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Herbivory and caterpillar regurgitants amplify the wound-induced increases in jasmonic acid but not nicotine in *Nicotiana sylvestris*

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Abstract. Both herbivory and mechanical damage result in increases in the concentration of the wound-signal molecule, jasmonic acid (JA), and the defense metabolite, nicotine, in native tobacco plants, *Nicotiana sylvestris* Speg. et Comes (Solanaceae). We found that higher concentrations of JA resulted from herbivory by *Manduca sexta* (L.) larvae than from the mechanical damage designed to mimic the herbivory. While both herbivory and mechanical damage increased JA concentrations in roots of wounded plants, herbivory did not induce either higher root JA or nicotine responses than mechanical damage. In a separate experiment in which mechanical damage was not designed to mimic herbivory, JA responses to herbivory were higher than those to mechanical damage, but the whole-plant (WP) nicotine responses were smaller. Furthermore, when regurgitants from *M. sexta* larvae were applied to standardized mechanical leaf wounds, leaf JA responses were dramatically amplified. However, neither the root JA response nor the WP nicotine response was comparably amplified by application of regurgitants. Our findings demonstrate that the response of *N. sylvestris* to herbivory is different from its response to mechanical damage; moreover, oral secretions from larvae may be partly responsible for the difference. During feeding, *M. sexta* larvae appear to modify the plant's normal defensive response to leaf wounding by reducing the systemic increase in root JA after leaf damage and the subsequent WP nicotine response.

Key words: Defense (induced) – Jasmonic acid – *Nicotiana* (wound response) – Nicotine – *Manduca* (oral secretion) – Regurgitant

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Abbreviations: ANOVA = anlysis of variance; JA = jasmonic acid; PLSD = protected least significant difference; WP = whole plant

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Introduction

Interactions between plants and their pathogens are often marked by a degree of specificity (Flor 1956, 1971) which has not been observed in inducible plant responses against insect herbivores that do not live inside plant tissues. Rhoades (1985) reviewed inducible plant defenses against non-endophytic herbivores and suggested that selection may favor both plants and herbivores that are able to respond differentially to each other. Since then, few studies have compared the induced defensive responses of plants to herbivore attack with their responses to mechanical simulations of such attack, the first step in documenting specificity in these plant-herbivore interactions (Baldwin 1990; Lin et al. 1990).

Two sets of observations bear on the specificity of plant defensive responses to herbivores. First, caterpillar oral secretions and regurgitants are known to elicit systemic emissions of volatiles from plants which, in turn, are thought to function as "alarm" calls from plants to the predators of herbivores (Turlings et al. 1990; Turlings and Tumlinson 1992; Mattiacci et al. 1995; Turlings et al. 1995). These systemic responses are generally not elicited by mechanical simulations of herbivore feeding unless caterpillar regurgitants are added to the wounds. A second set of related observations addresses the specificity of plant oxidative responses to herbivory which may be similar to the well-known "oxidative burst" response of plants to pathogens (Apostol et al. 1989). Herbivory (Hildebrand et al. 1986; Bi et al. 1994; Stout et al. 1994; Bi and Felton 1995) and mechanical damage (Hildebrand et al. 1989) can increase foliar lipoxygenase (LOX) activity and the magnitude of LOX induction varies with the type of damage inflicted (Stout et al. 1994). Stimulation of LOX activity by herbivory raises the possibility that LOX pathway products such as jasmonic acid (JA) (Vick and Zimmerman 1983) may be stimulated by herbivory as well. Jasmonic acid is a ubiquitous, damage-inducible compound which elicits a diverse suite of plant defense responses (Hamberg and Gardner 1992; Sembdner and Parthier 1993; Farmer 1994; Creelman and Mullet 1995).

In *Nicotiana sylvestris* plants, JA plays an important role as a wound-signal molecule in the wound-induced accumulation of nicotine, an important defensive compound in this plant. Mechanical leaf wounding results in a large, rapid increase in JA in wounded leaves and a smaller, delayed increase in the nicotine-synthesizing roots (Baldwin et al. 1994a, 1997). Direct transport of JA from leaves to roots can account for the systemic increase in root JA pools after leaf wounding (Zhang and Baldwin 1997). The exogenous addition of nanomolar amounts of JA increases endogeneous JA pools and de-novo nicotine biosynthesis as well as WP nicotine accumulation in a manner similar to that elicited by leaf wounding (Baldwin et al. 1994a, 1997). Folivory by larvae of the tobacco specialist, *Manduca sexta* (Lepidoptera: Sphingidae), also increases WP nicotine accumulations but not to the extent that would be expected from the amount of leaf damage resulting from larval feeding. Baldwin (1988) simulated the timing and spatial distribution of leaf damage caused by caterpillar feeding on *N. sylvestris* plants with microdissection scissors and found that induced levels of nicotine following damage were greater than the levels induced by caterpillar feeding. Microstructural differences between folivory and mechanical simulations of the folivory or differences in the temporal distribution of damage may have accounted for the differences in nicotine responses, but it is also possible that some factor in herbivore saliva suppressed the damage-induced accumulation of nicotine.

While observations of increased oxidative responses to herbivory raise the expectation that JA levels should be amplified, the apparent suppression of the induced nicotine response by *M. sexta* larval feeding on leaves indicates the alternative hypothesis that the JA response is suppressed as well. We test the hypothesis that JA is differentially induced by herbivory and mechanical damage and examine the role of regurgitants from *M. sexta* larvae on the wound-JA-nicotine signal cascade.

Materials and methods

Insect culture and collection of regurgitants. *Manduca sexta* (L.) larvae were hatched from eggs (Carolina Biological Supply, Burlington, N.C. USA) and reared en masse with fresh cut *N. sylvestris* Speg. et Çomes foliage at 26 °C with a 16-h photophase. After 2–3 d, larvae were reared individually in plastic soufflé cups. Regurgitants were collected from the oral cavity of fourth- to fifth-instar larvae with a 25-µl capillary and stored frozen at −60 °C until use.

Plant growth. To reduce between-plant genetic variance, seeds used in these experiments were full-sib progeny from greenhouse-grown plants used in Baldwin et al. (1990). Seeds were germinated in soil and grown individually in 1-L hydroponic chambers as described in Baldwin and Schmelz (1994). At the start of each experiment, plants were assigned to treatment groups by wet mass (see Ohnmeiss and Baldwin 1994) in order to reduce the variance in plant mass within and between treatment groups.

Nicotine and JA analysis. Whole-plant (WP) nicotine levels were determined by HPLC analysis of methanol: water extracts of homogenized, freeze-dried plants, as described in Baldwin and Schmelz (1994). Analysis of JA was determined by GC-MS with a doubly labeled JA ([1, 2-^{13}C]-JA) as an internal standard as described in Baldwin et al. (1997).

Induction of JA in wounded leaves by herbivory and a mechanical simulation. We compared the leaf JA response to caterpillar herbivory and a mechanical simulation which mimicked the spatial arrangement and timing of leaf removal by the herbivores. Larvae of *M. sexta* were reared on cut *N. sylvestris* leaves for 7 d after egg hatch. A third-instar larva was placed on a single leaf and allowed to feed 1 h; some plants were harvested at 30 min. Caterpillars were restricted to the leaf upon which they were placed but were allowed to move and feed freely on that leaf. These were large, fully expanded leaves, three or four nodes below the apical leaf. For each plant with caterpillars, two plants of similar wet mass were designated scissor-damaged plant and undamaged control, respectively. Plants had six to eight fully expanded leaves and weighed 11.20 ± 1.26 (SD) g wet mass at the start of the experiment. As caterpillars fed, matching areas of leaf tissue were removed with microdissection scissors from the corresponding leaf on the scissor-damaged plant. The progress of caterpillar feeding was checked, and caterpillar damage was progressively mimicked with scissors at several times during the feeding period. Three groups of plants were harvested at 240, 420 and 1440 min after the first non-exploratory bite was taken by a larva. For these groups of plants, herbivory-mimicking scissor damage was made at 15, 30, 45, and 60 min after the first bite. Three additional groups of plants were harvested at 30, 90, and 135 min after the first bite. For these plants, an extra application of scissor damage was made at 5 min after the first bite. At harvest, leaves from each of the three treatments were rapidly excised with a razor, weighed, and photographed so that the amount of leaf area removed, and length of the damaged edge, could be determined by image analysis. Plants in the 420- and 1440-min harvests were not photographed. There were 5 groups of replicate plants for each harvest time with the exception of the JA harvests at 420 and 1440 min which had only 2 and 3 plants per treatment group respectively; in total, 75 plants were used in this experiment.

Whole-plant nicotine and JA responses to herbivory and standardized mechanical damage. We allowed caterpillars to feed for 2 d in order to evaluate the plant responses to a sustained period of folivory. The mechanical damage treatment used a standard mechanical damage protocol which was known to induce increases in plant nicotine without significantly reducing plant growth (Baldwin and Schmelz 1994; Ohnmeiss and Baldwin 1994). A single third-instar *M. sexta* larva was placed on each of the two largest leaves on the plant and allowed to feed freely for 7 h. Plants had four to six fully expanded leaves and weighed 5.25 ± 1.26 (SD) g wet mass. After 7 h, caterpillars were removed from the plants overnight. At 21 h after the first bite – 14 h after the end of the first feeding period – the same caterpillars were placed on the same plants and allowed to feed for 5 h. At the end of this second period, caterpillars were removed from the plants. In the mechanical damage treatment, plants were wounded on leaves in the same position as those the caterpillars were eating by rolling a fabric pattern wheel over the leaf lamina parallel to the midrib. One roll of the tracing wheel on each side of the midrib was applied as the caterpillars began to feed; leaves were damaged five more times with the tracing wheel at 90 min, 180 min, 20 h, 22 h, and 25 h after the experiment began. Over the course of the experiment, damaged leaves from plants in both groups and equivalent leaves from undamaged control plants were harvested for JA analysis at 90 min, 180 min, 360 min, 18 h, 21.5 h, and 23 h. Baldwin et al. (1994b) and Ohnmeiss and Baldwin (1994) examined the time course of nicotine induction in *N. sylvestris* plants following damage; peak nicotine concentrations were reached in less than 5 d following a single episode of damage. We therefore used this schedule for our nicotine samples harvesting a seventh group for nicotine analysis 5 d after the start of damage. Each treatment by time combination was replicated 5 times, thus 90 plants were harvested for JA analysis and 15 plants were harvested for nicotine analysis.

Whole-plant nicotine and JA responses in roots and shoots to herbivory and a mechanical simulation. Here we extend our examination of differential induction by herbivory and mechanical simulations of this damage to the systemic JA response in the roots as well as to WP nicotine induction. Caterpillars used in the experiment were third- to fourth-instar larvae. Two caterpillars were placed on each of the two largest, fully expanded leaves on each plant. At the start of the experiment, plants had from four to six leaves and weighed 4.36 ± 0.79 (SD) g fresh mass. Caterpillars were allowed to feed for 1 h, and scissor damage was used to mimic the progressively increasing caterpillar damage at 5, 15, 30, 45, and 60 min after the first bite. Damaged leaves and roots were harvested for JA analysis at 135, 240, and 420 min after damage. A fourth group of plants was harvested after 5 d for nicotine analysis. Five replicate plants were harvested at each harvest time point for JA analysis and 10 replicates were harvested for nicotine analysis; 75 plants were used in total.

Whole-plant nicotine and JA responses in roots and shoots to caterpillar regurgitants. Here we apply either larval regurgitants or distilled water to a standardized leaf wound on two leaves and compare the JA and nicotine responses. Plants were wounded on equivalent leaves by rolling a fabric pattern wheel once over the leaf lamina parallel to the midrib to produce 4.5 1-mm^2 punctures per cm of leaf lamina. One-hundred microliters of either regurgitant or water was applied to these punctures at time 0. Five replicate plants were harvested from each of the regurgitant and water treatments and from an unwounded control treatment 90, 180 and 420 min after application. Plants had from four to six leaves and weighed 3.90 ± 0.79 (SD) g fresh mass at the start of the experiment. A separate group of 30 plants weighing 2.65 ± 0.87 (SD) g fresh mass (10 replicates/treatment) were treated as described above and harvested 5 d after application for nicotine analysis.

Statistical analysis. One-way analysis of variance (ANOVA) and protected contrasts with Fisher's protected least significant difference (PLSD) test within ANOVAs were used to analyze nicotine and JA pools.

Results

Caterpillar feeding induces higher levels of JA in damaged leaves than does a mechanical simulation of the herbivory. Caterpillars consumed an average of 3.42 ± 0.34 cm^2 of leaf area, leaving 10.27 ± 0.60 cm of damaged leaf perimeter; scissor-damaged plants had 3.49 ± 0.28 cm^2 of leaf area removed, leaving 10.11 ± 0.53 cm of damaged leaf perimeter. Neither the amount of leaf area removed, nor the length of cut edge exposed were significantly different ($F_{1,38} < 0.04$, $P > 0.84$).

Undamaged leaves had low levels of JA throughout the experiment, ranging between 15.24 ± 1.2 ng/g to 32.11 ± 7.02 ng/g (Fig. 1). Concentrations of JA were higher in damaged leaves than in undamaged leaves within 30 min of the first signs of caterpillar feeding (Fig. 1) and reached a maximum between 30 and 135 min after the start of feeding for both scissor-damaged and caterpillar-damaged plants. Caterpillar-damaged plants produced significantly greater amounts of JA in response to damage than did scissor-damaged plants (Fisher's PLSD, $P = 0.0001$; ANOVA for damage treatment $F_{2,60} = 46.6$, $P < 0.0001$). For both scissor-and caterpillar-damaged leaves, the largest JA concentrations were observed 90 min after the start of feeding. Caterpillar feeding resulted in JA concentrations which

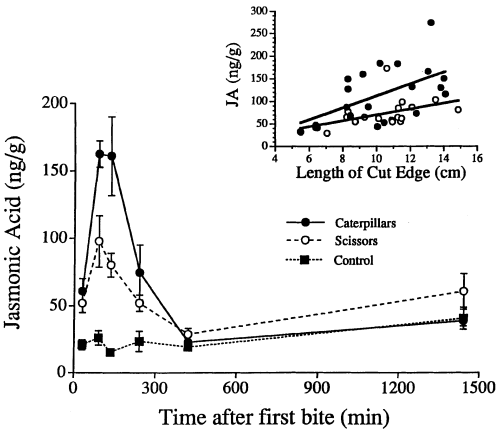

Fig. 1. Mean (±1 SE) JA concentrations in undamaged *Nicotiana sylvestris* leaves and leaves damaged by the feeding activity of third-instar *Manduca sexta* larvae (caterpillars) or by microscissors (scissors) mimicking the spatial and temporal characteristics of larval feeding. Scissor damage and harvests were coordinated with the first non-exploratory bite made by a caterpillar. Five replicate plants from each treatment group were analyzed at each harvest, except at the 420-min and 1440-min harvests which had 2 and 3 replicates, respectively. *Insert*. Each damaged leaf from the first four harvests was photographed and the length of the cut edge was measured and regressed against the JA concentrations in the leaf. Leaves damaged by caterpillars produced approximately twice as much JA per cm of cut edge ($y = 13.1 \text{ ng} \cdot \text{g}^{-1} \text{ cm} - 19.5$, $r^2 = 0.32$) as leaves damaged by microscissors ($y = 6.5 \text{ ng} \cdot \text{g}^{-1} \text{ cm} + 4.7$, $r^2 = 0.25$)

were 1.6 times higher than those in scissor-damaged leaves; caterpillar-damaged plants accumulated 162.6 ± 9.9 ng/g, scissor-damaged plants accumulated 97.8 ng/g. In both scissor-damaged and caterpillar-damaged plants, JA concentrations relaxed to values typical of undamaged leaves by 420 min (7 h) after damage had begun. A regression of JA concentrations of leaves harvested in the first four harvests against the length of the damaged leaf edge revealed that leaves damaged by caterpillars produced approximately twice as much JA per centimeter of cut edge compared with leaves damaged by microscissors (Fig. 1).

Despite higher JA concentrations in damaged leaves, herbivory induces lower nicotine levels than does standardized mechanical damage. Concentrations of JA in undamaged leaves varied from 33.6 ng/g to 63.6 ng/g. Damage from caterpillars and the pattern tracing wheel caused a significant increase in JA levels; both the main effects of damage treatment and time as well as the treatment by time interaction were highly significant (Fig. 2; F's$_{5;2;10,69} > 3.87$; P's < 0.0005). All pairwise comparisons of the three treatment groups were highly significant (all P's < 0.0001). Mean induced levels of JA increased to as much as 300 ± 60.1 ng/g in plants fed upon by caterpillars during the initial 3 h of the first feeding period and then reached a plateau. Overnight, JA in caterpillar-damaged leaves declined but remained at an elevated level relative to undamaged leaves. Resumption of feeding resulted in a second increase in JA concentrations; however, neither the relative increase in concentration nor the absolute concentrations

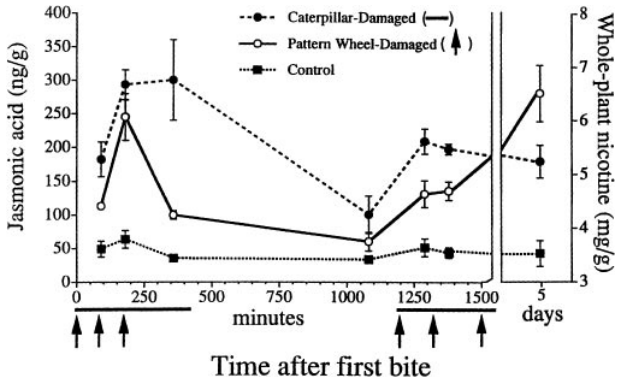

Fig. 2. Mean (±1 SE) JA and nicotine concentrations (in damaged leaves and whole plants, respectively) in undamaged plants and plants subjected to mechanical damage inflicted with a fabric pattern wheel and caterpillar feeding. Caterpillars were allowed to feed freely during the two intervals indicated by the *solid horizontal bars* below the x-axis; *arrows* represent incremental increases in the amount of pattern-wheel damage which were synchronized with the caterpillar feeding. Five replicate plants were harvested at each harvest time from each treatment group: six harvests were for jasmonic acid analysis and one harvest 5 d after the start of caterpillar feeding (after the axes break) was for nicotine analysis

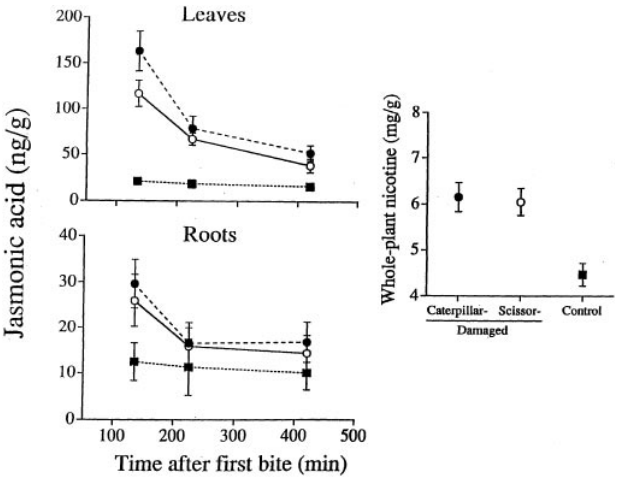

Fig. 3. Mean (±1 SE) JA concentrations in leaves and roots of undamaged *N. sylvestris* plants (■–■) and plants damaged by the feeding activity of third-instar *M. sexta* larvae (caterpillars; ●–●) or by microscissors; (scissors; ○–○) mimicking the spatial and temporal characteristics of larval feeding. Scissor damage and harvests were coordinated with the first non-exploratory bite made by a caterpillar. Five replicate plants from each treatment group were harvested 5 d after the start of caterpillar feeding for WP nicotine determinations

attained were as great as during the initial period of JA induction (Fig. 2). In leaves damaged with the pattern tracing wheel, the JA levels reached maximum values 180 min after the start of damage on the first day and subsequently relaxed. In contrast, induced levels remained elevated in caterpillar-damaged plants. Overnight, JA levels in pattern wheel-damaged plants declined to the concentration found in undamaged plants (60.3 ± 14.1 ng/g), and subsequent wounding on the second day also failed to raise JA to levels comparable to those of caterpillar-damaged plants. As with caterpillar-damaged plants, the amount and relative degree of JA increase caused by pattern wheel damage was lower on the second day than on the first.

Nicotine accumulation was significantly induced by both sources of damage (ANOVA for damage treatment $F_{2,12} = 15.82$, $P = 0.0004$). The increase in mean nicotine concentrations over controls ranged from 48% in caterpillar-damaged plants to 84% in plants damaged with the pattern wheel. In marked contrast to the differences in JA induction in damaged leaves, the mean induced nicotine level of caterpillar-damaged plants was only 80% of the level in plants damaged with the pattern tracing wheel (Fig. 2).

Despite higher JA induction in leaves damaged by caterpillar feeding, neither root JA induction nor WP nicotine induction are increased by caterpillars above that induced by a mechanical simulation of herbivory. Caterpillar feeding again produced higher JA concentrations in damaged leaves than did the same amount of scissor damage (Fig. 3, ANOVA for damage treatment $F_{2,36} = 27.9$, $P < 0.0001$, Fisher's PLSD, $P < 0.0001$). Mean levels of JA in undamaged plants ranged from 15 to 23 ng/g; scissor- and caterpillar-damaged plants had concentrations of 118.6 ± 14.8 ng/g and 167.1 ± 37.8 ng/g, respectively. Herbivory and mechanical damage increased root JA levels significantly above levels in controls (Fig. 3, ANOVA for damage treatment, $F_{2,35} = 3.4$, $P = 0.044$), but there was no significant difference between these two damage treatments (Fisher's PLSD, $P = 0.61$). Both damage treatments significantly increased WP nicotine concentrations ($F_{2,27} = 10.84$, $P = 0.0003$; Fig. 3) to levels that were 35–38% higher than undamaged controls, but there was no significant difference between the two damage treatments (Fisher's PLSD, $P = 0.61$).

Caterpillar regurgitants amplify the leaf JA response to wounding but not the WP nicotine responses. Water-treated wounded leaves had JA concentrations (96.5 ± 48.1 ng/g) that were 93% higher than those of unwounded controls (50.1 ± 18.2 ng/g) at the 90-min harvest. In contrast, regurgitant-treated wounded leaves at the 90-min harvest had JA concentrations (708.9 ± 130.9 ng/g) that were 13.1-fold higher than unwounded controls (Fig. 4). The systemic JA response in root tissues was much more attenuated. The systemic response in the roots is known to reach a maximum after a single damage event 180 min after wounding (Baldwin et al. 1997). At this harvest time, regurgitant-treated plants had JA concentrations (93.0 ± 22.0 ng/g) that were only 72% higher than those of unwounded controls (54.3 ± 4.3 ng/g), whereas water-treated damaged plants had concentrations (57.8 ± 3.4 ng/g) similar to those of unwounded controls (Fig. 4). In both root and leaf tissues, the responses to wounding were significant (ANOVA for damage treatment $F_{2,54} = 5.736$ and 41.76, respectively; P's < 0.0055). The wounding treatments increased nicotine concentrations 38–40% above the levels found in unwounded plants (Fisher's PLSD, P's < 0.0036), but concentrations in regurgitant-treated

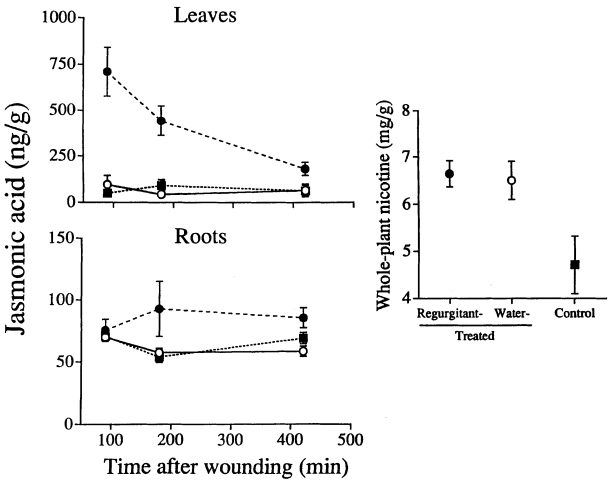

Fig. 4. Mean (±1 SE) JA concentrations in leaves and roots of undamaged *N. sylvestris* plants (■–■) and plants damaged at time 0 by two rolls of the fabric pattern wheel and treated with 100 µl of regurgitants from *M. sexta* larvae (●–●) or water (○–○). Five replicate plants from each treatment group were harvested for the three jasmonate harvests and for nicotine determinations at 5 d after treatment in a separate experiment

plants did not differ from water-treated plants (Fisher's PLSD, $P = 0.812$; Fig. 4).

Discussion

Our findings clearly demonstrate that the presence of caterpillars or their regurgitants dramatically amplifies increases in JA concentrations locally in wounded leaves, but this amplification is less than dramatic for the systemic JA response in the roots. The WP nicotine response is not amplified at all. While no studies have explicitly compared the JA responses to herbivory with careful simulations of the resulting damage as we have done here, the combined results of a number of studies indicate that biotic wounding agents may generally amplify the induced JA response in comparison with simple mechanical wounding. The JA concentrations in our experiments increased 7- to 8-fold when caterpillars caused the leaf damage compared with the 4- to 5.5-fold increases when scissors were the damaging agent (Figs. 1, 3). Similarly, treatment with regurgitants resulted in a 13-fold increase compared with the < 1-fold increase that resulted from the mechanical damage (Fig. 4). Blechert et al. (1995) found that JA increased by ca. 25-fold in *Vicia faba* leaves, but this study did not include a mechanical damage control. Jasmonic acid increases in mechanically wounded leaves from a variety of species can range from three- to nine-fold (reviewed in Farmer 1994; Bell et al. 1995; Peña-Cortes et al. 1995). In cell culture systems, JA increases triggered by fungal- and cell wall-derived elicitors (Farmer 1994; Mueller and Brodschelm 1994) are nearly always much higher (13- to more than 100-fold increases) than wound-induced JA increases in leaves. Thus, the nature of the inducing agent appears to influence the magnitude of the JA response.

Our findings (Figs. 1 and 3) indicate that herbivores amplify the JA response via some mechanism other than the macroscopic arrangement of leaf removal and that factors from the caterpillar's oral cavity are in part responsible for the amplification (Fig. 4). Whether the regurgitants are normally transferred from insect to plant during feeding is not clear. Microstructural differences may have contributed to the differences we saw, and it is helpful to compare nicotine and JA induction without the uncertainty involved in directly comparing caterpillar-damaged with scissor damaged-plants. Plants damaged with the pattern roller wheel attained lower JA concentrations (Fig. 2) but, unlike the pattern roller wheel, caterpillars consume leaf tissue as they damage plants. Thus, it is possible that the lower mass of tissue remaining in leaves from caterpillar-damaged plants might have produced smaller JA pools and thus a smaller damage signal. However, this was not the case; induced JA pools in the damaged leaves of caterpillar-damaged plants were always higher than those in pattern wheel-damaged plants, and this difference was statistically significant (Fisher's PLSD, $P < 0.0001$; overall ANOVA, $F_{2,69}$ (damage effect) = 82.32, $P < 0.0001$). Averaged over all sampling times, herbivory induced 105.4 ± 75.3 ng more JA per plant than did pattern-wheel damage.

A notable feature of our findings is that the quantitative relationships among wounding and JA and WP nicotine responses are altered by caterpillars and their secretions. We have investigated these relationships with a quantitative wounding protocol and found linear relationships among the number of leaf punctures, the levels of JA induced in wounded leaves, and the increases in WP nicotine (Baldwin et al. 1997; Ohnmeiss et al. 1997). The fact that the amplification of JA concentrations in the leaf does not result in greater nicotine responses indicates that relationships between JA induction and plant defense may be labile and that *M. sexta* may suppress the systemic defensive response of its host. The observation that the systemic JA response in the roots was not significantly increased by herbivory (Fig. 3) and only weakly increased in regurgitant-treated plants (Fig. 4) raises the possibility that caterpillars may interfere with the transport of JA (or some other systemic signal) between damaged leaves and roots. However, uncoupling between the root JA response and the induction of nicotine biosynthesis is not precluded. Furthermore, other wound signals may be involved, as has been shown for ethylene in the JA induction of proteinase inhibitor production in tomato (O'Donnell et al. 1996). For example, Creelman et al. (1992) suggested, on the basis of induction kinetics, that JA induction may help maintain the wound-induced expression of the defense-related gene *chs1* rather than directly trigger it.

Herbivory by *M. sexta* larvae has been independently shown to decrease induction of both nicotine (Baldwin 1988; this report) and proteinase inhibitors (Jongsma et al. 1994) relative to mechanical damage. Our data indicate that this herbivore is capable of suppressing the wound-induced responses of its hosts by altering the

relationship between signal induction and defense activation. Caterpillar regurgitants are known to elicit systemic emissions of volatiles from plants which, in turn, are thought to function defensively. In this regard, it is interesting to note that the exogenous application of jasmonates (albeit at high concentrations) have also been demonstrated to elicit sytemic volatile emissions (Boland et al. 1995). Perhaps the jasmonate cascade will prove to be an important intermediary of plant-insect interactions.

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