The jasmonate pathway alters herbivore feeding behaviour: consequences for plant defences

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Abstract

The jasmonate pathway is a highly conserved defensive cascade in plants that regulates the induction of resistance against herbivores; however, its role in herbivore feeding behaviour remains unknown. We used a mutant tomato plant (def-1) deficient in the production of jasmonate-related defensive proteins to test the hypothesis that genotypes with a reduced ability to induce resistance have a higher and more concentrated pattern of herbivore damage. Wild-type and def-1 plants received either damage by Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae) caterpillars or no damage. After treatment, we tested for systemic responses by allowing a free roaming S. exigua caterpillar to feed on the undamaged portions of plants. Weight-gain and leaf consumption of S. exigua were highest on def-1 plants, regardless of prior herbivore damage. Def-1 plants also had fewer numbers of leaves and leaflets eaten, and fewer feeding holes, which was indicative of a more concentrated distribution of damage on mutant compared to wild-type plants. Following these results, we mimicked the amount and distribution of feeding damage that wild-type or jasmonate-deficient plants would receive on wild-type plants to test whether changes in feeding behaviour may feedback to influence the expression of induced resistance. We mimicked the distribution of damage in wild-type and jasmonate-deficient plants by allowing caterpillars to feed on either one (leaf 1 or 2) or two leaves (leaf 1 and 2). Increased herbivore damage resulted in higher proteinase inhibitor (PI) activity, a jasmonate-regulated defensive protein, and lower S. exigua performance on wild-type but not jasmonate-deficient plants. Compared to undamaged plants, a concentrated pattern of herbivore damage increased systemic resistance; these induced responses were greater on leaflets with stronger vascular connections to the damaged leaf. A more dispersed pattern of caterpillar damage altered the expression of induced responses, but the outcome depended on the specific pattern of damage. When leaf 1 was damaged and then leaf 2, the undamaged (third) leaf (which is more strongly connected to leaf 1 than 2) expressed reduced the PI activity compared to plants receiving concentrated damage to leaf 1; whereas in plants where leaf 2 was first damaged and then leaf 1, there were no differences in PI activity in leaf 3 compared to plants receiving concentrated damage to leaf 2. Thus, induction of the jasmonate pathway may not only determine the amount and distribution of feeding damage by herbivores, but this may feedback to affect the subsequent expression of plant defence.

Introduction

Herbivores perceive variation in the chemical composition of plants, and modify their feeding behaviour in order to

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systemically on undamaged leaves (Karban & Baldwin, 1997). The strength of these plant responses can vary depending on the amount, concentration, and location of damage.

Spatial heterogeneity in plant resistance can affect herbivore feeding behaviour. Schultz (1983) argued that within-plant spatial heterogeneity due to induced defences may cause foraging herbivores to move more, generating a dispersed pattern of damage on plants. For instance, Edwards et al. (1991) showed that the common quaker moth (Orthosia stabilis Denis and Schiffermüller) moved more often, ate from more leaves, and removed less area per leaf when feeding on previously damaged leaves than on undamaged leaves.

The increased movement of herbivores on plants could affect the subsequent induction of chemical defences if it results in a reduced amount or distribution of feeding from the herbivore. Plant responses are often induced in a dose-dependent manner. For example, Underwood (2000) found that a greater amount of damage by the Mexican bean beetle, Epilachna varivestris Mulsant, on soybeans led to an increased level of induced resistance. Additionally, changes in the distribution of herbivore damage within plants may affect the expression of defensive chemicals for many reasons, one being a plant’s vascular architecture.

Plant vascular architecture refers to the degree of connectedness between leaves in a plant. In general, vascular connections between leaves are stronger between orthostichous leaves i.e., leaves vertically aligned along the stem (Sprugel et al., 1991; Orians et al., 2000). Vascular connections are known to restrict the movement of defensive chemicals (Jones et al., 1993; Orians et al., 2000; Schittko & Baldwin, 2003; Viswanathan & Thaler, 2004). Therefore, the strength of induced plant resistance can vary between leaves and create within-plant spatial heterogeneity in chemical defences, and thus food quality (Orians & Jones, 2001). Since herbivore feeding may induce responses that will affect its own feeding patterns and those of future colonizers, we hypothesized that these changes in herbivore feeding behaviour may ‘feedback’ to influence subsequent induced responses, such that plants respond to initial herbivore damage, these plant responses will affect further herbivore feeding, and these changes in herbivore feeding may ultimately affect the plant’s response.

Despite the potential effects of induced plant resistance on the within-plant foraging behaviour of herbivores (e.g., Watten et al., 1988; Edwards et al., 1991; Kaitaniemi et al., 2004), no study has yet linked a plant defensive pathway to these effects. The jasmonate pathway is a key pathway in signalling the induction of resistance against herbivores across the plant kingdom (Wasternack & Parthier, 1997). Activation of the jasmonate pathway leads to production of the hormone jasmonic acid (JA), a central signalling molecule in plant defence (Wasternack & Parthier, 1997). Induction of the jasmonate pathway by herbivore feeding or artificial treatment with JA leads to the synthesis of defensive proteins such as proteinase inhibitors (PIs) and oxidative enzymes (Farmer et al., 1992; Constabel et al., 1995; Koiva et al., 1997), which are linked to a decreased performance of generalist herbivores on plants (e.g., Felton et al., 1989; Thaler et al., 1996).

The use of genetically modified plants has proven to be a valuable tool for testing the role of the jasmonate pathway in plant resistance against herbivores (e.g., Li et al., 2002; Thaler et al., 2002; Kessler et al., 2004). In the present study we used a genetically modified tomato (defenceless-1 [def-1]), which lacks the ability to induce resistance due to a deficiency in the production of JA (Howe et al., 1996), to test the role of the jasmonate pathway on the feeding behaviour of a generalist caterpillar, Spodoptera exigua (Hübner) (Lepidoptera: Noctuidae). We hypothesized that genotypes with reduced induced defences (def-1) would receive a greater amount of herbivore damage, and in a more concentrated pattern, compared to wild-type plants. We also hypothesized that changes in the amount and distribution of herbivore feeding damage might alter subsequent induced plant responses. We predicted that the strength and pattern of induction would depend on the degree of vascular connectivity within and between leaves relative to the location of herbivore feeding (Orians et al., 2000).

**Materials and methods**

**Plants and insects**

Two tomato genotypes were used: a wild-type tomato, Lycopersicon esculentum Mill. var. Castlemart, and the def-1 tomato mutant derived from the same variety. Def-1 plants have a reduced ability to produce JA, and thus a lowered PI activity after herbivore damage compared to wild-type tomatoes (Howe et al., 1996; Li et al., 2002). The reduction in JA is caused by a lesion between the synthesis of hydroperoxylinolenic acid and 12-oxo-phytodienoic acid (Howe et al., 1996). As a consequence, def-1 plants were more damaged by M. sexta larvae (Howe et al., 1996) and supported increased survival and growth of S. exigua larvae (Thaler et al., 2002) compared to wild-types. The jasmonate pathway and resistance to herbivores on def-1 plants can be restored by exogenous applications of JA (Howe et al., 1996; Thaler et al., 2002). The production of PIs, and the jasmonate pathway, can be activated during the first 24 h of herbivore feeding on tomatoes (Kant et al., 2004).

The vascular architecture of tomato plants has been well characterized (Orians et al., 2000). Leaves 1 and 2 share weak vascular connections with each other. Although both...
of these leaves are connected to leaf 3, leaf 1 appears to have stronger vascular connections to leaf 3 than leaf 2 (Orians et al., 2000; Rodriguez-Saona et al., 2005) (Figure 1). In addition, Orians et al. (2000) showed that leaflets from one side of a leaf, closer in distance to an adjacent leaf, have stronger vascular connections to that particular leaf compared to leaflets on the opposite side of the leaf (Figure 1).

Plants were grown from seed for ca. 1 month (until they had four fully expanded leaves) in 500 ml pots containing soil mix (Premier Horticulture Ltd, Quebec, Canada) and 5–10 pellets of Nutricote (13-13-13 N-P-K; Chisso-Ashai Fertilizer Co., Tokyo, Japan). The plants were maintained in a greenhouse under natural lighting supplemented with 400 W sodium halide lamps and watered daily until the treatments were established. The light intensity in the greenhouse ranged from 700 to 1300 mE m$^{-2}$ s$^{-1}$, temperatures ranged from 19 to 24 ± 1 °C, with a humidity of ca. 40–50%.

*Spodoptera exigua* caterpillars, obtained from the USDA (Stoneville, Mississippi, USA), were maintained in a colony. Caterpillars were reared on an artificial diet (Southland Products, Lake Village, AR), and kept at room temperature (ca. 25 °C at 50–70% r.h.).

**Jasmonate pathway effects on *Spodoptera exigua***

To test the role of the jasmonate pathway on growth, leaf consumption, and the distribution of herbivore feeding on plants, we established four treatments in a 2 × 2 factorial design as follows: (1) insect-damaged wild-type plants, (2) insect-damaged *def*-1 plants, (3) undamaged wild-type plants, and (4) undamaged *def*-1 plants. Leaves 1 and 2 (oldest and second oldest, respectively) were bagged at the petiole using spun polyester sleeves (35 cm wide × 45 cm long) (Rockingham Opportunities Corporation, NC). Damaged *def*-1 plants had one 3rd instar *S. exigua* placed on leaf 2. To compensate for the higher amounts of herbivore damage on *def*-1 plants compared to wild-type plants, we placed two 3rd instar *S. exigua* (one on each bagged leaf) on the wild-type plants. To account for treatment differences from the initial amount of damage, we measured the amount of feeding (cm$^2$) by damagers by scanning the leaves and measuring the area consumed using the Scion Image software system (Scion Corporation, Frederick, MD). We analyzed the differences between genotypes in the initial amount of damage using t-tests (Systat ver. 9, 1998, SPSS Science, Chicago, IL). Two caterpillars feeding on wild-type plants caused a similar amount of leaf damage compared to one caterpillar feeding on *def*-1 plants (leaf consumed [cm$^2$ ± SE] for wild-types = 28.72 ± 3.18; *def*-1 plants = 23.13 ± 3.25; t = 1.22, d.f. = 22; P = 0.23), indicating that any treatment effects were not due to differences in the amounts of initial herbivore damage. We placed caterpillars on two separate leaves to avoid interference; placing two caterpillars on a single leaf did not result in a higher damage to plants compared to one caterpillar per leaf (C Rodriguez-Saona, pers. obs.). Plants were then placed for 2 days in a growth chamber (ca. 23 °C day temp, 25 °C night temp, L14:D10 photoperiod, and 60% r.h.) to allow for a systemic response (Stout et al., 1998; Thaler et al., 2002).

After 2 days of herbivore damage, individual plants were placed inside 30 × 30 × 30 cm polypropylene cages (Mega-view Science Education Services Co., Taichung, Taiwan). A newly molted 3rd instar *S. exigua* (hereafter ‘challengers’) was placed on the terminal leaflet of leaf 3 (undamaged leaf) of each plant, and allowed to feed ad libitum on the plant (except leaves 1 and 2) for 3 days at room temperature (ca. 25 °C and 50–70% r.h.). The caterpillars remained on bagged leaves 1 and 2 for the duration of the experiment. We measured the initial and final larval mass of the challengers, and the leaf area consumed, as previously described. In addition, the number of leaves and leaflets eaten by the challengers, as well as the number of independent feeding

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**Figure 1** Vascular connectivity of tomato leaves (after Orians et al., 2000; Rodriguez-Saona et al., 2005). Although leaves 1 and 2 are both connected to leaf 3, leaf 1 shares a stronger vascular connection with leaf 3 (leaves shown in grey), compared to leaf 2. Leaves 1 and 2 are weakly connected to each other. Leaflets from the left side of leaf 3 (closer to leaf 1) share a stronger vascular connection with leaf 1 (leaflets with dotted pattern); while leaflets from the right side of leaf 3 (closer to leaf 2) share a stronger vascular connection with leaf 2 (leaflets with dashed pattern).
holes on the plant were counted. Experiments were replicated five times (wild types, n = 27; def-1 plants, n = 28).

Data on larval mass gain, leaf consumption, and number of feeding holes were analyzed using a two-way ANOVA, with genotype (wild-type or def-1) and herbivory (damage or no damage) as the main effects. A non-parametric two-way ANOVA based on ranks was used for tests on the number of damaged leaves and leaflets (Shirley, 1981). Only those caterpillars found on plants at the end of the experiment were used in the analyses. Fewer than two larvae per treatment were found away from plants. Larval mass and consumption were log-transformed prior to analysis to increase the homogeneity of variances among treatments and the normality of residuals.

**Recovery of effects in jasmonate-deficient plants**

If the reduced ability of def-1 plants to produce JA is the causal factor for the differences in effects between the two types of plants, exogenous applications of JA should restore the plant’s induce response (Howe et al., 1996; Thaler et al., 2002). Therefore, we compared the growth and feeding behaviour of S. exigua larvae on untreated def-1 plants to those on plants treated with JA. Plants were sprayed with either a solution of water and acetone (controls) or a 0.8 mM solution of JA in water and acetone (ca. 0.2 mg of JA per plant). This dose increases resistance to herbivores in tomato plants (Thaler et al., 2001), but is below a level which is toxic to plants (Thaler et al., 1996). Jasmonic acid was synthesized from methyl jasmonate (Bedoukian Research, Danbury, CT), as described by Farmer et al. (1992). After treatment, plants were placed in the growth chamber for 1 day. A newly molted 3rd instar S. exigua was placed on the terminal leaflet of the third leaf of plants, as previously described, and allowed to feed for 3 days. The experiment was replicated four times (control plants, n = 13; JA-treated plants, n = 17).

The amount of larval food consumed, the initial and final larval mass, number of leaves and leaflets damaged, and the number of feeding holes on the plant were recorded, as described previously. The effects of treatment (JA vs. no JA) on food consumption, larval mass, and number of feeding holes per leaf were analyzed using t-tests. The number of leaves and leaflets damaged were analyzed using a non-parametric Mann–Whitney U-test statistic. Only larvae recovered on plants at the end of the experiment were used for analyses (larvae found away from plants: control = 2; JA-treatment = 5). Data were log-transformed to attain a homogeneity of variances between treatments.

After documenting the different amounts and patterns of damage on wild-type and jasmonate-deficient def-1 plants, we simulated these differences to test the dynamic consequences for subsequent responses. In addition, we sought to determine the importance of amount and pattern of damage, while considering vascular leaf connectivity.

**Amount of damage and induced defences**

We varied the amount of herbivore damage on the plants by altering the density of caterpillars on wild-type plants. Leaves 1 and 2 were bagged as previously described. Plants received either no damage (controls), damage by one caterpillar on leaf 1, or damage by two caterpillars simultaneously (one on leaf 1 and one on leaf 2), for 5 days. Plants in the two-caterpillar treatment received similar amounts of damage as def-1 plants and had ca. 50% more leaf area removed compared to plants with a single caterpillar (Rodriguez-Saona et al., 2005).

After the damaging period, leaflets from leaf 3 (undamaged leaf) of plants were excised to assay for PI activity and insect performance. Proteinase inhibitor activity is a good marker of resistance to S. exigua (Stout et al., 1996; Stout et al., 1998), and there is variation in PI activity within tomato plants (Stout et al., 1996; Orians et al., 2000). We assayed PI activity following the protocol of Rodriguez-Saona et al. (2005). Briefly, a sample (100 mg) of each leaflet was ground and centrifuged at 11 624 g for 10 min at 10 °C. Sixty µl of the supernatant was added to 20 µl of Tris buffer, 50 µl of 2% azocasein in Tris buffer, and 20 µl of a 0.001 M HCl containing 200 ng of trypsin. After incubation for 20 min at 28 °C, the reaction was stopped by adding 100 µl of trichloroacetate to each sample. One hundred µl of 1 M NaOH was added to 100 µl of the supernatant from each sample in individual microplate wells. Absorbance was read at 450 nm with a microplate reader. To measure insect performance, leaflets were placed with one S. exigua neonate in individual 90 mm Petri dishes (lined with moist filter paper), and kept at room temperature. Caterpillar mass was recorded after 5 days of feeding. The experiment was replicated four times (undamaged controls, n = 25; damaged by one caterpillar, n = 28; damaged by two caterpillars, n = 22).

Differences in percentage PI activity and larval mass among treatments were analyzed using ANOVA. A significant ANOVA was followed by Tukey tests. Mortality data were analyzed using G-tests. Data on larval mass were log-transformed prior to analysis. Percentage data were arcsine-square root transformed prior to analysis to fit assumptions of tests.

**Pattern of damage and induced defences**

To test the effect of different feeding patterns on induced defences, we simulated one aspect of the differences in the distribution of damage between wild-type and jasmonate-deficient plants to create patterns of attack that would mimic those received by a resistant or a susceptible genotype, respectively. We predicted that a susceptible genotype
would receive more concentrated damage compared to a resistant genotype. To create concentrated damage we allowed *S. exigua* to feed on one leaf of wild-type plants, whereas we created dispersed damage by allowing *S. exigua* to feed on leaf 1 and leaf 2 of the wild-type plant. In addition, we determined the effects of directionality of damage by comparing: (1) damage only on leaf 1 vs. damage on leaf 1 and then leaf 2; and (2) damage only on leaf 2 vs. damage on leaf 2 and then leaf 1. Plants that had a single leaf damaged received damage by a 3rd instar *S. exigua* for 2 consecutive days. In contrast, plants that were damaged on two leaves received damage by *S. exigua* on either leaf 1 or 2 for 24 h, after which the caterpillars were moved to an undamaged leaf (2 and 1, respectively) and allowed to feed for an additional 24 h (total of 2 days of damage).

To test for the confounding effect of differences in the amount of herbivore damage between treatments, we compared the leaf area consumed by the damaging caterpillars when feeding on a single leaf (concentrated damage) compared to those feeding on two leaves (dispersed damage). The dispersed pattern yielded ca. 25% herbivore damage to the first leaf consumed (leaf consumed [cm\(^2\) ± SE] for dispersed damage to leaf 1 and then leaf 2 on leaf 1 = 1.26 ± 0.20 and leaf 2 = 3.78 ± 0.27, and for dispersed damage to leaf 2 and then leaf 1: leaf 2 = 1.13 ± 0.14, leaf 1 = 3.83 ± 0.13). Control plants received no herbivore damage. All treatments were run concurrently on a particular day. We measured the response of plants to herbivore damage by measuring PI activities and insect performance on leaf 3 (systemic response), as described above. We sampled leaflets from both sides of leaf 3, because leaflets from opposite sides have different vascular connections, whereas leaflets from the same side share a similar connectivity (Orriens et al., 2000). Treatments were replicated four times (undamaged controls, n = 15; plants damaged on leaf 1, n = 13; plants damaged first on leaf 1 then leaf 2, n = 14; plants damaged on leaf 2, n = 13; plants damaged first on leaf 2 then leaf 1, n = 15).

Differences across treatments on larval mass and percentage PI activity were transformed and analyzed using ANOVA, as previously described. A significant ANOVA was followed by Tukey tests. Differences in leaf area consumption between treatments were examined using t-tests. Data for leaflets from each side of leaf 3 (i.e., more connected to either leaf 1 or 2; Figure 1) were analyzed separately.

**Results**

**Jasmonate pathway effects on Spodoptera exigua**

There was a significant effect of genotype on larval mass-gain (F\(_{1,49} = 26.594, P<0.001\)) and leaf consumption (F\(_{1,47} = 4.03, P = 0.05\)) by the free-ranging challenge herbivores. On average, challenge caterpillars fed on def-1 plants gained threefold more mass and ate 1.6-fold more leaf material compared to wild-type plants. There was also a significant effect of previous herbivore damage on larval mass-gain (F\(_{1,49} = 12.077, P = 0.001\)) and leaf consumption (F\(_{1,47} = 4.574, P = 0.038\)). This effect of damage on larval mass and consumption, however, was influenced by genotype (damage × genotype interaction; mass-gain: F\(_{1,49} = 8.337, P = 0.006\); leaf consumption: F\(_{1,47} = 5.397, P = 0.025\)). Caterpillar growth and consumption were similar on def-1 plants, regardless of whether the plants were damaged or not (mean larval mass-gain [mg ± SE] on damaged def-1 plants: 3.69 ± 0.68; undamaged: 3.86 ± 0.55; leaf consumption [cm\(^2\) ± SE] on damaged plants: 0.95 ± 0.12; undamaged: 1.01 ± 0.17); whereas larval mass and consumption on damaged wild-type plants decreased by 3.7 and 2.5-fold, respectively, compared to undamaged plants (mean larval mass-gain [mg ± SE] on wild-type damaged plants: 0.55 ± 0.18; undamaged: 2.06 ± 0.53; leaf consumption [cm\(^2\) ± SE] on damaged plants: 0.35 ± 0.08; undamaged: 0.85 ± 0.13).

We found a significant effect of genotype on the pattern of damage. When comparing previously damaged plants, wild-type plants had twice the number of leaves (χ\(^2 = 7.43,\ d.f. = 1,\ P = 0.006;\ Figure 2A) and leaflets damaged (χ\(^2 = 4.34,\ d.f. = 1,\ P = 0.037;\ Figure 2B), and twice the number of feeding holes (F\(_{1,47} = 16.09,\ P<0.001\); Figure 2C) as jasmonate-deficient plants. There was no effect of previous damage, or damage-by-genotype interaction, on any of these behavioural parameters (all P-values>0.05).

**Recovery of effects in jasmonate-deficient plants**

Caterpillars on def-1 tomato plants sprayed with JA had decreased larval growth (mean larval mass-gain [mg ± SE] on JA-treated plants = 0.26 ± 0.15; controls = 2.48 ± 1.02; t = 4.17, d.f. = 20, P<0.001) and lowered leaf consumption (leaf consumption [cm\(^2\) ± SE] on JA-treated plants = 0.28 ± 0.05; controls = 0.71 ± 0.15; t = 3.60, d.f. = 20, P = 0.001) compared to unsprayed plants. These negative effects of JA-sprayed def-1 plants on caterpillar performance were similar to those observed on wild-type damaged plants, indicating that induced resistance to caterpillars on def-1 plants was recovered with the JA treatment. The number of leaves damaged on JA-treated plants was almost twice that on untreated plants (JA-treated plants = 1.91 ± 0.37; controls = 1.09 ± 0.09; U-test = 89.5, P = 0.021). Similarly, the number of leaflets damaged on treated plants was 1.6-fold higher than on untreated plants (JA-treated plants = 3.27 ± 0.59; controls = 2.00 ± 0.27; U-test = 88.0, P = 0.064). This magnitude of effect of the JA treatment on larval feeding behaviour was similar to the effect of wild-type plants in the free-range experiment (see above).
Amount of damage and induced defences
There was a dose-dependent relationship between damage and resistance in wild-type tomatoes (Figure 3). Increased amounts of herbivory led to increased levels of PI activity ($F_{2,72} = 25.0, P < 0.001$; Figure 3A) and decreased larval survival ($G$-test = 6.10, $P = 0.047$) and growth ($F_{2,35} = 6.14, P = 0.005$; Figure 3B,C).

Pattern of damage and induced defences
Different patterns in the distribution of herbivore damage simulating the patterns on wild-type and jasmonate-deficient plants.

**Figure 2** (A) Number of leaves eaten; (B) number of leaflets eaten; and (C) total number of feeding holes by *Spodoptera exigua* on wild-type or jasmonate-deficient *def-1* plants that had either no previous damage or previous damage by *S. exigua*. Bars indicate means ± 1 SE.

**Figure 3** (A) Activities of proteinase inhibitors; (B) % larval mortality; and (C) larval mass of *Spodoptera exigua*, on the undamaged leaf 3 of wild-type plants damaged either by one *S. exigua* larva, damaged by two *S. exigua* larvae, or none. Bars indicate means ± 1 SE. Different letters above the bars indicate significant differences among treatments ($P \leq 0.05$).
Jasmonate pathway and distribution of insect feeding

131

levels of PI activity and larval performance on leaflets from leaf 3 that are more connected to leaf 1 varied among treatments (PI: $F_{4,67} = 2.83, P = 0.03$; larval performance: $F_{4,67} = 6.12, P < 0.001$; Figures 4 and 5A). Similarly, induced responses in leaflets from leaf 3, which are more connected to leaf 2, were influenced by treatment (PI: $F_{4,67} = 3.51, P = 0.011$; larval performance: $F_{4,67} = 3.34, P = 0.015$; Figures 4 and 5B).

Concentrated damage to leaf 1 compared to dispersed damage to leaf 1 and then leaf 2 (Figures 4 and 5, bars in black). Following concentrated herbivore damage to leaf 1, PI activities increased twofold in leaf 3 compared to undamaged controls; but this effect was only significant on the leaf side more strongly connected to leaf 1 (Figure 4A, B). Damage to leaf 1 also reduced larval performance 2.6-fold on both sides of leaf 3 compared to controls (Figure 5A, B). In contrast, dispersed damage to leaf 1 and then leaf 2 did not induce PI activity compared to control plants (Figure 4). Nonetheless, in spite of the lack of PI induction on plants with dispersed damage, dispersed damage did reduce larval performance by nearly the same amount as plants with concentrated damage (Figure 5). The differences between concentrated and dispersed damage treatments were not due to differential amounts of total damage inflicted by the damaging caterpillars (leaf consumed [cm$^2$ ± SE]: concentrated damage = $4.14 ± 0.63$; dispersed damage = $5.03 ± 0.35$; $t = 1.264$, d.f. = 25, $P = 0.217$).

Concentrated damage to leaf 2 vs. dispersed damage to leaf 2 and then leaf 1 (Figures 4 and 5, bars in grey). Similar to leaf 1, herbivore damage to leaf 2 increased PI activities on leaf 3 compared to controls; however, this effect was stronger on leaflets with a higher connectivity to the damaged leaf (Figure 4A, B). Concentrated damage to leaf 2 also reduced larval performance on the connected leaflets 1.9-fold.

**Figure 4** Activities of proteinase inhibitors on leaflets from leaf 3 of wild-type plants that are more connected to (A) leaf 1, or (B) those more connected to leaf 2. Treatments to plants were: no damage (undamaged) (white bars), damage on leaf 1 for 2 consecutive days or sequential damage to leaf 1 on day 1 and to leaf 2 on day 2 (black bars), and damage on leaf 2 for 2 consecutive days or sequential damage to leaf 2 on day 1 and to leaf 1 on day 2 (grey bars) by *Spodoptera exigua*. Bars indicate means ± 1 SE. Different letters above the bars indicate significant differences among treatments ($P ≤ 0.05$).

**Figure 5** Larval mass of *Spodoptera exigua* on leaflets from leaf 3 of wild-type plants that are more connected to (A) leaf 1, or (B) those more connected to leaf 2. Treatments to plants are the same as described in Figure 4. Bars indicate means ± 1 SE. Different letters above the bars indicate significant differences among treatments ($P ≤ 0.05$).
compared to controls, but had no effect on the less connected leaflets (Figure 5A, B). Similar to the results from dispersed damage mentioned above, caterpillar feeding on leaf 2 and then leaf 1 altered induced responses on leaf 3, but in the opposite direction: dispersed damage increased the induced resistance in some parts of leaf 3 compared to concentrated damage. Leaflets from leaf 3 sharing weak connections to leaf 2 had 16% higher PI activity (Figure 4A) and 47% lower caterpillar performance (Figure 5A) when the plants received a dispersed pattern of damage on leaf 2 and then leaf 1, compared to those that received concentrated damage on leaf 2 only. There were no differences in PI activity (Figure 4B) or larval performance (Figure 5B) between these patterns of damage for leaflets with a strong vascular connection to leaf 2. This increase in induced resistance in leaf 3 could have been due to an increased total damage in the dispersed damage treatment compared to the concentrated damage treatment (leaf consumed [cm² ± SE]: concentrated damage to leaf 2 = 3.94 ± 0.31; dispersed damage to leaf 2 and then leaf 1 = 4.95 ± 0.24; t = 2.595, d.f. = 28, P = 0.015).

Discussion

Previous studies have causally linked activation of the jasmonate pathway with a reduced preference and performance of herbivores on plants (Orozco-Cardenas et al., 1993; Howe et al., 1996; Thaler et al., 1996). The present study shows the importance of the jasmonate pathway for within-plant patterns of damage and feedbacks for subsequent induction. It has been suggested that changes in herbivore movement on induced plants may not have a chemical basis and might be caused simply by the visual or tactile responses of herbivores to a cut edge (Edwards et al., 1991). Our results contradict this, and indicate that activation of the jasmonate pathway results in more dispersed patterns of herbivore feeding on plants. Damaged wild-type plants had more than twice as many leaves and leaflets damaged, and twice as many feeding holes eaten by S. exigua larvae compared to jasmonate-deficient plants. The finding that S. exigua feeding damage was dispersed on def-1 plants that were sprayed with JA further supports the hypothesis that the jasmonate pathway, and not other factors, modified herbivore food preference.

Why was there a significant effect of plant type but not damage to the distribution of feeding? Herbivore damage reduced larval growth and consumption of challenge herbivores on wild-type plants, but did not affect the pattern of damage, compared to undamaged wild-type plants. Similar amounts of damage to def-1 plants did not increase any measure of resistance. Other studies of S. exigua feeding on tomato plants have shown that herbivores move away from damaged leaves (e.g., Edwards et al., 1991; Barker et al., 1995). Both of these studies found that caterpillars would move away from leaves they themselves had damaged. Thus, the response to self-feeding may be sufficient to overwhelm a response to feeding by a previous caterpillar. In this study, it is possible that the induction of jasmonate-regulated defences on wild-type plants over the 3 days the challenge herbivores fed on the plant were sufficient to eliminate the differences between undamaged and damaged wild-type plants. This may be likely, given that the damage-inducing herbivores fed on the plant for 2 days and the challenge herbivores fed for 3 days, enough time for the initially undamaged plants to ‘catch up’. The fact that responses to herbivory are observed in tomato plants within 1 day of herbivore feeding supports this possibility (Edwards et al., 1991; Stout & Duffy, 1996; Kant et al., 2004). Alternatively, differences in the constitutive defences of the two plant types could overwhelm the effect of damage on herbivore feeding patterns. In the absence of herbivory, S. exigua caterpillars have a higher mortality and lower mass when reared on wild-type compared to jasmonate-deficient plants (this study; Thaler et al., 2002). However, while the main effect of plant type on the pattern of damage was significant and the effect of previous damage alone was not, the difference between feeding patterns was always larger when comparing damaged wild-type and jasmonate-deficient plants than when comparing undamaged wild-type and jasmonate-deficient plants, suggesting an effect of damage.

Implications for plant defence

The expression of induced responses in plants can be influenced by the amount and distribution of herbivory (e.g., Jones et al., 1993; Stout et al., 1996; Orians et al., 2000; Underwood, 2000). There are many ways in which the distribution of damage could influence subsequent induction, including the amount of damage each leaf receives, the number of leaves damaged, the number of cells or surface area damaged, damaging leaves with a different source/sink status, and damaging plant parts that differ in their vascular connectedness. For instance, Viswanathan & Thaler (2004) found that the amount of herbivore damage and degree of connectivity between leaves combines to determine the pattern of induction in leaves. Induced responses were observed in leaves of Solanum dulcamara L. that were weakly connected to the damaged leaf only following high levels of herbivory. In contrast, induced responses in strongly connected leaves were found following both high and low levels of herbivore damage. We extend these findings by showing that the inducibility of the plant can alter the pattern of feeding damage and subsequent induction.
In fact, changes in the pattern of caterpillar damage from a more connected leaf (1) to a less connected leaf (2), led to reduced PI activity in undamaged leaf 3 compared to plants that received concentrated damage to leaf 1; although these two patterns of damage resulted in similar amounts of herbivore damage. In contrast, a change in the pattern of feeding damage by sequentially damaging leaf (2) and then leaf (1), led to increased resistance compared to concentrated damage to leaf 2. This effect could be due to an increased amount of herbivore damage compared to damage concentrated on leaf 2 only. This increase in induced resistance by dispersed damage only occurred on leaflets of leaf 3 (undamaged) weakly connected to the damaged leaf 2, compared to concentrated damage to leaf 2. Leaflets from leaf 3 more connected to the damaged leaf 2 had increased resistance irrespective of any changes in the pattern of herbivore damage. Therefore, changes in the patterns of herbivore damage can affect the amount of damage that different plant parts receive, another way for the pattern of damage to influence the plant’s induced responses. Furthermore, the fact that PI activities were not always correlated with larval performance indicates that spatial patterns of other defences may change in a different manner than PIs (Stout et al., 1996).

Dispersed damage may increase a plant’s fitness, compared to those with a more concentrated pattern of damage (Mauricio et al., 1993; Marquis, 1996; Meyer, 1998). This could happen for several reasons. For example, Zangerl et al. (2002) demonstrated that herbivory can depress levels of photosynthesis within a leaf in areas distant to the actual damage. Therefore, dispersed or concentrated damage could influence nearby tissues differently. From the herbivore’s perspective, in some cases movement away from an induced leaf can improve the herbivore’s food consumption, and could be a counter-defence by the herbivore to avoid a plant’s induced defences (Karban & Agrawal, 2002), but in other cases movement will reduce herbivore performance. Increased movement can be costly to herbivores if it results in increased exposure to their natural enemies (Kaitaniemi et al., 2004). Since dispersed patterns of herbivore damage on plants are common in nature, and such patterns of damage can influence the interactions between plants and organisms at higher trophic levels (Kaitaniemi et al., 2004), studies on the foraging behaviour of herbivores might prove critical in explaining the general effects of induced plant defences.

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