

Andrew C. McCall · Richard Karban

Induced defense in *Nicotiana attenuata* (Solanaceae) fruit and flowers

Received: 6 October 2005 / Accepted: 12 October 2005 / Published online: 3 December 2005
© Springer-Verlag 2005

Abstract Plants protect themselves against herbivory using a continuum of strategies, ranging from constitutive defenses to intermittent induced responses. Induced defenses may not provide immediate and maximum protection, but could be advantageous when continuous defense is either energetically or ecologically costly. As such, induced defenses in flowers could help defend relatively valuable tissue while keeping reproductive structures accessible and attractive to pollinators. Thus far, no one has demonstrated the efficacy of induced defenses against floral herbivores (florivores) in the field. Here we show that mechanical leaf damage in wild tobacco, *Nicotiana attenuata* (Solanaceae), reduced both flower and fruit herbivory in the field and that exogenous application of methyl jasmonate, a potent elicitor of induced responses, reduced both leaf and floral damage in natural populations. This result is consistent with a survey of damage in the field, which showed a negative relationship between leaf damage and flower and fruit damage. Although optimal defense theory predicts that induced defenses should be rare in reproductive tissues, owing to their high fitness value, our results suggest otherwise. Induced defenses in leaves and reproductive tissues may allow plants to respond effectively to the concomitant pressures of defending against herbivory and attracting pollinators.

Keywords Florivory · Induced defense · *Nicotiana* · Optimal defense theory

Introduction

Plants are not passive players in interactions with herbivores; researchers have identified a large number of chemical and physical defenses that are effective in deterring herbivory (Zangerl and Berenbaum 1993; Karban and Baldwin 1997; Kessler and Baldwin 2002). Anti-herbivore defenses are usually characterized as either constitutive or induced, although many mechanisms may be expressed both ways. Constitutive defenses are constantly expressed while induced defenses are typically characterized by prior tissue damage that reduces or deters further herbivory, and have been the topic of much recent work (Agrawal et al. 1999).

Induced plant defenses have mainly been studied in leaves (Karbon and Baldwin 1997; Agrawal et al. 1999; Agrawal 2001) and only a few researchers have investigated induced responses in flowers. This disparity may be due to predictions based on theory. For example, optimal defense theory (ODT), a common theory invoked to explain patterns of plant chemical defense, predicts that valuable structures, such as flowers and fruit, should be protected by constitutive defenses that operate at a high level before any initial damage occurs (McKey 1974; Rhoades and Cates 1976; Rhoades 1979).

There are few studies of putative defenses in plant reproductive tissues. A seminal investigation by Zangerl and Rutledge (1996) found low levels of induced resistance and high levels of constitutive resistance in *Pastinaca sativa* reproductive tissues.

A few studies, however, have documented significant induced responses in reproductive tissues. In *Raphanus sativus*, leaf damage increased the concentration of petal glucosinolates in anthocyanin-dominant morphs (Strauss et al. 2004). In *Nicotiana attenuata*, artificial leaf damage increased nicotine content in corolla tissue fourfold, and damage to individual flowers doubled the nicotine concentration in the same flower (Euler and Baldwin 1996). Similarly, nicotine content in *N. attenuata* fruits increased following leaf damage (Baldwin and

Communicated by Jim Ehleringer

A. C. McCall (✉) · R. Karban
Center for Population Biology, Department of Entomology,
University of California, Davis, CA 95616, USA
E-mail: acmccall@ucdavis.edu
Tel.: +1-530-5743624
Fax: +1-530-7521449

Karb 1995), and Adler, Wink, and Lentz have found that nicotine concentration in *N. tabacum* nectar increased following leaf damage (unpublished manuscript).

The mechanisms of induced resistance are particularly well characterized for *N. attenuata* and many of these are elicited by methyl jasmonate. Methyl jasmonate is produced by plants and can affect their responses to many environmental stimuli. Methyl jasmonate is involved in the regulation of induced responses to herbivore attacks in many plants (Reinbothe et al. 1994; Creelman and Mullet 1997; Reymond et al. 2000; Schaller 2001). In *N. attenuata* foliar nicotine induction in response to herbivory is mediated by methyl jasmonate (Baldwin 1998) although it is not clear if methyl jasmonate also affects floral defense.

Regardless of the mechanism, there clearly are examples of inducible defenses in plant reproductive tissues, although the ecological and evolutionary consequences of induction are unknown. We know that nicotine concentrations in *N. attenuata* floral structures can increase in response to either leaf or floral herbivory (hereafter termed florivory), but there is no evidence that this mechanism actually deters subsequent florivory in nature.

Given these gaps in our knowledge, we use the *N. attenuata* system to ask and answer the following questions: (1) Is natural leaf damage associated with reduced flower or fruit damage?, (2) Does simulated leaf damage deter natural floral or fruit damage in *N. attenuata*?, and (3) Can exogenous chemical elicitors of induced defense (methyl jasmonate) reduce florivory in the field? This study, to our knowledge, is the first to determine the efficacy and consequences of induced defense against florivores in a field setting.

Materials and methods

The study plant, wild tobacco, *N. attenuata* (Solanaceae), is a widespread annual in the Great Basin of the Western United States that is often found in disturbed areas like roadsides and burned areas (Wells 1959; Baldwin 1998). *N. attenuata* foliage at our study site is damaged by six species of grasshopper (Orthoptera), in order of abundance: *Cratypedes neglectus*, *Trimerotropis fontana*, *Conozoa sulcifrons*, *Cratypedes lateritius*, *Melanoplus sanguinipes*, and *Cordillacris occipitalis*. It is also attacked by two common noctuid cutworms: *Peridroma saucia* and *Agrotis ypsilon* (Karban et al. 2003). In addition, the larvae of *Manduca quinquemaculata* (Lepidoptera: Sphingidae) readily consume entire plants, including reproductive tissues; flowers and developing buds are eaten by *Cratypedes neglectus* (A. McCall and R. Karban, personal observation). Fruit and seeds are consumed by the seed predator *Corimelaena virilis* (Heteroptera: Cormelaenidae) (Wells 1959; Karban et al. 2003). Pollinators of wild tobacco include one of its main herbivores, *Manduca quinquemaculata*, and migrating *Selasphorus* hummingbirds (Aigner and Scott

2002), although we have not observed any birds visiting plants at our sites over 3 years of casual observations (A. McCall, personal observation).

Survey of damage to leaves and to flowers

On August 1, 2002, we surveyed a population of 80 *N. attenuata* plants near the Sierra Nevada Aquatic Research Lab of the University of California Natural Reserve System (SNARL) (N37°36'57", W118°49'47") for signs of leaf herbivory, florivory, and herbivore damage to fruit. To determine if natural leaf damage is associated with decreased flower or fruit damage, we correlated the proportion of leaves suffering from natural damage with the proportion of flowers and fruits damaged. In order to understand if flower damage is associated with fruit damage, we correlated the proportion of flowers damaged with the proportion of fruits damaged. Since there were large deviations from normality in the frequency distribution of the response variables, we used the non-parametric Spearman's rank correlation test to determine these relationships (Conover and Iman 1981).

Induced defense in response to leaf clipping

On August 3, 2002 we marked 80 individual *N. attenuata* plants on a glacial outwash plain near SNARL to experimentally evaluate the effects of leaf damage. Prior to imposing treatments, we measured the percentage of leaves already damaged by natural herbivory for use as a covariate. Cutworms caused most of the leaf damage at the rosette stage (A. McCall, personal observation).

On experimental plants we damaged 30% of all leaves by cutting the distal quarter of the leaf with a pair of scissors. Leaves with previous natural herbivory were also cut. Our aim was to induce resistance without changing the visual appearance of the leaf for herbivores and pollinators, so we cut the midrib of each damaged leaf without completely severing the blade or actually removing any leaf tissue. This type of damage induces nicotine production in leaves (Baldwin 1988). Control plants were only handled, with no artificial leaf damage.

Two weeks after treatment application to leaves, we measured the percentage of flowers with florivory and fruit with insect damage. All percentages were arcsine-transformed and both measures of reproductive damage were incorporated in a protected MANCOVA analysis to control for type I experiment-wide error rates (Scheiner 2001). The percentage of leaves damaged on each plant prior to treatment application was used as a covariate in the analyses, since prior leaf damage may affect the induction of defensive compounds in the flowers or capsules (Euler and Baldwin 1996). As required in ANCOVA models (Neter et al. 1996), there was no significant interaction between the covariate and the treatment (Two-tailed ANOVA, $F_{1,72} = 1.64$, $P = 0.204$).

Induced defense in response to pattern wheel damage

On June 1, 2003, we marked 87 plants at SNARL to randomly receive either 3 rows of damage by a pattern wheel used by seamstresses to mark fabric or to receive no damage as controls. This technique can induce chemical changes in leaves without removing any leaf area (Strauss 1997). Controls were not damaged, only handled. At a second population, Moran Springs, in the Benton Range of the Eastern California (N37°39'21", W118°35'19"), we repeated the experiment with 49 plants. On June 15, 2003 we repeated the damage treatment to experimental plants in both populations to elicit the maximum level of response. We also measured the proportion of flowers damaged by herbivory and the maximum width and length of the two most apical flowers on each plant on July 5, 2003, since damage with a pattern wheel can result in reduced corolla size (Strauss 1997).

We used the MANCOVA method to determine if damage treatment (fixed effect), or population (random effect) affected the percent leaf damage, percent flower damage, or flower size. We used the initial proportion of basal leaf damage as a covariate in the model in order to control for possible prior induction. Although we began the experiment with 136 plants, 44 of these plants died or were not flowering when we recorded the flowering data; there was no significant effect of treatment ($\chi^2 = 0.725$, 1 df, $P = 0.39$) or population ($\chi^2 = 2.21$, 1 df, $P = 0.13$) on mortality.

Defense in flowers triggered by methyl jasmonate application

To determine if methyl jasmonate controls defenses in flowers, we conducted an experiment using exogenous application of methyl jasmonate. Induction with methyl jasmonate allowed us to induce plants without removing leaves and thus changing the appearance of the plants. On May 19, 2003, we marked 40 plants at SNARL for control and experimental treatments (20 plants per treatment). Experimental plants received 100 μg methyl jasmonate (Bedoukian, Danbury, CT, USA) loaded into 25 mg lanolin (Sigma-Aldrich, St. Louis, MO, USA) and rubbed onto the abaxial side of a single leaf; 25 mg of lanolin alone was placed on the abaxial side of a single leaf on the control plants. To test if methyl jasmonate induced resistance in leaves, we measured the proportion of leaves with damage on June 20, 2003. On August 8, 2003 we measured the percentage of flowers with floral damage on each plant. We included only those plants that were flowering at this time ($n = 20$ total). For the statistical analysis, we used the Wilcoxon rank sum one-tailed test, since residual errors were not normal, variances were not homoscedastic, and transformations failed to alleviate these problems. We employed a one-tailed test since we had an a priori expectation that methyl jasmonate application would reduce florivory, based on our previous work showing

induction of defense in flowers in response to leaf damage (A. McCall, unpublished data).

Results

Patterns of natural florivory, fruit herbivory, and leaf herbivory

There was a wide range in the amount of natural herbivore damage in *N. attenuata*, depending on the type of tissue examined. The mean percent of damaged leaves per plant was 25%. In contrast, the mean percent of flowers per plant with damage was only 4%, and the mean percent of fruits with damage per plant was nearly twice that rate, at 7%.

We found a significant negative relationship between percent leaf damage and percent flowers damaged (Spearman's $\rho = -0.22$, $P = 0.04$, $n = 79$, Fig. 1a) and a negative but non-significant relationship between percent leaf damage and percent fruit damage (Spearman's $\rho = -0.17$, $P = 0.12$, $n = 79$, Fig. 1b). Additionally, there was a strong positive relationship between percent of flowers and fruits damaged (Spearman's $\rho = 0.355$, $P = 0.001$). These results suggest that natural leaf damage is associated with lower rates of florivory and that defense in both types of reproductive tissue may be correlated. Alternatively, the same insects responsible for florivory could also be damaging fruit, although our observations suggest that different herbivores are found on the different tissues (Karban et al. 2003; A. McCall, personal observation).

Florivory and fruit herbivory in response to clipping

Plants that we experimentally damaged by clipping leaf midveins received significantly less subsequent natural damage to flowers and fruits compared to undamaged controls (MANCOVA results in Table 1). Univariate ANCOVA showed that leaf clipping significantly reduced florivory by 40% relative to the undamaged control plants (Two-tailed test, $F_{1,72} = 4.08$, $P = 0.046$, Fig. 2a). Similarly, leaf clipping reduced capsule herbivory by 20% relative to the control plants with undamaged leaves (ANCOVA, Two-tailed test, $F_{1,72} = 5.46$, $P = 0.022$, Fig. 2b).

Florivory, capsule herbivory, and corolla size in response to pattern wheel damage

In contrast to leaf clipping, leaf damage with a pattern wheel had no significant effect on percent of flowers per plant with florivory ($F_{1,91} = 2.14$, $P = 0.146$), percent of leaves per plant with herbivory ($F_{1,117} = 0.0005$, $P = 0.984$), mean corolla length ($F_{1,79} = 1.58$, $P = 0.214$), or mean corolla width ($F_{1,79} = 0.295$, $P = 0.589$). The two populations, however, did differ in the proportion of flowers damaged ($F_{1,91} = 15.46$, $P < 0.001$), and the per-

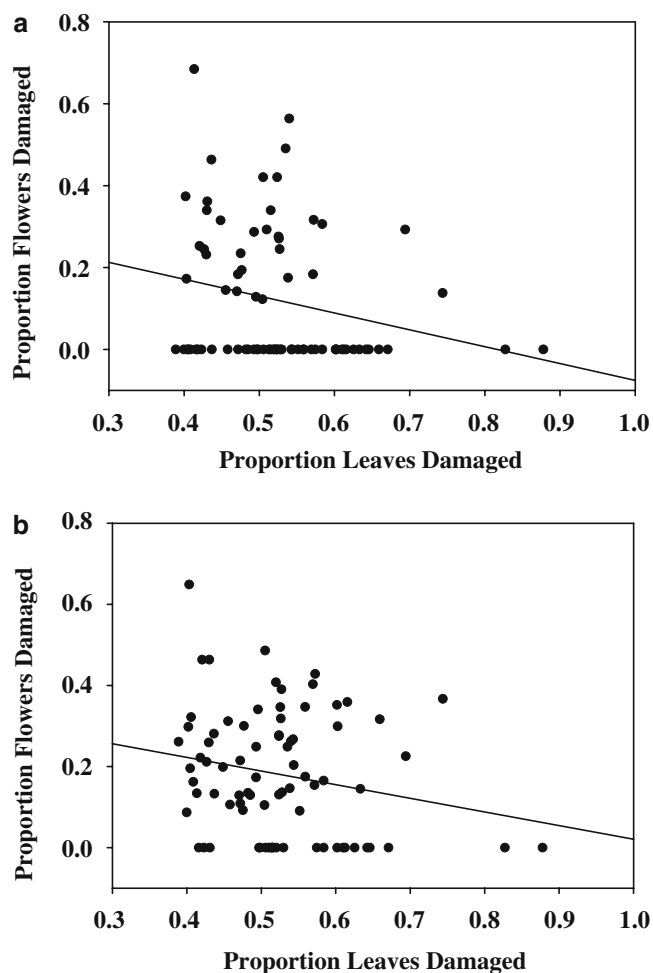


Fig. 1 The relationship between the percentage of leaves damaged and **a** the percentage of flowers damaged (Spearman rank correlation, $\rho = -0.22$, $P = 0.05$) and **b** the percentage of fruits damaged (Spearman rank correlation, $\rho = -0.17$, $P = 0.12$) in a survey of 79 naturally-occurring *N. attenuata* plants

cent of leaves damaged ($F_{1,117} = 30.36$, $P < 0.001$), regardless of the treatments.

Leaf herbivory and florivory in response to methyl jasmonate application

One month after methyl jasmonate treatment, treated plants accumulated roughly 70% less leaf damage than lanolin-only control plants (Wilcoxon rank sum test,

Table 1 Results from a MANCOVA with damage as a treatment effect and percentage flowers damaged and percentage capsules damaged as response variables

	Wilk's λ	F	Num. df	Den. df	P
Whole model	0.716	6.34	4	140	<0.001
Damage treatment	0.114	3.99	2	70	0.022
% Initial damage	0.286	10.02	2	70	<0.001

The percentage of leaves damaged prior to treatment application was used as a covariate in the model

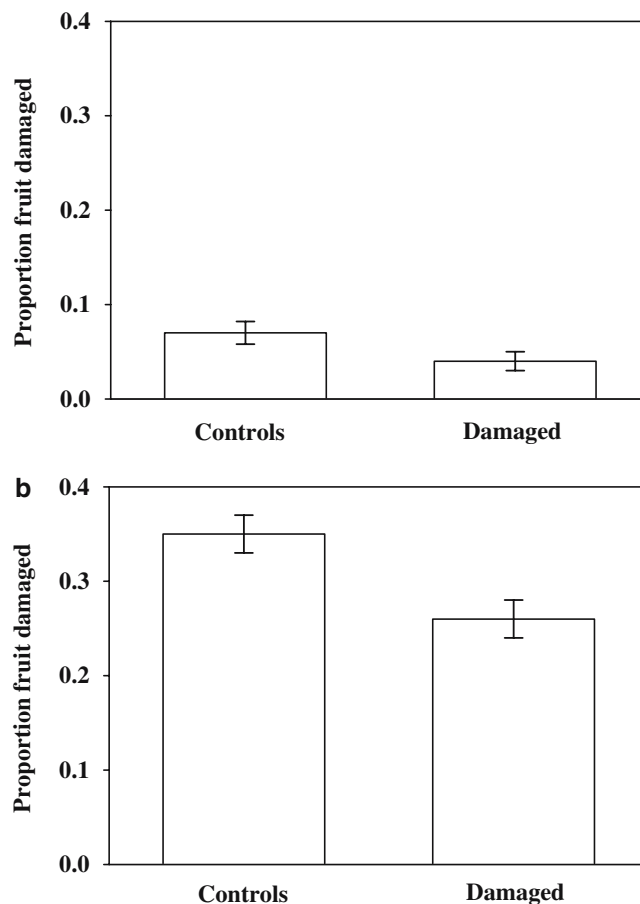


Fig. 2 The effects of mechanical damage on the distal portion of 30% of all leaves on wild tobacco plants. **a** Shows the effects of damage on florivory (one-way ANCOVA, $F_{1,72} = 4.08$, $P = 0.046$) and **b** shows the effects of damage on fruit damage (one-way ANCOVA, $F_{1,72} = 5.46$, $P = 0.023$)

$Z = -1.96$, 24 df, $P = 0.023$, Fig. 3a). This result is consistent with previous findings about the role of methyl jasmonate in the stimulation of induced foliar resistance. More surprisingly, methyl jasmonate-treated plants received much less damage to flowers than control plants (Wilcoxon rank sum test, $Z = -1.75$, 14 df, $P = 0.034$, Fig. 3b). Indeed, none of the plants treated with methyl jasmonate suffered any florivory.

Discussion

We found that experimentally damaging leaf tissue of *N. attenuata* reduced levels of naturally-occurring damage to reproductive tissues. We also observed a negative correlation between levels of foliar damage and reproductive tissue damaged in unmanipulated survey plants. Plants that received more leaf herbivory received less damage to flowers and marginally less damage to fruits. This relationship in unmanipulated individuals was consistent with the results of two of three experimental manipulations to leaves to induce resistance. When we severed the leaf midvein with scissors we caused plants

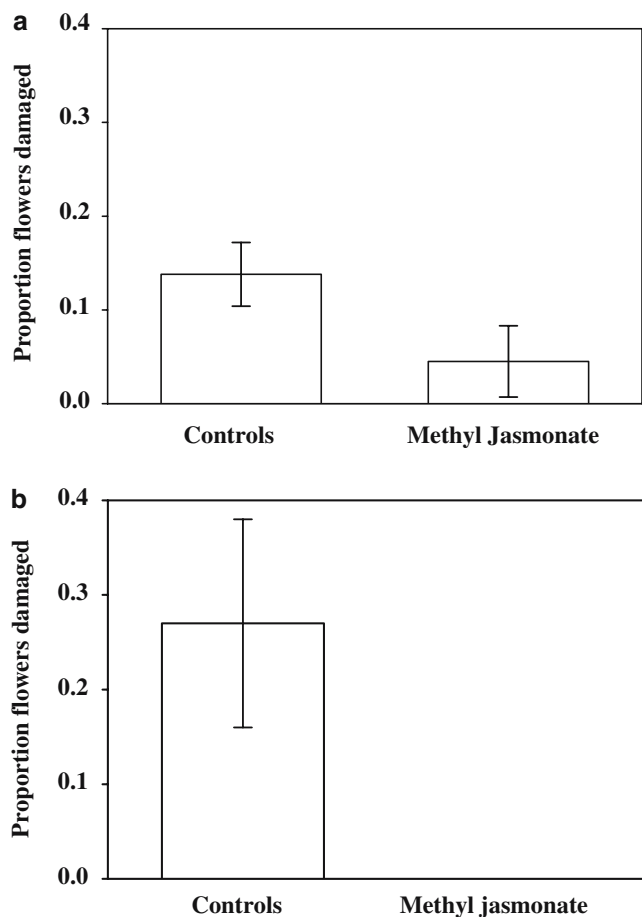


Fig. 3 The effect of exogenous methyl jasmonate application on the percentage of **a** leaves damaged by herbivores (Wilcoxon rank sum test, $Z = 0-1.96$, $P = 0.023$), and the **b** percentage of flowers consumed by florivores (Wilcoxon rank sum test, $Z = -1.75$, $P = 0.034$)

to receive less flower damage and less fruit damage relative to control plants. Furthermore, exogenous application of methyl jasmonate also reduced natural damage to flowers. Artificial leaf damage using a pattern wheel that punctured leaf tissue had no measurable effects on subsequent leaf or floral or fruit herbivory. A large literature documents the inconsistent consequences of different forms of experimental damage (e.g. Baldwin 1988), although most of our data indicate a negative association between leaf damage and subsequent damage to reproductive tissues.

Most studies of plant defense have studied only leaf tissue although some notable exceptions exist (e.g. Euler and Baldwin 1996; Gronquist et al. 2001). This is surprising since many plants suffer from herbivore damage to floral tissue (Karban and Strauss 1993; Breadmore and Kirk 1998; Krupnick and Weis 1999) and flowers may be critical in sexually-reproducing plants such as *N. attenuata*. Since flowers are so valuable, ODT predicts that flowers should be more constantly defended than other tissues, all other things being equal (McKey 1974; Rhoades and Cates 1976). However, ODT also predicts

that tissues with low risk of being discovered and attacked by herbivores should be less well defended. Flowers are usually more ephemeral than leaf tissue and therefore less likely to be discovered and damaged by herbivores. However, flowers may also be more conspicuous than leaves and may represent a higher quality resource, especially if pollen and nectar are taken into account (Mattson 1980). Predictions of ODT are thus ambiguous with respect to flowers.

Optimal defense theory also assumes that leaf traits and floral traits are independent of each other and that a plant is able to allocate resources to these organs independently. This may not be true for at least two reasons. First, traits are not inherited independently of one another but rather may show some degree of linkage in co-adapted gene complexes (Fenster and Galloway 2000). Second, a particular gene may provide several different consequences as a result of interactions among loci. As a result, leaf defensive traits and floral defensive traits may be associated. For example, Adler, Wink, and Lentz (unpublished manuscript) have shown that alkaloid content in leaves and nectar are positively correlated across individuals in *N. tabacum*. Furthermore, Irwin and Adler (2005) have shown that alkaloid concentrations in leaves and corollas are highly correlated with each other across populations in the distylous vine, *Gelsemium sempervirens*.

These correlations between floral and leaf traits may be the result of common mechanisms operating in both types of tissue. For example, Fineblum and Rausher (1997) suggested that the flavonoid pathway in plants may affect both flower color and resistance to herbivory, since this pathway produces both anthocyanins, which are ubiquitous floral pigments, and anti-herbivore compounds, such as flavones and tannins. Similarly, petal color and the amount of leaf herbivory plants receive are highly correlated in wild radish (Irwin et al. 2003; Strauss et al. 2004). Herbivores and pollinators may also be linked in *N. attenuata*. In this system *Manduca quinquemaculata* is both a pollinator as an adult and a major herbivore as a larva, so leaf traits that attract these specialist herbivores may also increase pollinator service and flower traits that attract pollinators may also increase herbivory.

In our population of *N. attenuata*, leaves suffered higher rates of herbivory than flowers. Since flowers can escape damage by being ephemeral in time, and constitutive defense may deter pollinators, theory predicts that flowers may be able to rely on less costly induced defenses rather than constitutive defenses. Since fruits are both more valuable and suffer higher rates of attack than flowers, we might predict that fruits employ more constitutive defenses than flowers. In fact, we found evidence for induced resistance in both the flowers and fruits of *N. attenuata*. Perhaps flowers and fruits showed induced responses when leaf tissue was removed because wild tobacco is developmentally constrained to use the same defense in all of its plant tissues. However, there are relatively little data in the literature regarding the

conservation of either the mode or quantity of defense among different plant tissues.

In conclusion, we found strong evidence for induced resistance in the flowers and fruits of *N. attenuata* when leaf tissue was damaged through experimental clipping, or experimental addition of methyl jasmonate. This evidence corroborated the results of a survey of damage in the field, which showed negative correlations between leaf damage and both floral and fruit damage. These results, to our knowledge, are the first to show that induced defenses in flowers operate effectively in a field setting with natural florivores. As a first step, this study points to the tempting idea that induced defenses may provide plants with a way to escape florivory through intermittent defenses when pollination is possible. An alternate and equally interesting possibility is that plants, being integrated organisms, employ similar methods of defense in different tissues throughout growth and reproduction.

Acknowledgements We thank Dan Dawson for allowing the use of SNARL facilities and property for our experiments. Mikaela Huntzinger, Rebecca Balogh, and Sarah Elmendorf all helped with field work. Comments on this manuscript were contributed by Lynn Adler, Judie Bronstein, Jay Rosenheim, Sharon Strauss, and two anonymous reviewers. We thank Lynn Adler and Rebecca Irwin for access to unpublished works. A.M. was supported with a Ford Foundation Predoctoral Grant, a UC-Davis Jastro-Shields award, a University of California NRS Mathias Grant, the UC-Davis Entomology Department, and the UC-Davis Center for Population Biology. R.K. was supported by an NSF grant # DEB 0121050. We also thank the UC-Davis Plant-Insect group for stimulating comments during the project.

References

- Agrawal AA (2001) Transgenerational consequences of plant responses to herbivory: an adaptive maternal effect? *Am Nat* 157:555–569
- Agrawal AA, Strauss SY, Stout MJ (1999) Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution* 53:1093–1104
- Aigner PA, Scott PE (2002) Use and pollination of a hawkmoth plant, *Nicotiana attenuata*, by migrant hummingbirds. *Southwest Nat* 47:1–11
- Baldwin IT (1988) The alkaloidal responses of wild tobacco to real and simulated herbivory. *Oecologia* 77:378–381
- Baldwin IT (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proc Natl Acad Sci USA* 95:8113–8118
- Baldwin IT, Karb MJ (1995) Plasticity in allocation of nicotine to reproductive parts in *Nicotiana attenuata*. *J Chem Ecol* 21:897–909
- Breadmore KN, Kirk WDJ (1998) Factors affecting floral herbivory in a limestone grassland. *Acta Oecol* 19:501–506
- Conover WJ, Iman RI (1981) Rank transformations as a bridge between parametric and nonparametric statistics. *Am Stat* 35:124–129
- Creelman RA, Mullet JE (1997) Biosynthesis and action of jasmonates in plants. *Annu Rev Plant Physiol Plant Mol Biol* 48:355–381
- Euler M, Baldwin IT (1996) The chemistry of defense and apparency in the corollas of *Nicotiana attenuata*. *Oecologia* 107:102–112
- Fenster CB, Galloway LF (2000) Inbreeding and outbreeding depression in natural populations of *Chamaecrista fasciculata* (Fabaceae). *Conserv Biol* 14:1406–1412
- Fineblum WL, Rausher MD (1997) Do floral pigmentation genes also influence resistance to enemies? The W locus in *Ipomoea purpurea*. *Ecology* 78:1646–1654
- Gronquist M, Bezzerides A, Attygalle A, Meinwald J, Eisner M, Eisner T (2001) Attractive and defensive functions of the ultraviolet pigments of a flower (*Hypericum calycinum*). *Proc Natl Acad Sci USA* 98:13745–13750
- Irwin RE, Strauss SY, Storz S, Emerson A, Guibert G (2003) The role of herbivores in the maintenance of a flower color polymorphism in wild radish. *Ecology* 84:1733–1743
- Irwin RE, Adler LS (2005) Correlations among traits associated with herbivore resistance and pollination: implications for pollination and nectar robbing in a distylous plant. *Am J Bot* 93(1) (in press)
- Karban R, Baldwin IT (1997) Induced responses to herbivory. University of Chicago Press, Chicago
- Karban R, Strauss SY (1993) Effects of herbivores on growth and reproduction of their perennial host, *Erigeron glaucus*. *Ecology* 74:39–46
- Karban R, Maron J, Felton GW, Ervin G, Eichenseer H (2003) Herbivore damage to sagebrush induces resistance in wild tobacco: evidence for eavesdropping between plants. *Oikos* 100:325–332
- Kessler A, Baldwin IT (2002) Plant responses to insect herbivory: the emerging molecular analysis. *Annu Rev Plant Biol* 53:299–328
- Krupnick GA, Weis AE (1999) The effect of floral herbivory on male and female reproductive success in *Isomeris arborea*. *Ecology* 80:135–149
- Mattson WJ (1980) Herbivory in relation to plant nitrogen content. *Annu Rev Ecol Syst* 11:119–161
- McKey D (1974) Adaptive patterns in alkaloid physiology. *Am Nat* 108:305–320
- Neter J, Kutner MH, Wasserman W, Nachtsheim CJ (1996) Applied linear statistical models, 4th edn. McGraw-Hill, Irwin
- Reinbothe S, Mollenhauer B, Reinbothe C (1994) Jips and rips: the regulation of plant gene expression by jasmonates in response to environmental cues and pathogens. *Plant Cell* 6:1197–1209
- Reymond P, Weber H, Damond M, Farmer EE (2000) Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. *Plant Cell* 12:707–719
- Rhoades DF (1979) Evolution of plant chemical defense against herbivores. In: Rosenthal GA, Janzen DH (eds) *Herbivores: their interaction with secondary plant metabolites*, 1st edn. Academic, New York
- Rhoades DF, Cates RG (1976) Toward a general theory of plant antiherbivore chemistry. *Recent Adv Phytochem* 10:168–210
- Schaller F (2001) Enzymes of the biosynthesis of octadecanoid-derived signalling molecules. *J Exp Bot* 52:11–23
- Scheiner SM (2001) MANOVA: multiple response variables and multispecies interactions. In: Scheiner SM, Gurevitch J (eds) *Design and analysis of ecological experiments*. Oxford University Press, New York, pp 99–115
- Strauss SY (1997) Floral characters link herbivores, pollinators, and plant fitness. *Ecology* 78:1640–1645
- Strauss SY, Irwin RE, Lambrix VM (2004) Optimal defence theory and flower petal colour predict variation in secondary chemistry of wild radish. *J Ecol* 92:132–141
- Wells PV (1959) An ecological investigation of two desert tobaccos. *Ecology* 40:626–644
- Zangerl AR, Berenbaum MR (1993) Plant chemistry, insect adaptations to plant chemistry, and host plant utilization patterns. *Ecology* 74:47–54
- Zangerl AR, Rutledge CE (1996) The probability of attack and patterns of constitutive defense: a test of optimal defence theory. *Am Nat* 147:599–608