MEASURING TOLERANCE TO HERBIVORY WITH NATURAL OR IMPOSED DAMAGE: A REPLY TO LEHTILÄ

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The ability of plants to tolerate herbivory without a decrease in plant fitness has important implications for the ecology and evolution of plant herbivore interactions (Rosenthal and Kotanen 1994; Strauss and Agrawal 1999; Stowe et al. 2000; Juenger and Lennartsson 2000). Because tolerance is a measure of the effect of herbivore damage on plant fitness, it cannot be measured on a single plant. Rather, tolerance is usually measured as the slope of a linear regression of fitness versus herbivory for a related group of individuals (i.e., families, clones, or inbred lines). Researchers have used both naturally occurring herbivory and imposed herbivory to evaluate the effects of different levels of damage on plant fitness.

In our previous paper, we drew attention to some of the biological, logistical, and statistical advantages and disadvantages of using natural or imposed damage for estimating tolerance (Tiffin and Inouye 2000). We also pointed out that covariances between unmeasured environmental variables, fitness, and herbivory can affect estimates of tolerance. Specifically, because experimentally imposed damage removes the covariance between herbivore damage and unmeasured environmental variables, randomly assigned imposed damage can provide more accurate (unbiased) estimates of tolerance. However, under some conditions, this greater accuracy comes at the expense of a less precise estimate.

Lehtilä (2003) pointed out that Tiffin and Inouye (2000) assumed the distribution of levels of herbivory (H) is the same for naturally occurring and imposed herbivory. Relaxing this assumption questions the conclusion that estimates of tolerance based on naturally occurring damage will often be more precise than estimates based on experimentally imposed damage. We agree with Lehtilä that the statistical power of experiments can often be improved through the thoughtful allocation of experimental effort to different treatments (Inouye 2001). An experimenter who uses imposed herbivory can choose an efficient allocation of levels of herbivory, whereas an experimenter who uses natural levels of herbivory must accept whatever distribution the herbivores provide. Thus, experiments that use imposed herbivory can potentially gain enough statistical power to provide more precise and more accurate estimates of tolerance.

Lehtilä (2003) makes a valuable contribution to the discussion of methods for estimating tolerance. We think, however, that the importance of relaxing the assumption about the distribution of levels of herbivory was overstated in concluding ''the precision of experiments with natural damage are usually very low compared to experiments with imposed

damage'' (without citations). In cases in which natural levels of herbivory are mostly concentrated near their mean, which Lehtilä (2003) claims are frequent, imposing a more uniform distribution of herbivory may indeed have a large effect on the precision of tolerance estimates. However, few data are available on the distribution of levels of herbivory in natural populations.

There are several reasons that the distribution of herbivory levels may not be concentrated near their mean in many study systems. Herbivores that lay eggs in clutches and/or feed gregariously can lead to bimodal distributions of damage— a very good distribution of herbivory from the standpoint of increasing the precision of an estimate for a linear model. Recent modeling results suggest that induced plant defenses can also lead to bimodal distributions of herbivory, even when herbivores are not initially aggregated (N. Underwood et al., unpubl. ms.). Furthermore, some important kinds of herbivory cannot be measured on a continuous scale, such as apical-meristem damage, in which both natural and imposed herbivory can only consist of 0s and 1s. In these cases, using imposed damage will not improve statistical properties much, unless the distribution of natural herbivory is severely unbalanced.

Stinchcombe (2002) provides an example of an experiment in which imposed herbivory could provide little increased statistical power. In this experiment, which estimated tolerance to naturally occurring deer herbivory, almost all plant families had individuals that experienced no herbivory and others that were nearly completely eaten. Within treatments, families experienced an average of 81% of the range of possible damage (between 0 and 100% herbivory), and herbivory levels had high variance and were not obviously unimodal (J. R. Stinchcombe, unpubl. data). When distributions of natural herbivory levels have a large range and variance, imposed distributions of herbivory are unlikely to provide much of an increase in the precision of estimates.

Regardless of the distribution of levels of herbivory, correlations between unmeasured environmental variables, herbivory, and plant fitness will also affect the relative precision of tolerance estimates using natural or imposed herbivory (Tiffin and Inouye 2000, eq. 6; Lehtilä 2003, eq. 11). If these correlations are large, then they may be more important than controlling the distribution of levels of herbivory. Although there is no doubt that environmental variables can affect fitness and herbivory, the interrelationships between environmental variables, herbivory, and plant fitness are largely un-

explored. Empirical studies that investigate these interrelationships are needed to determine the importance that environmental factors, such as nutrient levels or water stress, have on estimates of tolerance.

In addition to comparing the precision of estimates of tolerance made with natural versus imposed damage, Tiffin and Inouye (2000) listed other advantages and disadvantages of natural and imposed herbivory. For example, for studies that aim to compare selection on both resistance and tolerance, using natural damage allows one to quantify both strategies of plant defenses on the same set of plants. Because levels of resistance measured in different environments are not always correlated (Pilson 1992; Bowers and Stamp 1993; Stinchcombe and Rausher 2001; Tiffin 2002), comparing values of tolerance and resistance estimated from experiments conducted at different times or in different places may be problematic. Using simulated herbivory also requires caution because many aspects of natural herbivory are impossible to recreate artificially, such as the chewing actions and application of herbivore saliva that seem to be critical for eliciting a plant's response to damage in some cases (Detling and Dyer 1981). Even when damage is done by real herbivores, constraining the herbivores may alter their behavior and feeding patterns.

Perhaps a valuable empirical approach would be to conduct experiments in which naturally and artificially damaged plants are grown together in a randomized experimental design. These experiments could be used to compare the precision of estimates of tolerance made using naturally occurring and imposed damage, which to our knowledge has not yet been done. Such experiments would also allow resistance and tolerance to be measured on plants grown under the same environmental conditions. Moreover, having plants that incur natural damage intermixed with plants with manipulated damage would provide an internal control on the appropriate levels and distribution of damage individual plants receive. Unfortunately, these experiments may not come without certain disadvantages. First, there are logistical issues that arise from trying to exclude herbivores from some plants, while other plants experience natural levels of damage. Second, experiments would have to be larger than experiments that examine only natural or imposed damage—a real concern given that estimating tolerance already requires large experiments. The results from these experiments may also be difficult to interpret. For example, if estimates from naturally damaged and artificially damaged plants are significantly different, it is not possible to know if the differences are due to environmental factors that have affected herbivory and fitness, artifacts arising from methods used to impose damage and/or exclude herbivores, or artificial damage serving as a poor proxy to natural damage.

In conclusion, we agree there are serious nonstatistical reasons to be cautious about using imposed herbivory. We also agree that using an efficient experimental design can improve the precision of tolerance estimates over estimates based on a natural distribution of herbivory (Lehtilä 2003), at least in those situations in which naturally occurring damage has poor sampling properties. Published data on distribution of naturally occurring damage in plant populations are surprisingly rare. The magnitude of the potential improvement in statistical power from imposed herbivory may also be small, relative to the unknown effects of environmental covariances. Results from empirical studies that measure the magnitude of these covariances and directly compare the precision of estimates of tolerance using naturally occurring and imposed herbivore damage are necessary before the relative merits of these approaches can be thoroughly evaluated.

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