

## Density dependence in induced plant resistance to herbivore damage: threshold, strength and genetic variation

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The density-dependent effect of induced plant resistance on herbivore populations depends on the relationship between the amount of herbivore damage and the level of induced resistance produced by the plant. This relationship should influence the interaction of induced resistance and herbivore population dynamics, and if the relationship varies among plant genotypes, it could be subject to natural selection by herbivores. In this study the relationship between percent leaf area damaged and level of induced resistance was characterized for four genotypes of soybeans grown in a greenhouse. Damage ranging from 8 to 92% of leaf area was imposed using Mexican bean beetle larvae, and induced resistance was measured by bioassay using Mexican bean beetle adults. The level of induced resistance was significantly affected by the amount of damage, and the level of induced resistance varied significantly among the four genotypes. There was also a marginally significant interaction of damage and plant genotype, suggesting that the form of density dependence varies among these four genotypes of soybeans. These results suggest that these genotypes of plants might affect herbivore populations differently. If this variation is heritable, the form of density-dependent effects of induced resistance has the potential to evolve in this system.

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Induced plant resistance to herbivores has received a great deal of attention over the past 20 years, in part because it has been thought that induced resistance might contribute to the regulation and/or cyclic fluctuation of insect herbivore populations (Benz 1974, Haukioja 1980, Rhoades 1985, Myers 1988, Karban and Baldwin 1997). In order for induced resistance to affect herbivore population dynamics in this way, it must be able to provide density-dependent negative feedback to insect population sizes. Assuming that the amount of damage to a plant increases as the herbivore population increases, the relationship between amount of damage and the strength of the induced response should be an indicator of the type of density dependence in the induced response.

Several aspects of the relationship between damage and induced resistance may be important for determin-

ing how induced resistance affects herbivore population dynamics. Many mathematical models predict that density dependence which is delayed can drive cycles in herbivore populations (May 1973, Berryman et al. 1987), and in particular that lags in the production of induced resistance can contribute to fluctuations in herbivore populations (Edelstein-Keshet and Rausher 1989, Lundberg et al. 1994, Underwood 1999a). If there is a high threshold level of damage necessary to provoke a significant induced response, this could cause a delay in the plant's response to herbivore attack, thus contributing to the production of cycles in the herbivore population. Also, the maximum strength of induced resistance is predicted to affect the size of herbivore populations (Edelstein-Keshet and Rausher 1989, Underwood 1999a). For instance, Underwood (1999a) predicts a negative exponential relationship be-

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tween the strength of induced resistance and herbivore population size. Induced resistance that responds to increases in damage quantitatively should provide more sensitive feedback to herbivore populations than a qualitative (all-or-nothing) induced response. Thus to understand how induced resistance may interact with herbivore population dynamics in any specific system, it is important to understand the relationship between induced resistance and the amount of damage the plant receives.

Interest has also focused on whether and how induced resistance can evolve in response to herbivory (e.g. Rhoades 1979, Baldwin et al. 1990, Frank 1993, Adler and Karban 1994, Padilla and Adolph 1996). Heritable genetic variation for induced resistance is necessary for induced resistance to evolve by natural selection. Because induced resistance is a plastic trait, to fully characterize variation in the trait requires measuring it over a variety of environments (i.e. characterizing the norm of reaction) (Karbon and Baldwin 1997). This means that induced resistance should ideally be measured over a range of damage levels, representing a range of environments differing in herbivore loads. Of the relatively few studies that have looked for variation in induced resistance among more than two genotypes (Shapiro and DeVay 1987, Anderson et al. 1989, Zangerl and Berenbaum 1991, Raffa 1991, Brody and Karban 1992, Bi et al. 1994, van Dam and Vrieling 1994, English-Loeb et al. 1998) none have measured induced resistance over more than two damage levels (damaged versus undamaged). Thus we still know little about how much variation in the relationship between induced resistance and herbivore load exists within plant-herbivore systems. Characterizing the shape of this relationship will allow consideration of the evolution not only of the ability to induce, but of the form of induced resistance as well.

Here I report the results of an experiment designed to characterize the relationship between Mexican bean beetle damage and induced resistance in soybeans, and to determine if the form of this relationship varies among soybean genotypes.

## Methods

Soybean seed (*Glycine max*) for this experiment was obtained from T. Carter and J. Burton (North Carolina State University, NC). Soybean plants were grown in a mixture of soil, perlite, peat, sand (2:2:1:1) and lime (to correct pH) in 4-inch plastic pots in the Duke University greenhouses. The plants were provided with a 14-h day length, including supplemental lighting (430-W high-pressure sodium lamps). This day length was sufficient to keep the plants vegetative throughout the experiment. Plants were watered as needed and fertilized

weekly with Peters soluble fertilizer (20-10-20). Although plants were not inoculated with *Rhizobium*, haphazard sampling of roots indicated that plants often had nodules that appeared to be active. Mexican bean beetles (*Epilachna varivestis*: Coccinellidae) were obtained from T. Dorsey (New Jersey Department of Agriculture, Trenton, NJ) and reared in an environmental chamber at 28°C, under a 14-h day length. Beetles were reared on common snap bean plants (*Phaseolus vulgaris*) to prevent them from developing preferences for any particular soybean genotype.

I tested four genotypes of soybeans (Bragg, Williams, Clark and Young) for their induced responses to each of five levels of Mexican bean beetle damage (8, 25, 50, 75 and 92% leaf area damaged). This experiment was carried out in four temporal blocks. For each combination of soybean genotype and damage level, I grew 24 plants (six in each block) in the greenhouse until the plants had one partially expanded and three fully expanded trifoliates. Half the plants in each combination of genotype, damage level and block were assigned to be damaged and the other half were kept undamaged as controls. I determined percent leaf area to be damaged roughly as the ratio of damaged to total number of leaflets on the plant  $\times 100$  (including the first true leaves, which are single leaflets, and considering the newly expanding leaf to be equal to one leaflet). Damage treatments were created by placing 3rd and 4th instar Mexican bean beetle larvae on varying numbers of the plant's leaflets, from one through 11 leaflets out of 12 leaflets. Larvae were confined to individual leaves or leaflets using mesh bags. Control plants had equal numbers of leaves bagged. Enough larvae were used to result in the desired leaf area being damaged within 48 h. After 48 h, the larvae were removed. Induced resistance was measured by bioassay 3 d after damage ceased. If the timing of induced resistance varies among genotypes, comparing induced resistance among genotypes at one time could produce apparent differences in induction due only to catching the genotypes at different times in their induced responses. However, previous work indicates that soybean genotypes do not vary in the time course of their induced responses to Mexican bean beetle damage (Underwood 1998) so timing should not be a confounding variable in this experiment. I used a bioassay to measure induced resistance rather than a chemical analysis, in order to obtain a direct measure of the effect of induced resistance on the beetle. Because the induced response of soybeans to beetle damage is complex and not well-understood (Wheeler and Slansky 1991, Felton et al. 1994), it is not yet clear what specific chemicals should be measured to best represent effects on the beetles.

For the bioassay, each damaged plant was paired with a control plant, and induced resistance was measured as the relative preference of a beetle for the control over the damaged plant. Leaf disks were cut

with a cork borer from the most recently expanded and undamaged leaves of both the damaged and control plants. Two disks from the damaged plant and two from the control plant were then placed in a Petri dish lined with wet filter paper. Control and damaged disks were arranged alternately at the edges of the dish, and one adult female Mexican bean beetle was placed in the dish. The beetle was allowed to feed until it had eaten 25% of the leaf area in the dish, or for 24 h, whichever came first. Dishes in which no disk had greater than 3% of its area damaged (about 19% of all dishes) were not included in the analyses. I measured the area of each disk eaten by the beetle using the Image 1 computer image analysis system (Universal Imaging Corporation, 1991) on an IBM Gateway 486 computer with a BURLE black-and-white video camera. Two choice tests (two Petri dishes) were run for each plant pair to minimize lost data due to some beetles choosing not to eat at all. The measures of induced resistance from the two dishes from each pair of damaged and control plants were averaged to form a single observation.

### Analysis

For constancy with previous work on induced resistance in soybeans (Kogan 1972), I measured induced resistance as a preference index ( $PI = 2(c/(c+d))$ ) where  $c$  and  $d$  indicate the amount of damage to the control and damaged disks in each dish. A PI of 1 indicates no preference (no difference in feeding between the damaged and control plants) and PI's higher than 1 indicate a preference for the control (rejection of previously damaged plants).

Statistical analyses were performed on the arcsine square-root transformed ratio of amounts of control versus damaged disks eaten ( $c/(c+d)$ ) rather than the PI's (Zar 1984). I used ANOVA (GLM procedure of SAS, SAS 1989) to determine if % leaf area damaged, soybean genotype and the interaction between genotype and damage significantly affected the amount of induced resistance in the plants. One-tailed  $t$ -tests were used to determine if each level of damage induced significant resistance in each genotype (that is, whether the PI for each damage level was significantly greater than 1). The alpha levels used for these  $t$ -tests were corrected for five comparisons within each genotype using the Bonferroni correction.

To determine if the responses of these genotypes were qualitative (all-or-nothing) or quantitative (amount of induced resistance varying with damage level), I compared the fit of an estimated step-function (qualitative) versus a linear function (quantitative) to the data. The step-function was estimated by finding the sum of squared deviations from the mean PI for levels of damage that did not produce significant induced responses, and from the mean for levels of damage producing significant induced resistance. To compare the fit of this step-function to a linear function, I constructed an  $F$  statistic consisting of the mean squared deviations from the step function divided by the mean squared deviations from the linear function.

### Results

The relationships between percent leaf area damaged and induced resistance for four genotypes of soybeans are shown in Fig. 1. Analysis of variance indicates that

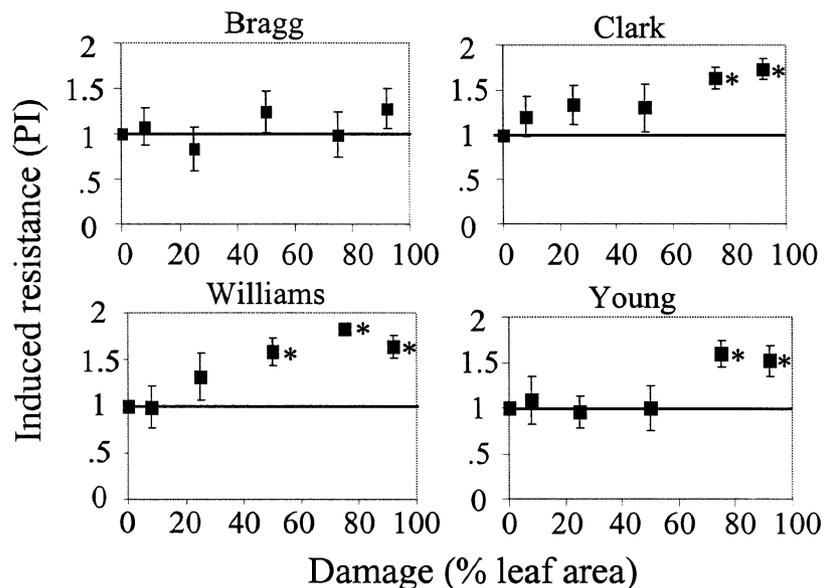


Fig. 1. Relationship between % leaf area damaged and level of induced resistance for four genotypes of soybeans. Induced resistance is measured as beetle preference for undamaged over damaged plants. A preference index (PI) value of 1 indicates no preference (no induced resistance).  $N \approx 12$  for each point, error bars indicate 1 standard error. Points with asterisks indicate significant beetle preference for undamaged over damaged plants (one-tailed  $t$ -test).

genotypes varied in their induced responses to beetle damage (significant effect of genotype,  $F_{3,193} = 12.19$ ,  $P = 0.0001$ ). The induced response also varied with the percent leaf area damaged (significant effect of damage level,  $F_{4,193} = 4.83$ ,  $P < 0.001$ ), with increasing levels of damage generally leading to increased levels of induced resistance. The relationship between amount of damage and induced resistance also varied among genotypes of soybeans (marginally significant interaction of plant genotype and % leaf area damaged,  $F_{12,193} = 1.77$ ,  $P < 0.055$ ). The genotypes appear to differ somewhat in the level of damage necessary to produce significant induced responses. For example, Young and Clark both require 75% of their leaf area to be damaged before they produce significant induced resistance, while Williams responds significantly at only 50% damage, and Bragg does not respond significantly at all (Figs 1 and 2). Because Bragg did not exhibit any significant induced resistance, only the other three genotypes were tested for a qualitative versus a quantitative response to damage. There was no significant difference in the fit of the step-function and linear function to the data for any of these three genotypes (Clark:  $F_{54,54} = 1.5$ , Young:  $F_{51,51} = 1.25$ , Williams:  $F_{56,56} = 1.54$ ). Thus, these data do not allow distinction between a qualitative and a quantitative model of the relationship between damage and induced resistance.

## Discussion

In this study genotypes of soybeans varied not only in their overall levels of induced resistance, but in the form of the relationship between damage and induced resistance (the norm of reaction to damage) as well. While constitutive resistance is known to be heritable in soybeans (Sisson et al. 1976) the heritability of induced resistance is unknown. If this variation is heritable, these results suggest that natural and artificial selection could change not just whether a plant is inducible, but also some of the details of how induced resistance responds to herbivore damage. Plant genotypes that respond at lower levels of damage (such as Williams), might have an advantage over plants that do not respond until higher levels of damage (such as Clark), since herbivores could be driven off to other, not-yet-induced, plants. Alternatively, plants that respond to low levels of damage could suffer from costs of inducing resistance when herbivore attack is not detrimental enough to strongly reduce plant fitness. The relative advantages of higher or lower "thresholds" (levels of damage provoking a significant induced response), qualitative vs quantitative responses and varying strengths of induced resistance should depend on the pattern and predictability of herbivore attack, the cost of inducing resistance and the responses of other plants in the population.

Many discussions of the evolution of induced resistance have focused on the relative advantages and disadvantages of inducible vs constitutive resistance (e.g. Åström and Lundberg 1994, Padilla and Adolph 1996, Agrawal and Karban 1999). However, results of this study suggest that there is genetic variation not just for inducibility (plasticity) but for the shape of the relationship between damage and induced resistance as well. If future studies indicate that heritable variation for the relationship of induced resistance to damage is a common feature of inducible plant-insect systems, discussions of the evolution of induced resistance should be broadened to include the evolution of the form of the induced response (i.e. what kind of induced response is optimal, see for example Adler and Karban 1994), rather than focusing only on whether an induced response is favored over a constitutive response. There has been a burst of recent interest in the evolution of norms of reaction (Schlichting and Pigliucci 1998). Induced resistance could be a particularly interesting character for studies of the evolution of reaction norms, since induced resistance may influence herbivore population dynamics (Haukioja 1980, Rhoades 1985, Karban and Baldwin 1997), and has potential practical significance for the control of agricultural pests (Karban 1991, Thaler 1999).

Several models predict that aspects of the relationship of damage to induced resistance should be important for determining the effect of induced resistance on herbivore population dynamics (Edelstein-Keshet and Rausher 1989, Underwood 1999a). The significant effect of damage on induced resistance found in this study is consistent with the results of many other studies showing that induced resistance increases in a density-(damage) dependent fashion (Karban and Baldwin 1997). The fact that induced resistance in soybeans is density dependent suggests that it has the potential to influence regulation and cycles in herbivore populations. In general, if induced responses are quantitative, they should provide more precise regulation of herbivore populations than qualitative (all-or-nothing) responses would. Results of this experiment do not allow distinction between a qualitative and a quantitative model, because there is no significant difference between the fit of a step-function and a linear function to the relationship between damage and induced resistance (Fig. 2). Data from two previous studies examining the effect of damage on induced resistance in soybeans suggest that induced resistance in soybeans may be a quantitative response (Iannone 1989, Brown et al. 1991), although neither of these studies discusses whether the response is qualitative or quantitative. While previous studies of the relationship between damage and induced resistance in other systems have not made a distinction between qualitative and quantitative responses, this would be a valuable characteristic of the relationship to measure where data are available.

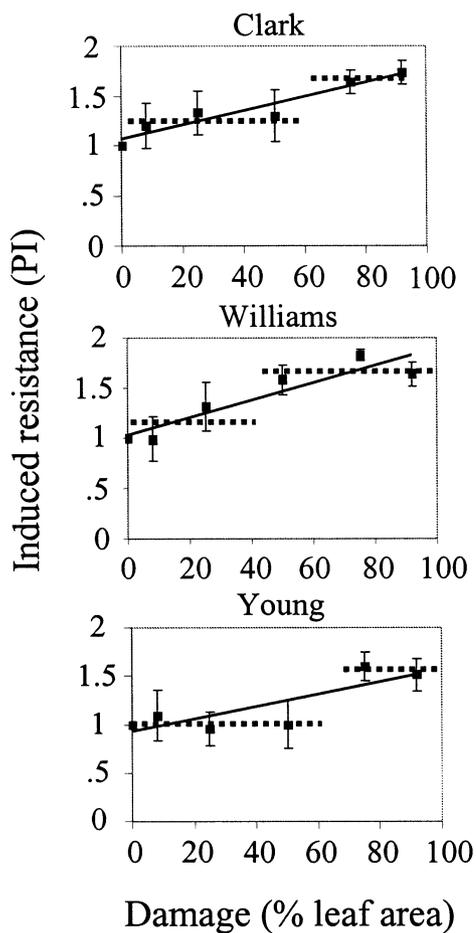


Fig. 2. Linear (—) and step (----) functions fit to the relationship between % leaf area damaged and induced resistance for three soybean genotypes. Induced resistance is measured as beetle preference for undamaged over damaged plants. A preference index (PI) value of 1 indicates no preference (no induced resistance).  $N \cong 12$  for each point, error bars indicate 1 standard error. There is no significant difference between the fits of the two functions to the data.

The effect of induced resistance on herbivore populations should depend strongly on whether induced resistance is lagged with respect to herbivore generation times (Edelstein-Keshet and Rausher 1989, Lundberg et al. 1994, Underwood 1999a). Previous work indicates that there is very little, if any, lag in the timing of induced resistance to a single level of damage (60% of leaf area) in this system (Underwood 1998). However, the high level of damage needed to induce significant resistance in the soybean genotypes tested here (between 50 and 75% leaf area damaged) suggests that induced resistance could effectively be lagged in this system simply due to this high threshold. The variation in the strength, threshold level and form of density dependence of induced resistance among these genotypes suggests that these genotypes may have different effects on herbivore populations. Results of field exper-

iments (Underwood 1997) indicate that herbivore population dynamics do differ among these genotypes, although it has not yet been determined whether these differences are directly attributable to induced resistance.

Induced resistance is thought to be more likely to influence long-term herbivore population dynamics than other plant characters because induced resistance can provide a density-dependent response to herbivores (Berryman et al. 1987, Underwood 1999b). This study indicates that the form of density dependence of induced resistance can vary among plant genotypes and thus could be subject to selection. Studies of the form of the density-dependent relationship between damage and resistance could thus help link population dynamic and evolutionary phenomena in plant-insect systems, since changes in this plant character are particularly likely to be important both for plant fitness and herbivore population dynamics.

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