *et al.*, 1997). Moreover, hybrid *Populus* plants varied greatly in translocation patterns (Scarascia-Mugnozza *et al.*, 1999). Secondly, in an *Artemisia* hybrid system, F<sub>2</sub> progeny were significantly more variable than either parental subspecies for biomass production (Messina *et al.*, 1996), suggesting that hybridization can increase variance in plant growth and biomass accumulation. Finally, F<sub>2</sub> hybrid plants generally showed greater variation than F<sub>1</sub> hybrids and parental species for another physiological trait, secondary chemicals (Orians, 2000; see the following section for more detail). Taken together, these lines of evidence suggest that variability in tolerance to damage by F<sub>2</sub> hybrids can be expected to be greater than tolerance expressed by parental species. Thus, recombination in interspecific hybrids may eliminate some drawbacks that single-lineage evolution places on natural selection, thereby potentially providing a more effective evaluation of the evolution of tolerance.

*Detecting tolerance mechanisms, allocation costs, and tradeoffs with defense*

Even if hybridization did not increase variability in tolerance and its traits, recombination in interspecific hybrids would have a second, possibly more important effect – a tolerance trait can become dissociated from other tolerance traits through segregation and recombination during hybridization. Provided that species have large numbers of chromosomes, most pairs of segregating loci will be on different chromosomes, diminishing the effects of linkage (Lynch, 1991). Therefore, multicollinearity among traits that confer tolerance will be reduced. Often, multicollinearity can limit the ability to determine the importance of individual factors for explaining a response variable (Philippi, 1993). Consequently, interspecific hybridization can provide a greater ability to determine the independent contribution of traits to tolerance and to ascertain the costs associated with those tolerance traits.

Evaluating secondary metabolites may also sharpen our understanding of the evolution of plant tolerance to damage (see Mauricio, 2000). In hybrid systems, chemical compounds are often inherited additively in interspecific F<sub>1</sub> hybrids (reviewed by Rieseberg and Ellstrand, 1993; Orians, 2000). In cases where two hybridizing species differ in their chemical constituents, additive patterns of inheritance should result in F<sub>2</sub> hybrid plants expressing greater variance for defensive compounds than either parental species. In studies addressing this question, F<sub>2</sub> hybrids have been found to be quantitatively more variable than F<sub>1</sub> hybrids (Orians, 2000). If both chemical defenses and tolerance mechanisms (e.g., storage of nitrogen or carbohydrates) incur allocation costs (*sensu* Simms, 1992), then the resources available for tolerance would be inversely proportional to resources used by defenses (Simms and Triplett, 1994). Therefore, increased variation in investment to secondary chemistry in recombinant hybrids could also increase the variance in tolerance.

In our hybrid system, *Salix sericea* produces high concentrations of phenolic glycosides and low concentrations of condensed tannin, while *S. eriocephala* produces high concentrations of condensed tannin, but no phenolic glycosides (Orians and Fritz, 1995). For naturally occurring hybrids (hybrids of unknown pedigree), the concentrations of both phenolic glycosides and condensed tannins were intermediate (Orians and Fritz, 1995). Similarly, condensed tannins of F<sub>1</sub> hybrids did not show a deviation from the mid-parent value, suggesting additive inheritance for these secondary compounds (Orians *et al.*, 2000). However, the concentration of phenolic glycosides for F<sub>1</sub> hybrids was lower than the midpoint of the parental species, indicating incomplete dominance (i.e., possible recessive control at one or more alleles controlling phenolic glycoside production) (Orians *et al.*, 2000). In addition, F<sub>1</sub> hybrids showed a negative correlation between tannin concentration and phenolic glycoside concentration (Orians *et al.*, 2000), suggesting that a tradeoff caused by

resource allocation costs for both defenses may occur. Finally, F<sub>1</sub> hybrids showed greater variation in expression of chemical phenotypes than did either parental species for both tannin concentration and phenolic glycoside concentration (Orians *et al.*, 2000).

We have found variation in chemical expression of phenolic glycosides in F<sub>2</sub> seedlings to be exceptionally great, as well; some F<sub>2</sub> seedlings expressed less than 10 mg/g of salicortin, while others expressed nearly 90 mg/g (Fig. 1). Thus, F<sub>2</sub> variability spanned almost the entire range of concentrations expressed by the parental species. Not surprisingly, F<sub>2</sub> hybrids showed significantly greater variance in phenolic glycosides than did F<sub>1</sub> hybrids or *S. sericea* plants (variance tests:  $F_{79,69} = 2.2$ ;  $p < 0.005$  and  $F_{79,37} = 1.7$ ;  $p < 0.05$ , respectively) (Fig. 1).

A great benefit of creating F<sub>2</sub> hybrids may therefore be that F<sub>2</sub> plants will experience recombination. For plants with a high haploid chromosome number, such as our system ( $n = 19$  for both *S. sericea* and *S. eriocephala*), many intra-specific gene combinations can be broken up, causing the creation of more trait combinations. With respect to defensive chemicals, the findings from our system, as well as findings from other hybrid systems, support this viewpoint. Greater independence of tolerance traits may also be the rule in F<sub>2</sub> hybrid populations. In addition, greater independence between tolerance traits and defense traits may be achieved through recombination in interspecific hybrid plants. Greater independence of the traits that confer tolerance and traits that confer defense can potentially increase the range over which tradeoffs between these two strategies would be evaluated. Thus, by evaluating F<sub>2</sub> hybrids, we may have an increased chance of detecting tradeoffs between defense and tolerance.

As we mentioned, a negative correlation between defense and tolerance could be due to underlying allocation costs (i.e., due to antagonistic pleiotropy) or it could be due to linkage disequilibrium caused by correlational selection.

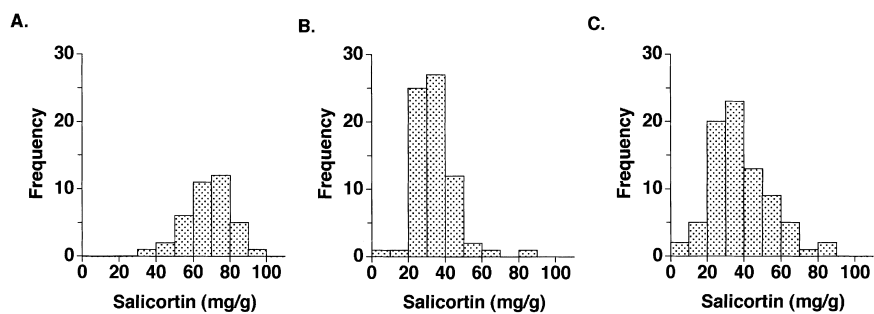


Figure 1. Frequency distributions for salicortin (a phenolic glycoside) concentration for three genetic classes of willow. (A) *S. sericea*. (B) F<sub>1</sub> hybrids. (C) F<sub>2</sub> hybrids. *S. eriocephala* has no phenolic glycosides in its leaves.

To the extent that recombination would disrupt negative correlations between tolerance and defense, tradeoffs detected between tolerance and defense would be due to underlying allocation costs for each strategy. Recombinant hybrid plants, then, can provide an useful evaluation of fundamental constraints that exist between these alternative strategies.

#### *Inheritance of tolerance to damage*

Detailing the genetic architecture of tolerance to damage can clarify the evolution of tolerance. Using methods developed by Fritz *et al.* (1994, 1996), inheritance patterns of tolerance to damage can be detailed when just parent species and F<sub>1</sub> hybrids are examined. For example, in the *Salix* hybrid system that we study, several mammals often browse willow plants heavily during the winter, potentially making tolerance to damage an important evolutionary response to herbivores in this system. (For general information regarding this hybrid system, see Fritz *et al.*, 1998.) To examine tolerance to damage, we paired plants for size after one year of growth for 9–10 full-sib families for *S. eriocephala*, *S. sericea*, and their interspecific F<sub>1</sub> hybrids. One of each pair was randomly chosen as an undamaged control plant. Using the other plant, we simulated browsing damage by clipping the upper 50% of main stem and 50% of all side shoots (approximately  $30.6 \pm 0.2\%$  (mean  $\pm$  SE) of the above ground biomass). Using a pair of contrasts and visual inspection of its graphical pattern (Fritz *et al.*, 1996), we analyzed patterns of inheritance of tolerance to damage for this hybrid complex. Plant size typically influences probability of survival, especially during early stages (Arendt, 1997), so our use of biomass as a estimate of fitness for these year old woody perennials is reasonable. In addition, plant size is positively correlated with catkin production during the first years of growth for these species (unpublished data), further supporting our use of biomass to measure tolerance.

For both parental species, *S. eriocephala* and *S. sericea*, damaged plants produced less biomass ( $95.7 \pm 2.9$  g (mean  $\pm$  SE) and  $79.6 \pm 2.8$  g, respectively) than undamaged plants ( $100.2 \pm 2.3$  g and  $83.7 \pm 2.7$  g, respectively), suggesting that parental species expressed under-compensating tolerance to damage (Fig. 2). In contrast, for F<sub>1</sub> hybrids, damaged plants produced more biomass than undamaged plants ( $87.5 \pm 3.0$  g and  $84.2 \pm 3.2$  g, respectively), suggesting over-compensating tolerance to damage (Fig. 2). The F<sub>1</sub> hybrid vs. mid-parent contrast was significant ( $F_{1,185} = 7.5$ ;  $p = 0.007$ ), indicating heterosis for tolerance. Our second contrast was one between the F<sub>1</sub> hybrids and *S. sericea*, the parent with the nearest mean. This second contrast was also significant ( $F_{1,157} = 5.1$ ;  $p = 0.03$ ), suggesting that dominance or epistatic interactions may have been involved (Lynch, 1991; Edmands, 1999). If alleles expressing dominance provided tolerance, introgression of tolerance traits is

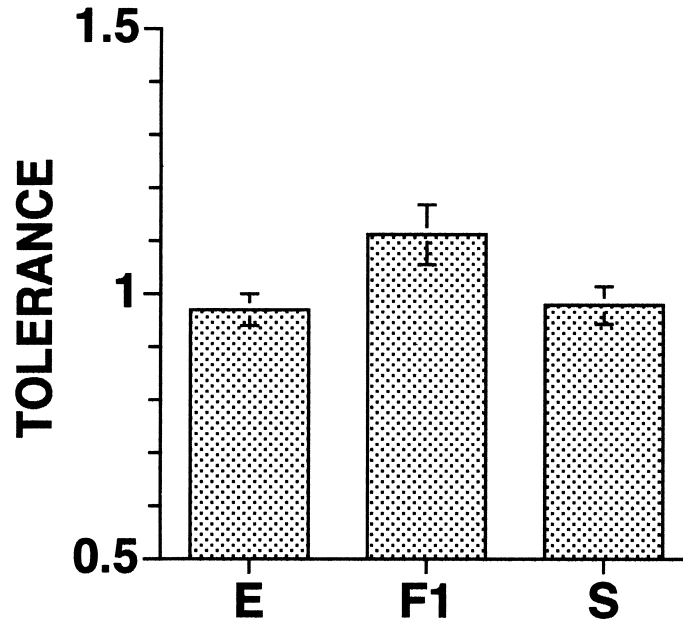


Figure 2. Tolerance (measured as the ratio of biomass for damaged to undamaged plants paired by size) of hybrid and parental willow species. E = *S. eriocephala*, S = *S. sericea*, F1 = F<sub>1</sub> hybrids. Error bars =  $\pm 1$  SE.

feasible. In other words, if F<sub>1</sub> hybrids showed dominance to *S. sericea* or if traits for tolerance were due to dominant alleles for both species (i.e., if hybrid vigor occurred due to the masking of deleterious recessive alleles), then introgression of traits that confer tolerance would be feasible. If the traits that conferred tolerance in our F<sub>1</sub> hybrids involved epistatic interactions instead of dominant traits, however, the genetic background within which alleles are expressed will be of much greater importance. Clearly, distinguishing the mode of inheritance is important to understanding the evolution of tolerance to damage.

In cases where recombinant hybrid plants (e.g., F<sub>2</sub> and backcross hybrids) are incorporated into the experimental design, more complex genetic analyses can be performed, thereby providing a means to evaluate the importance of additive, dominance, and epistatic effects for tolerance to damage (see Lynch and Walsh, 1998). If tolerance represents an adaptation to selection by herbivores, then evidence for evolution of different tolerance strategies among species (or among subspecies, races, or populations) can be determined by documenting whether epistatic effects are important for tolerance. In our own research, evaluating resistance to herbivores, we have found large and varied epistatic effects (R.S. Fritz *et al.*, unpublished data), leading us to expect that

epistatic components are also likely to be found for tolerance. With regard to studying the evolution of plant tolerance to damage, such analyses could provide insight into how tolerance evolves as a function of evolutionary divergence, population subdivision, and population distribution across environmental conditions (e.g., across environments that vary in predictability or intensity of herbivory).

#### *Selection experiments*

Because interspecific hybridization can generate a broader range of phenotypes than a single parental species can, hybrid complexes almost beg to be used as an experimental approach to examine trait selection. The general question that can be addressed by such studies is, which sets of gene combinations are favored by selection. Are parental combinations of genes favored over novel combinations? Or do certain novel assortments of genes have greatest fitness? Although such methods have been ignored by researchers examining tolerance to damage, research in other fields suggest that the potential for the evolution of tolerance via hybridization exists.

In a study by Burke *et al.* (1998), epistatic interactions were found to play a major role in determining F<sub>2</sub> hybrid viability in crosses between two *Iris* species. Their findings revealed a general pattern of parental-like genotypes being favored over intermediate hybrid genotypes. In some cases, though, beneficial epistatic interactions caused the heterozygous condition to be favored over the parental condition, suggesting that recombination can provide a basis for adaptive evolution. The benefit observed depended upon which species contributed cytonuclear material. Thus the probability of adaptive evolution via introgression may not be reciprocal in this system (one *Iris* species may be less permeable to introgression than the other).

Rieseberg *et al.* (1996) give a second example where positive epistatic interactions may provide a basis for adaptive evolution, in this case leading to the generation of a new species through hybridization. Using genome mapping, they compared the genomic composition of three experimentally synthesized hybrid lineages to each other and to an ancient hybrid-derived species. Interspecific gene interactions were common in hybrid lineages. Most epistatic interactions were unfavorable, but a few combinations of genes had favorable epistatic interactions. The similarity of all three experimental hybrid pedigrees to the hybrid-derived species supports the viewpoint that interspecific, epistatic interactions can provide the basis for adaptive evolution of a novel hybrid taxa.

The results of these studies suggest that epistatic interactions are likely to influence the patterns of tolerance to damage that will be seen in naturally occurring hybrid plants. Because epistatic interactions may be disruptive, selection is anticipated to favor parental combinations of tolerance traits

overall. Still, lower mean fitness does not result in all hybrids within a lineage having lower fitness (Arnold, 1997; Rieseberg and Carney, 1998). Hence, placing alleles from one species into a new genetic environment will not necessarily lead to selection against and elimination of those alleles. Selection may act such that introgression of tolerance traits is non-reciprocal. In addition, the benefit of epistatic interactions for tolerance to damage may be contingent upon the environment in which the plants are found. Novel gene combinations may be more beneficial in novel environments than in parental environments (Lewontin and Birch, 1966; Stebbins, 1973; Wang *et al.*, 1997).

### **Conclusions**

Experiments utilizing interspecific hybridization can provide a great opportunity to expand our understanding of the mechanisms, the costs, and the evolutionary tradeoffs involved with tolerance. Moreover, studies using hybrid complexes can extend our knowledge regarding the adaptive nature of tolerance. For example, patterns of defense against herbivores suggest that backcrossing of interspecific hybrids to parental species has led to the adaptive transfer of genes from one species to another (Stutz and Thomas, 1964; Manley and Fowler, 1969; Wu *et al.*, 1996). Similarly, introgression may be a mechanism for evolution of tolerance in plants, thereby influencing the genetic diversity within plant species. Further, evaluating the genetic architecture of tolerance can suggest the degree to which traits conferring tolerance are coadapted. In turn, the level of coadaptation of tolerance traits can suggest the degree to which local adaptation of tolerance occurs within a species and the extent that tolerance is differentiated between species pairs. To evaluate the adaptive role of tolerance to damage, more research is still needed to determine the selective importance of tolerance, to detail the patterns of inheritance of tolerance traits, and to elucidate the genetic architecture of plant species involved.

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