Induced Responses to Herbivory and Increased Plant Performance

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Plant resistance to herbivores was induced in a field experiment to evaluate the consequences of induced responses for subsequent herbivory and plant fitness. Induction early in the season resulted in halving of herbivory by chewing herbivores and a reduction in the abundance of phloem-feeding aphids when compared with controls. A correlate of lifetime plant fitness, seed mass, was enhanced by over 60 percent for individuals that were induced.

Herbivores can reduce seed production and other correlates of plant fitness, and this reduction can result in natural selection for either constitutively expressed or inducible plant defenses (*1*, *2*). Induced plant responses to herbivory are "immune-like" responses that reduce the performance or preference of herbivores (or both) and have been reported from over 100 plant-herbivore systems (*2*). These responses are assumed to benefit plants, although such benefits have never been demonstrated in a field experiment (*2*, *3*). Previous experiments have demonstrated that initial herbivore attack increased levels of chemical (*4*), physical (*5*, *6*), and biotic defenses (*7*) in a wide variety of plants ranging from unicellular algae to acacia trees (*2–11*). Such induction negatively affects plant attackers, including pathogens (*9*), insect herbivores (*10*), and vertebrate grazers (*11*). However, to comprise a true plant defense, the male or female fitness (or both) of plants must be enhanced by virtue of reduced herbivory. Here, I report on an experimental evaluation of the hypothesis that induced responses to herbivory increase plant performance.

Lifetime plant performance was evaluated for wild radish (*Raphanus sativus* L. Brassicaceae) plants assigned to one of three treatments: induced plants, leaf damage controls, and overall controls. Plant fitness was assessed by an index calculated by multiplying seed number by mean seed mass. Because mean seed mass significantly affected plant fitness in previous experiments with *Raphanus* (*12*), this index of total seed mass is superior as an indicator of female fitness to seed number alone. Early season flower number is a correlate of male reproductive success in this species and was used as an indicator of male fitness (*13*). I induced plants by caging a caterpillar larva (*Pieris rapae*) on one leaf at the four-leaf stage. Damage-induced responses in wild radish included increased concentrations of defensive mustard oil glycosides (glucosinolates) and increased densities of setose trichomes (*14*). Two control treatments were also established: leaf damage control and overall control plants (*15*). Leaf damage controls had one leaf clipped off at the petiole with a scissors. Such clipping resulted in an equal amount of leaf tissue removed as in the induced treatment but without the associated induced plant response. Induced plant responses are thought to be minimized by clipping damage because of the absence of herbivore saliva and the greatly reduced area of actual leaf tissue that is damaged (*16*). All naturally occurring herbivores were picked off of the plants by hand daily until treatments had been imposed. This removal effectively created treatments in which both sets of control plants were denied the natural inducing signal by early season herbivores.

Naturally occurring insect herbivores colonized the plants early in the season. Plants that were experimentally induced consistently received less herbivory than plants in both control treatments. Earwigs (*Forficula* sp.) and other chewing herbivores caused 100% more leaf damage on control and clipped plants than on induced plants (Fig. 1A). Plant colonization by green peach aphids (*Myzus persicae*) was affected by induction in a similar manner, with 30% more aphids on controls compared with induced plants (Fig. 1B). Aphids were intermediate on clipped plants, suggesting that plant size affected colonization by aphids (*17*). The abundance of ladybird predators (Coccinellidae) that feed on aphids was not affected by my treatments (*18*). Flea beetles (*Phyllotreta* spp.) emerged later in the season and caused large amounts of plant damage and mortality of many plants. Plant mortality of the leaf damage control and overall control plants was 46% and 50%, respectively, and mortality of induced plants was significantly reduced to 36% (*19*). These results indicate that the induced plants were more resistant to herbivores than the uninduced controls were. Induced plant resistance was not species-specific and affected earwigs, aphids, grasshoppers, flea beetles, and lepidopteran larvae (*20*).

Induction of resistance early in the season significantly increased the index of lifetime female fitness by over 60% compared with controls (Fig. 1C) (*21*). Leaf damage controls (plants damaged without induction) had 38% less fitness than controls, indicating that these plants suffered because of the loss of leaf tissue without the associated benefits of induction (*21*). Although induced plants suffered a loss of leaf tissue equal to that of the leaf damage controls, the benefits of induction outweighed these costs. Early season flower number, an indicator of male fitness in this species, showed the same pattern of increased production in induced plants

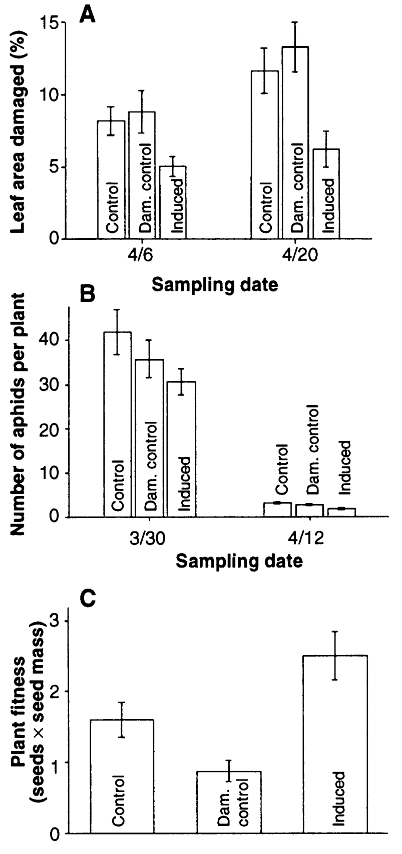

Fig. 1. (**A** and **B**) Herbivory on plants measured on two sampling dates (in month/day) (mean ± SEM) (*31*). (A) The percentage of leaf area consumed by chewing herbivores. Dam. control, leaf damage control. (B) The number of aphids infesting plants. (**C**) Fitness of control plants, leaf damage controls, and induced plants (mean ± SEM). Female fitness was calculated for each plant by multiplying the number of seeds produced by the mean seed mass (in milligrams).

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(*22*). This increase in the fitness of induced plants was found even though wild radish can tolerate large amounts of herbivory without reductions in fitness (*23*, *24*). Only two previous experiments have attempted to detect fitness benefits of induced responses to herbivory; these experiments failed to detect fitness benefits, probably because they involved long-lived perennial plants (*25*).

Induced responses to herbivory appear to be an example of adaptive plasticity in plants. Induced responses are nearly ubiquitous in the plant kingdom, yet it is still not understood why plants have evolved facultative defenses instead of constitutive defenses. Energetic costs associated with plant defense have been suggested as the primary force favoring facultative defenses because of energetic savings when the defenses are not needed (*26*). However, evidence for such costs is weak (*2*, *27*). Recent work has implicated a broad array of other ecological and physiological trade-offs that may be important constraints on plant defense favoring inducible strategies (*2*, *27*). Although the benefits of induction have received relatively little attention, inducible defenses may have many benefits for plants that constitutive defenses lack (*28*). For example, induced responses may attract predators and parasites of herbivores (*29*). In addition, because some plant resistance traits attract specialist herbivores, inducible defenses may make plants less obvious targets for these adapted herbivores when the plants are not induced (*30*).

Determining the ultimate fitness consequences of plant defense traits bears directly on the long-standing view of plant parasites as selective agents and plants as active participants in the evolutionary process (*1*). Induced plant responses to herbivory provided high levels of plant defense that were reflected in increased plant performance. Leaf damage controls had lower fitness than overall controls, indicating that early season herbivory was costly. In this experiment, fitness benefits of induced defenses outweighed the costs associated with loss of leaf tissue and induction itself. The excess of benefits to costs makes induced defense a strategy that may be favored by natural selection in environments with herbivory.

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14. Chemical analyses indicated that leaf damage by *Pieris rapae* caterpillars induced high concentrations of indole glucosinolates, whereas leaf damage by scissors alone did not induce indole glucosinolates [$F(1,30) = 6.480$, $P = 0.016$] (*24*). Setose trichomes were also found to be inducible (increase in density and total number) on newly formed leaves of previously damaged plants [$F(1,36) = 10.208$, $P = 0.003$] (*6*).
15. A total of 454 plants were divided into three treatments as follows: 151 induced plants, 150 leaf damage controls, and 153 overall (unmanipulated) controls. Half of the leaf damage control plants were clipped at the start of the treatments (that is, when caterpillars were placed on induced treatment plants), and the other half were clipped when the caterpillars on the induced treatments had finished consuming the leaf (3 to 4 days later). The research plot was located in a plowed old field at the University of California Student Experimental Farm (Davis, CA). Seeds were collected from several wild populations of *R. sativus* in Davis, and cotyledonary plants were transplanted from plug trays during the typical period of juvenile growth. The plot was not irrigated.
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18. From the analysis of variance for the effect of treatments on abundance of ladybird predators, $F(2,451) = 0.118$, $P = 0.889$.
19. From the likelihood ratio χ^2 test for the effect of flea beetles on plant mortality, $G = 7.532$, degrees of freedom = 2, $P = 0.023$.
20. In identical experiments performed on a sibling species (*Raphanus raphanistrum*) at a site in the Sierra Nevada foothills, I found similar results (*6*). In these experiments, induced responses to herbivory protected plants from herbivory by grasshoppers and lepidopteran larvae and increased flower production and seed set compared with control plants.
21. Overall effects of treatments on female plant fitness are significant [$F(2,445) = 11.004$, $P < 0.001$]: For the contrast of induced plants compared with overall controls, $F(1,445) = 6.567$, $P = 0.011$; and for the contrast of leaf damage controls compared with overall controls, $F(1,445) = 4.954$, $P = 0.027$.
22. From the analysis of variance on early season flower production, $F(2,451) = 3.470$, $P = 0.032$.
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31. From the analysis of variance for the effects of experimental induction on (i) chewing herbivory and (ii) plant colonization by aphids measured on two sampling dates with multivariate analysis of variance, Wilks' lambda = 0.972, $F(4,888) = 3.23$, $P = 0.012$ for chewing herbivory; and Wilks' lambda = 0.956, $F(4,876) = 4.985$, $P = 0.001$ for plant colonization by aphids. Half-sib families also varied in their susceptibility to herbivory and attack by aphids [Wilks' lambda = 0.834, $F(30,1694) = 2.625$, $P < 0.001$].
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