Ecology Letters, (2002) **5**: 764–774

REPORT

Jasmonate-deficient plants have reduced direct and indirect defences against herbivores

Jennifer S. Thaler,[1] Mohamed A. Farag,[2] Paul W. Paré[2] and Marcel Dicke[3]
[1]*Department of Botany, University of Toronto, 25 Willcocks Street, Toronto, ON M5S 3B2, Canada*
[2]*Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, Texas 79409, USA*
[3]*Laboratory of Entomology, Wageningen University, PO Box 8031, 6700 EH, Wageningen, the Netherlands*
Correspondence: E-mail: thaler@botany.utoronto.ca

Abstract

Plants employ a variety of defence mechanisms, some of which act directly by having a negative effect on herbivores and others that act indirectly by attracting natural enemies of herbivores. In this study we asked if a common jasmonate-signalling pathway links the regulation of direct and indirect defences in plants. We examined the performance of herbivores (direct defence) and the attraction of natural enemies of herbivores (indirect defence) to wild-type tomato plants and mutant plants that are deficient in the production of the signalling hormone jasmonic acid. Wild-type plants supported lower survivorship of caterpillars compared with jasmonic acid-deficient plants. Damaged wild-type plants were more attractive to predaceous mites compared with undamaged wild-type plants, whereas damaged jasmonate-deficient plants were not more attractive to predators. Damaged wild-type plants induced a greater production of volatile compounds (primarily the sesquiterpene β-caryophyllene and the monoterpenes α-pinene, β-pinene, 2-carene and β-phellandrene) compared with damaged jasmonate-deficient plants. Treating jasmonate-deficient plants with exogenous jasmonic acid restored both the direct and indirect defence capabilities, demonstrating that jasmonic acid is an essential regulatory component for the expression of direct and indirect plant defence.

Keywords

Direct defence, indirect defence, induced resistance, jasmonate-deficient, jasmonate, *Lycopersicon esculentum*, *Phytoseiulus persimilis*, plant–insect interactions, *Spodoptera exigua*, tritrophic interactions.

Ecology Letters (2002) 5: 764–774

INTRODUCTION

Should plants employ several lines of defence to protect themselves against herbivorous insects? If one defence strategy reduces the benefits that a plant will derive from a second defence strategy, multiple defences will have diminishing returns for the plant. The energetic cost of defences should disfavour redundant defence strategies. For example, if direct plant defences are effective at reducing herbivore numbers, then there will be fewer herbivores for natural enemies of herbivores to remove. In such a case, the accumulation of plant supplied toxins and the attractants of natural enemies may be negatively associated (Steward & Keeler 1988; Dicke 1999a) and independently or antagonistically regulated at the biosynthetic level (Kahl *et al.* 2000). However, if multiple strategies increase the reliability or effectiveness of defence, then multiple strategies should be deployed simultaneously (Steward & Keeler 1988; Berenbaum & Zangerl 1996; Dyer *et al.* 2001) and perhaps be coregulated at the biosynthetic level.

Plant defence responses can be organized into groups based on biosynthetic pathways and the hormones that regulate these pathways (Schneider *et al.* 1996; Creelman & Mullet 1997). The strength of these categories will be determined by the degree to which responses within a category are coregulated (positively or negatively) and the degree to which they are influenced by other hormones. Thus, the deployment of an individual defence can influence the deployment of other defences. The coregulation and coordination of defence responses in a multispecies environment is critical for successful plant defence. Indirect evidence has suggested that common signalling pathways regulate functionally divergent defence mechanisms. For example, the jasmonate pathway in plants has been implicated in coordinating production of direct defences such as proteinase inhibitors and oxidative enzymes (Thaler

et al. 1996; McConn *et al.* 1997; Staswick & Lehman 1999) as well as the production of volatile compounds that can function as indirect defences (Hopke *et al.* 1994; Boland *et al.* 1995; Dicke *et al.* 1999; Gols *et al.* 1999; Thaler 1999). Herbivore-induced plant volatile compounds are released by many plants following damage (Dicke 1999b) and can serve to attract natural enemies (Dicke *et al.* 1990a,b; Turlings *et al.* 1990; Drukker *et al.* 1995; De Moraes *et al.* 1998; Thaler 1999; Kessler & Baldwin 2001). Increased predation or parasitism of herbivores has the potential to increase plant fitness (Gomez & Zamora 1994; van Loon *et al.* 2000; Fritzsche-Hoballah & Turlings 2001).

Tomato plants (*Lycopersicon esculentum*) are known to release volatile compounds that play a role in indirect defence following herbivory and mechanical damage (Takabayashi & Dicke 1993). The predatory mite, *Phytoseiulus persimilis* prefers the volatiles from tomato plants damaged by spider mites compared with those from undamaged plants (Takabayashi & Dicke 1993). Moreover, there is evidence from the field that attraction or retention of natural enemies of herbivores caused by jasmonate-induced volatile compounds of tomato plants results in higher rates of parasitism of herbivores (Thaler 1999).

In this study we employed tomato plants that were genetically deficient for the production of jasmonic acid and jasmonate-dependent defence compounds. Jasmonate-deficient plants were produced from mutagenized seed (var. Castlemart) and identified based on their reduced ability to induce proteinase inhibitor II activity following mechanical damage (Lightner *et al.* 1993). The jasmonate-deficient plants produce 40% of the proteinase inhibitor activity in damaged leaves and 5% in systemic undamaged leaves compared with damaged wild-type plants. Jasmonate-deficient plants also received more damage by *Manduca sexta* than wild-type plants (Howe *et al.* 1996). The mutant has a similar growth form to the wild type in several traits, including height, number of leaves and dry mass (G. Howe & J. Thaler, unpublished data). The genes associated with the mutation follow simple Mendelian inheritance suggestive of a single locus, two-allele system (G. Howe, personal communication). The mutants used in this experiment were homozygous and backcrossed twice in the Castlemart variety. Using pharmacological experiments, the location of the mutation has been localized to an area of the jasmonate pathway between the intermediates 13(S)-hydroperoxylinolenic acid and pentadienoic acid. Consequently, jasmonic acid is produced in lower quantities by the mutant plants. Addition of jasmonic acid to the plant restores proteinase inhibitor production (Howe *et al.* 1996).

The release of volatile compounds following herbivory may be affected by the jasmonate pathway in several ways. The location of the lesion in the biochemical pathway of mutant plants is upstream of both pentadienoic acid and jasmonic acid synthesis (Howe *et al.* 1996). In some plant species, including corn and lima bean, both compounds are signals for volatile production (Koch *et al.* 1999). We do not know if either compound is involved in volatile production by tomato plants. Nevertheless, as both of these putative signals are reduced in this jasmonate-deficient mutant, we expected volatile production to be reduced.

The goal of this study is to link the biochemical basis of direct and indirect plant defence to ecological and behavioural effects on the target organisms. In doing so, we unequivocally implicate the jasmonate pathway in direct and indirect induced plant defence. Jasmonate-deficient mutant tomato plants and wild-type controls were damaged by herbivores and then assayed for caterpillar performance and production of oxidative enzymes (direct defence) and attractiveness to natural enemies of herbivores (indirect defence). We further examined the volatile compounds released by jasmonate-deficient and wild-type plants that are putatively involved in indirect defence. Lastly, we tested if exogenous application of jasmonic acid would alleviate the loss of direct and indirect defence in jasmonate-deficient plants.

METHODS

Plants and insects

Wild-type (*L. esculentum* cv Castlemart) and jasmonate-deficient mutant tomato plants (def-1) were grown in 500-mL pots in potting soil in a glasshouse (natural light supplemented with high pressure mercury lamps, 16 : 8, day : night, 25 ± 5 °C). When plants had three fully expanded leaves and the fourth was almost fully expanded, we moved them to a controlled environment chamber (27 ± 1 °C, 14 : 10, light : dark) and imposed the damage treatments.

Spodoptera exigua caterpillars were hatched from eggs and used when they were 1 day old. *P. persimilis* predators were obtained from a laboratory colony reared on lima bean plants infested with spider mites, *Tetranychus urticae*. Predators were maintained on pieces of infested bean leaves inside Petri dishes with approximately 10 adults and their juvenile offspring. The colony was maintained by transferring 10 adult predators to new spider mite-infested leaves once a week. Females had no experience with tomato plants prior to experiments.

Induction of direct defence

To test for the role of jasmonate in direct defence we established four treatments: insect-damaged wild-type plants, undamaged wild-type plants, insect-damaged

jasmonate-deficient plants, and undamaged jasmonate-deficient plants. Insect damage was achieved by confining four *S. exigua* neonates in a clip cage on the terminal leaflet of the third leaf. The clip cages were 2.5 cm in diameter and constructed out of clear plastic held together with a hair clip. The point where the clip cage clamps the leaf was padded with foam and the cages were supported with a wooden stick. Control plants received an empty clip cage. We allowed the caterpillars to feed for 2 days, at which point they were moved to the terminal leaflet of the fourth leaf for two additional days of feeding. This treatment resulted in approximately 0.5 cm^2 tissue damage to the third leaf and 1 cm^2 damage to the fourth leaflet for wild-type plants, and 1 cm^2 tissue damage to the third leaf and 1.5 cm^2 damage to the fourth leaf for the jasmonate-deficient plants (*c.* 5% total leaf area).

Excized leaflets from each plant were used for assays of mortality and growth of *S. exigua* caterpillars. The damaged terminal leaflet of the fourth leaf (local, $n = 124$), the undamaged leaflet adjacent to the terminal leaflet on the fourth leaf (leaf systemic, $n = 124$) and the undamaged terminal leaflet of the fifth leaf (plant systemic, $n = 192$) were collected for a bioassay. Each leaflet was placed in a 90-mm Petri dish lined with moist filter paper and a freshly hatched *S. exigua* caterpillar was placed in each dish. The caterpillars fed on the leaflets at 25 °C for 5 days, after which they were scored for mortality and weighed. At most, one caterpillar per plant per leaf position (local, leaf systemic, plant systemic) was tested; in some cases not each leaf position was used, accounting for the variation in sample size. We analysed the effects of plant variety and damage treatment using *G*-tests. Mass of the survivors was analysed for each leaf position using two-way ANOVA with plant type and damage treatment as main effects.

In a separate experiment, we measured polyphenol oxidase activity to determine constitutive and inducible levels of jasmonate-based direct chemical defences in wild-type and jasmonate-deficient plants. Polyphenol oxidase is induced to a similar degree by caterpillar damage and exogenous jasmonic acid application (Thaler *et al.* 1996) and has been causally linked to resistance to herbivores (Felton *et al.* 1989; Stout *et al.* 1998). Using the same protocol as in the above experiment we established the same four treatments. The same methods were employed as in the direct defence experiment, except that the *S. exigua* caterpillars damaged the terminal leaflet of the fifth and sixth leafs. The leaf systemic leaflet of the sixth leaf was collected for chemical analysis. Polyphenol oxidase activity was measured using a caffeic acid substrate and the reaction was measured at 470 nm with a microplate reader (modified from Thaler *et al.* 1996). Two trials of this assay were conducted ($n = 25$–30 per treatment per trial).

Induction of indirect defence

We tested the attractiveness of plants to females of the predatory mite, *P. persimilis*. Our hypothesis was that wild-type plants would become more attractive to predatory mites when the plants were damaged by herbivores but that the jasmonate-deficient plants would not become more attractive when damaged. *S. exigua* caterpillars were used to damage the plants. Although *S. exigua* is not a prey of *P. persimilis*, these caterpillars were purposefully employed because we were able to control/quantify the amount of damage imposed, which would not have been possible with spider mites (a preferred prey item of *P. persimilis*). It has been demonstrated that *P. persimilis* can be attracted to volatiles produced by plants damaged by the non-host *S. exigua* (Shimoda & Dicke 1999, 2000).

Four binary predator choice comparisons were conducted:

1 wild-type undamaged vs. jasmonate-deficient undamaged;
2 wild-type undamaged vs. wild-type damaged;
3 jasmonate-deficient undamaged vs. jasmonate-deficient damaged; and
4 wild-type damaged vs. jasmonate-deficient damaged.

Comparison 1 tests the relative attractiveness of the two plants in the undamaged state; comparison 2 tests whether herbivore damage increases the attractiveness of the wild-type plant; comparison 3 tests whether damage increases the attractiveness of the jasmonate-deficient plant; and comparison 4 tests whether damaged wild-type plants are more attractive than damaged jasmonate-deficient plants.

We assayed predator choice using a closed system Y-tube olfactometer (Takabayashi & Dicke 1992). In this system, the air flows over a charcoal filter and then over the two odour sources, through the arms of the Y, and into the base of the Y where they mix. A metal wire runs from the base of the Y to the two arms. The predator is placed on the wire at the base of the Y and given 5 min to walk upwind towards the odour sources in each arm. The airflow was 4 L/min in each arm. The position of the odour sources was switched after five predators made a choice.

The plants used for assessment of indirect defence were also used for assessment of direct defence, and were treated as described above. After collecting the leaflets for the direct defence measures, we excized the terminal leaflet of the third leaf (the remaining leaflet with direct herbivore contact, the terminal leaflet of the fourth leaf was removed earlier that day for herbivore bioassays) and the plant was cut at the base of the stem. We were able to easily remove all of the caterpillars and frass from the plant prior to the assay to ensure that the volatile cues were coming from the plants

themselves. Each cut stem was inserted into a water pick sealed with parafilm and six plants were combined as an odour source for each treatment. Each odour source was placed in a 5-L glass jar with a wide mouth, an inlet and an outlet. We allowed the air to flow for 1 h after placing plants in the jars and before collection to let the flush of volatile compounds released due to handling of the plants pass. Each odour source had approximately the same mass (means ± SE from all days: wild-type undamaged, 116.8 ± 5.6 g; wild-type damaged, 117.2 ± 2.6 g; jasmonate-deficient undamaged, 117.8 ± 4.2 g; jasmonate-deficient damaged, 119.2 ± 3.2 g; $P \gg 0.05$). Adult female predators were starved 1–6 h before use and each individual was used only once.

Three of the four comparisons were performed per day on four separate days with independent sets of plants. Each comparison on each day was performed using 10–30 predators. Because results were qualitatively the same for each day, the days were combined for analysis. The total number of replicates per comparison is given in the figures. The number of predator choices for each odour source was compared using a Pearson's chi-square test. Predatory mites that did not make a choice were not included in the statistical analyses.

Volatile collection

Volatile organic compounds were collected and quantified from a separate set of plants treated in the four groups described above. These experiments were conducted to determine whether damage causes the same amount and composition of volatile emissions in jasmonate-deficient plants compared with wild-type plants. Larvae were placed on plants and adjustments to insect numbers were made as necessary to insure that damaged plants were exposed to commensurate amounts of feeding. The third and fourth leaves from the base of six-leaf plants were damaged with 10-second instar caterpillars on the wild-type plants and 4-second instar caterpillars on the jasmonate-deficient plants. Herbivores were allowed to feed continuously for 5 days. Volatiles were collected for 10-h intervals from each plant on days 1, 3 and 5. Volatiles were collected from leaves still attached to the plant. The third and fourth leaves were placed in a Teflon framed square chamber. The sandwich glass plate design (18 × 18 × 3 cm) has a fixed and sliding glass plate fitted with Teflon moulding. A slit in the Teflon allows the petiole to exit the chamber. Charcoal-purified air passed over the leaves at a rate of 1 L/min and exited the chamber through a second port at a rate of 0.5 L/min. Collection chambers were placed under metal halide and sodium lamps for a 16 h : 8 h, light : dark photoperiod with a total light intensity of 700 μmole m^{-2} s^{-1}, and temperature in the chambers was held at 28 °C.

Volatiles were collected on the Super-Q adsorbent traps for 10-h intervals and then eluted with 150 μL of dichloromethane; 800 ng of nonyl acetate was added as an internal standard. Extracts were analysed by capillary GC on a 15 mm × 0.25 mm (i.d.) fused silica column with a 0.25-μm thick bonded methyl siloxane (Quadrex, New Haven, CT, USA). Injections were made in the splitless mode for 30 s, and the gas chromatograph (GC) was operated under the following conditions: injector 230 °C, detector 250 °C, column oven 40 °C for 0.5 min, then programmed at a rate of 12 °C until 180 °C and finally ramped at a rate of 40 °C to 220 °C for 2 min; He carrier gas linear flow velocity was 50 cm s^{-1}. The components of the plant volatile emission were identified by comparison of GC retention times with those of authentic standards and by comparison of mass spectra with spectra of an EPA/NIH data base. Quantification was based on comparison of area under the GC-FID peak, with the internal standard added at an amount of 800 ng. For comparisons of the same compound under different treatments, response factors for individual compounds were assumed to be equal. The abundant volatile compounds are reported and statistically analysed.

Volatiles from three replicates of each treatment were collected on three separate days. The amounts of each abundant volatile compound were analysed using two-way MANOVA with plant type and damage as the main effects. Compounds were classified by biosynthetic origin so that the C-6, C-15 and C-10 compounds were analysed as groups. The results of MANOVA on volatiles collected on days 1, 3 and 5 are reported. As the results were similar, only the volatiles collected on day 5 are presented in graphs.

Restoration of defence in the jasmonate-deficient plants

If the deficiency in jasmonic acid is what decreases the defence of the mutant plants, treating the plants with exogenous jasmonic acid should restore the defended phenotype. To assess this, we repeated our measures of direct and indirect defence on additional sets of wild-type and jasmonate-deficient plants that were either untreated or sprayed with jasmonic acid. The plants in the jasmonic acid spray treatment received two treatments of 0.5 mM jasmonic acid. The entire plant was misted with 0.7 mL of 0.5 mM jasmonic acid 4 and 2 days before use in olfactometer experiments. Jasmonic acid was synthesized from methyl jasmonate according to the methods of Farmer & Ryan (1992). We established four treatments: wild-type control, wild-type plants treated with jasmonic acid, jasmonate-deficient control, and jasmonate-deficient plants treated with jasmonic acid. We examined direct defence by measuring the performance of *S. exigua* caterpillars on the excised terminal leaflet of the fourth leaf of plants in these

four treatments. We also measured the indirect defences by assaying predator choice for these plants as in the indirect defence experiments described above. We compared the attraction of predatory mites to (i) wild-type control vs. wild-type plants treated with jasmonic acid, and (ii) jasmonate-deficient control vs. jasmonate-deficient plants treated with jasmonic acid. Lastly, we tested for attractiveness of the jasmonic acid itself to predatory mites. Six 10-cm^2 pieces of filter paper were misted with 0.7 mL of 0.5 mM JA and six pieces were misted with water as a control and allowed to sit for 2 days in a growth chamber (the time between jasmonic acid treatment of plants and use in assays in the previous experiments). They were subsequently tested for attractiveness to *P. persimilis* using the same methods as described above. The number of replicates per comparison is shown in the figures. Preference for each odour source was analysed using a Pearson's chi-square test.

RESULTS AND DISCUSSION

Induction of direct defence

Direct defence was induced in wild-type plants when they were damaged by *S. exigua* but not in the jasmonate-deficient plants when they were damaged. Independent of damage, herbivores had 30% lower mortality (Fig. 1) and survivors had over double the mass (Table 1) when reared on jasmonate-deficient plants compared with wild-type plants. Mortality of caterpillars on wild-type foliage increased following damage on the local leaflet (mortality: $G = 14.2$, $P < 0.001$) and on the leaf systemic leaflet (mortality: $G = 3.76$, $P = 0.052$), but not on the plant systemic leaflet compared with undamaged wild-type plants ($G = 1.33$, $P = 0.25$; Fig. 1). In contrast, we did not detect increased mortality in any of the damaged jasmonate-deficient plants compared with undamaged plants (local leaflet, $G = 1.84$, $P = 0.17$; leaf systemic leaflet, $G = 0.06$, $P = 0.80$; systemic leaf, $G = 0.02$, $P = 0.88$).

The effects of plant type and damage on the growth of the surviving caterpillars followed the same patterns as the effects on mortality (Table 1). The effects of plant type and damage on caterpillar growth are expected to be weaker than the effects on mortality because only the surviving caterpillars can be assayed. Independent of damage, mass of *S. exigua* was higher on the jasmonate-deficient plants compared with wild-type plants (effect of plant: local leaflet, 1.8 times heavier on jasmonate-deficient plants compared with wild-type plants; leaf systemic leaflet, 3.3 times heavier on jasmonate-deficient plants; plant systemic leaflet, 2.1 times heavier on jasmonate-deficient plants). The mass of *S. exigua* caterpillars was lower on damaged plants of both plant types, although this was only marginally significant (damage: local leaflet, 86% mass on damaged compared with undamaged plants; leaf systemic leaflet, 81% mass on damaged compared with undamaged plants; systemic leaflet, 97% mass on damaged compared with undamaged plants) (Table 1).

Polyphenol oxidase activity (Δ optical density g^{-1} leaf tissue min^{-1}) was not different between the wild-type plants and the jasmonate-deficient plants when both plant types were undamaged (Fig. 2). However, polyphenol oxidase activity nearly doubled in the leaf systemic leaflets when wild-type plants were damaged, but did not increase when the jasmonate-deficient plants were damaged (plant, $F_{1,100} = 1.27$, $P = 0.26$; damage, $F_{1,100} = 7.17$, $P = 0.008$;

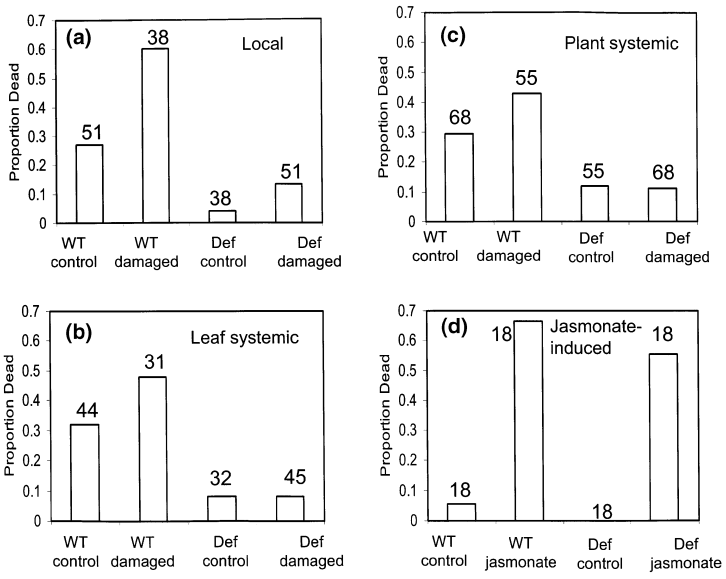

Figure 1 Mortality of *Spodoptera exigua* caterpillars growing on wild-type (WT) and jasmonate-deficient plants (Def). In the local (a), leaf systemic (b), and plant systemic panels (c), half of the plants were damaged by *S. exigua*. In the jasmonic acid-induced panel (d), half of the plants were treated with jasmonic acid. The numbers in each bar represent the number of treated plants.

Table 1 Effect of *Spodoptera exigua* feeding damage on subsequent larval mass (mg) gain of *S. exigua* neonates that survived. Note that there was differential survival among the treatments causing variation in sample size (see text and Fig. 1). Separate two-way ANOVA tests for each leaf position are reported. Non–significant interaction terms included in the model are not reported.

	Local		Leaf systemic		Plant systemic	
Plant/treatment	*n*	Mean ± SE	*n*	Mean ± SE	*n*	Mean ± SE
Wild-type undamaged	38	0.355 ± 0.030	27	0.254 ± 0.034	48	0.335 ± 0.030
Wild-type damaged	13	0.325 ± 0.113	18	0.178 ± 0.024	29	0.339 ± 0.045
Jasmonate-deficient undamaged	34	0.792 ± 0.074	28	0.598 ± 0.061	48	0.742 ± 0.084
Jasmonate-deficient damaged	44	0.511 ± 0.079	39	0.507 ± 0.047	58	0.643 ± 0.067
2-way ANOVA	Effect of plant			Effect of damage		
	F	d.f.	*P*	*F*	d.f.	*P*
Local	16.6	1,125	< 0.001	2.84	1,125	0.095
Leaf systemic	45.3	1,108	< 0.001	2.83	1,108	0.096
Plant systemic	28.2	1,179	< 0.001	0.51	1,179	0.44

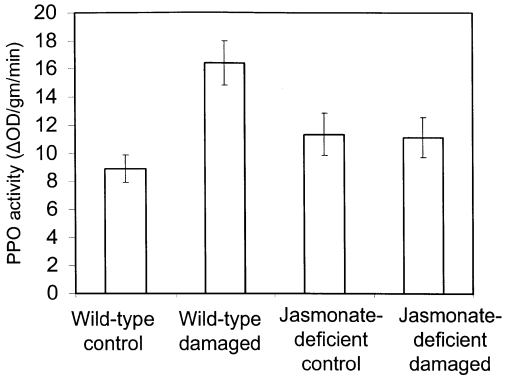

Figure 2 Polyphenol oxidase activity (ΔOD g^{-1} min^{-1}) for plants in four treatments: wild-type control, wild-type damaged with *Spodoptera exigua* caterpillars, jasmonate-deficient control, and jasmonate-deficient plants damaged with *S. exigua* caterpillars. Mean ± standard error is shown.

trial, $F_{1,100} = 5.10$, $P = 0.026$; damage × plant, $F_{1,100} = 7.64$, $P = 0.007$).

Why was the growth of caterpillars higher on the jasmonate-deficient plants compared with the wild-type plants when the plants were undamaged? It may be that during the 5-day assay period of the experiment the wild-type leaflets responded to the assay caterpillar whereas the jasmonate-deficient leaflets did not. There are likely to be other unmeasured properties of the jasmonate-deficient plants that are altered due to differences in basal jasmonate pathway expression. However, in several other traits that were measured, we did not find a significant difference between undamaged wild-type and jasmonate-deficient plants. For instance, polyphenol oxidase activity, one reliable measure of the jasmonate pathway in tomato plants, was not different in wild-type and jasmonate-deficient plants when both were undamaged. Biomass of the undamaged jasmonate-deficient plants did not differ from the wild-type plants (see mass of the odour source in the indirect defence methods; Thaler, unpublished data). Finally, there was no difference between volatile production and attraction of predators to wild-type and jasmonate-deficient plants when both were undamaged (see data below). Thus, differences in caterpillar mass between plant types were likely to be caused by rapid induction of direct defences in excized wild-type leaves.

Induction of indirect defence

S. exigua feeding induced indirect defence in wild-type plants, but not in jasmonate-deficient plants (Fig. 3a). The predatory mites did not differentiate between the wild-type and jasmonate-deficient plants when both types were undamaged ($G = 0.63$, $P = 0.43$). Predatory mites were more attracted to wild-type plants when they were damaged compared with undamaged wild-type plants ($G = 4.76$, $P = 0.029$), but they did not discriminate between volatiles from damaged and undamaged jasmonate-deficient plants ($G = 0.262$, $P = 0.608$). When plants of both types were damaged, wild-type plants were more attractive than the jasmonate-deficient plants ($G = 11.84$, $P = 0.006$). The overall rate of non-responding by predators was low (10.9%), indicating that the predators were stimulated by the odour sources we presented. The non-responding predators were excluded from the analysis.

Volatile collection

Wild-type and jasmonate-deficient plants released the same levels of volatile compounds in the absence of insect damage (Fig. 4, Table 2). Jasmonate-deficient plants pro-

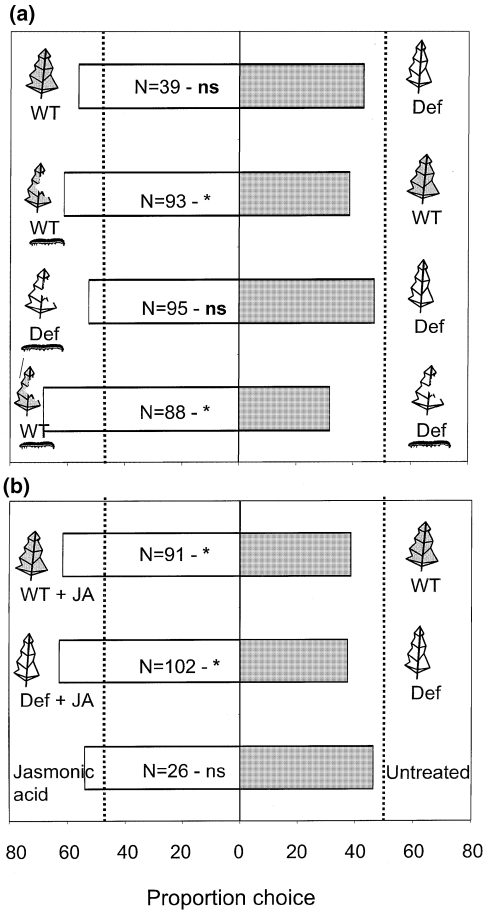

Figure 3 (a) Proportion of female predatory mites, *Phytoseiulus persimilis*, choosing volatiles from plants in each arm of the Y-tube olfactometer. Only predatory mites making a choice were included in the analyses. Bar 1: wild-type undamaged vs. jasmonate-deficient undamaged; 24% of predators did not make a choice in this comparison. Bar 2: wild-type caterpillar damaged vs. wild-type undamaged; 3% of predators did not make a choice in this comparison. Bar 3: jasmonate-deficient damaged vs. jasmonate-deficient undamaged; 20% of predators did not make a choice in this comparison. Bar 4: wild-type damaged vs. jasmonate-deficient damaged; 17% of predators did not make a choice in this comparison. The numbers inside each bar represent the sample size and the stars indicate level of significance. n.s. = non-significant, $*P < 0.05$. The vertical dashed lines indicate 50% choice. (b) Proportion of female predatory mites, *Phytoseiulus persimilis*, choosing plants in each arm of the Y-tube olfactometer. Bar 1: wild-type sprayed with jasmonic acid vs. wild-type untreated; 5% of predators did not make a choice in this comparison. Bar 2: jasmonate-deficient sprayed with jasmonic acid vs. untreated; 5% of predators did not make a choice in this comparison. Bar 3: jasmonic acid alone vs. untreated paper towel; 13% of predators did not make a choice in this comparison. The numbers inside each bar represent the sample size and the stars indicate level of significance. n.s. = non-significant, $*P < 0.05$. The vertical dashed lines indicate 50% choice.

duced higher levels of some volatile compounds when damaged, particularly the C-6 compounds (Z)-3-hexenal and (E)-2-hexenal, compared with undamaged jasmonate-deficient plants. As predicted, damaged jasmonate-deficient plants released 34% less monoterpenes and 51% less sesquiterpenes compared with damaged wild-type plants; these two groups comprise the major components of the volatile blend (Fig. 5).

We now know the composition of the volatile blend produced by tomato plants following damage by several herbivore species, including *S. exigua* (this study), *Manduca sexta* (M. Farag and P. Pare, unpublished data), the spider mite *T. urticae*, and mechanical damage (Dicke *et al.* 1998). Damage by different insect herbivores causes the release of remarkably similar volatile blends. The most variable compound induced by different types of damage was methyl salicylate (MeSA), a known attractant of *P. persimilis* (Dicke *et al.* 1990a). Spider mite damage and mechanical damage caused the release of similar compounds, except for MeSA and (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene, which were only released from plants damaged by spider mites (Dicke *et al.* 1998). Release of volatile compounds by tomato following damage by *S. exigua* was similar to spider mite damage, except that MeSA was again only released following spider mite damage. This pattern is similar to that of lima bean, where the spider mite *T. urticae* induces MeSA whereas the caterpillar *S. exigua* does not (Dicke *et al.* 1990a; Ozawa *et al.* 2000). Differences in the induction of MeSA have been correlated with stronger induction of the jasmonate pathway by *S. exigua* and the salicylate pathway by spider mites (Ozawa *et al.* 2000). Damage by another lepidopteran, *Manduca sexta*, did cause emission of methyl salicylate by tomato plants (M. Farag and P. Pare, unpublished data). In summary, these data confirm that there is some level of specificity in the volatile response of plants to different insect attackers even within the same feeding guild (De Moraes *et al.* 1998; Du *et al.* 1998; Dicke 1999a).

Restoration of defence in the jasmonate-deficient plants

Application of exogenous jasmonate restored both direct and indirect defences in the jasmonate-deficient plants (Figs 1d and 3b). The mortality of herbivores on the jasmonate-deficient plants increased from 0% mortality on controls to 58% mortality on jasmonate-treated plants ($G = 12.67$, $P < 0.001$). Similarly, mortality was 8% on control wild-type plants and 75% on jasmonate-treated plants ($G = 12.22$, $P < 0.001$). Rates of mortality on jasmonate-treated plants were too high to meaningfully measure herbivore growth rate. We have also shown that application of exogenous jasmonate restores expression of defensive proteins, e.g. polyphenol oxidase, to wild-type levels in jasmonate-deficient plants (Thaler and Higgins, unpublished manu-

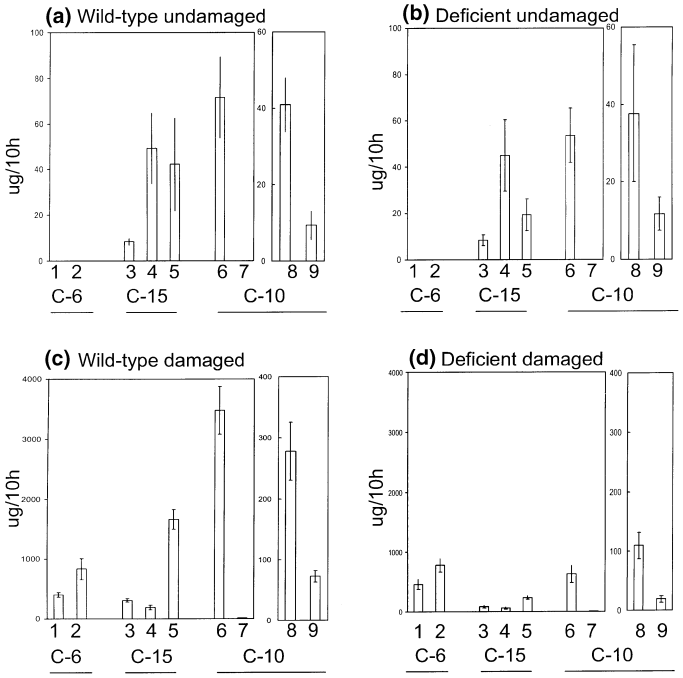

Figure 4 Volatile compounds collected on day 5 for a 10-h interval from leaves of wild-type and jasmonate-deficient plants: (a) wild-type undamaged plants, (b) jasmonate-deficient undamaged plants, (c) wild- type plants damaged by *Spodoptera exigua*, and (d) jasmonate-deficient plants damaged by *Spodoptera exigua*. Compounds are grouped as C6-volatiles (C-6), sesquiterpenes (C-15) and monoterpenes (C-10) and include (*Z*)-3-hexenal (1), (*E*)-2-hexenal (2), β-caryophyllene (3), α-humulene (4), δ-elemene (5), α-pinene (6), β-pinene (7), 2-carene (8), β-phellandrene (9), (_) = not detected. Bars represent mean ± standard error ($n = 3$).

Table 2 Two-way MANOVA for effects of plant type (wild type and jasmonate deficient) and damage treatment (*Spodoptera exigua* or undamaged) on the volatile compounds produced. Results are given for collections on days 1, 3 and 5. Volatiles were analysed in groups based on biosynthetic class, C-6, C-15 and C-10. d.f. = 2.7 for all C-6 comparisons, 3.6 for all C-15 comparisons and 4.5 for all C-10 comparisons.

	Plant			Damage			Plant × Damage		
	Wilk's λ	*F*	*P*	Wilk's λ	*F*	*P*	Wilk's λ	*F*	*P*
Day 1									
C-6	0.856	0.587	0.581	0.038	89.75	< 0.001	0.856	0.587	0.581
C-15	0.012	159.72	< 0.001	0.009	212.3	< 0.001	0.013	151.5	< 0.001
C-10	0.005	233.77	< 0.001	0.002	652.6	< 0.001	0.005	237.7	< 0.001
Day 3									
C-6	0.510	3.358	0.095	0.009	370.7	< 0.001	0.510	3.358	0.095
C-15	0.068	27.514	0.001	0.037	51.84	< 0.001	0.083	22.19	0.001
C-10	0.026	46.909	0.029	0.018	67.4	< 0.001	0.027	44.48	< 0.001
Day 5									
C-6	0.931	0.261	0.7	0.060	54.37	< 0.001	0.931	0.261	0.778
C-15	0.042	45.4	< 0.001	0.020	96.99	< 0.001	0.043	44.04	< 0.001
C-10	0.007	189.83	< 0.001	0.002	579.2	< 0.001	0.006	196.9	< 0.001

script). When treated with jasmonic acid, both the wild-type ($G = 4.86$, $P = 0.027$) and the jasmonate-deficient plants ($G = 6.67$, $P = 0.01$) became more attractive to predatory mites than the respective untreated plants. Predators were not attracted to the jasmonic acid itself or its breakdown products ($G = 0.151$, $P = 0.697$) (Fig. 3b).

CONCLUSIONS

Our results demonstrate that the jasmonate pathway is essential for both direct and indirect defence in tomato plants. Herbivore survivorship and growth rate were lower on damaged compared with undamaged wild-type plants but were not different on the jasmonate-deficient plants. This decrease in herbivore performance was correlated with an increase in polyphenol oxidase activity in damaged wild-type plants, but not in the damaged jasmonate-deficient plants. Predators were more attracted to damaged wild-type plants compared with undamaged controls and damaged jasmonate-deficient plants. Predator attraction correlated with differential changes in volatile production following damage by wild-type and jasmonate-deficient plants. Jasmonate

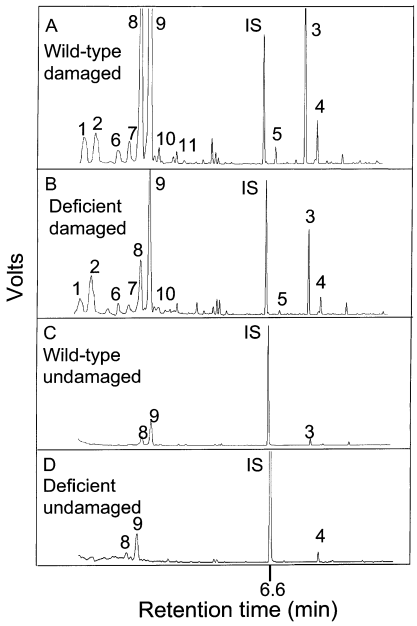

Figure 5 Chromatographic profiles of volatile compounds collected on day 5 for a 10-h interval from leaves of wild-type and jasmonate-deficient plants: (A) wild-type damaged by *Spodoptera exigua*, (B) jasmonate-deficient damaged by *Spodoptera exigua*, (C) wild-type undamaged, and (D) jasmonate-deficient undamaged. Compounds include (*Z*)-3-hexenal (1), (*E*)-2-hexenal (2), β-caryophyllene (3), α-humulene (4), δ-elemene (5), α-pinene (6), β-pinene (7), 2-carene (8), β-phellandrene (9), γ-terpinene (10), terpinolene (11). IS is the internal standard.

treatment restored the attractiveness of the jasmonate-deficient plants to the predator.

Coregulation of direct and indirect defences may result in interference with natural enemy effectiveness (Thaler 1999; Havill & Raffa 2000; Thaler 2002). The compounds or structures that function as direct defences against herbivores can also reduce the performance of natural enemies (Campbell & Duffey 1979; van Haren *et al.* 1987; Barbosa *et al.* 1991). Natural enemies can contact the plant secondary compounds inside their host or through the plant itself. For instance, parasitoids that pupate on the leaf surface can come into contact with toxins in the trichomes of plants (Kauffman & Kennedy 1989). Some compounds in glandular trichomes, such as polyphenol oxidase, are induced by jasmonate treatment (Thipyapong & Steffens 1997). Thus, although the increased attraction of natural enemies to induced plants can result in more offspring of the natural enemy, these offspring may have reduced performance compared with natural enemies reared in hosts from uninduced plants. Coregulation of direct and indirect defences could also interfere with the host location process of natural enemies searching for prey. If the direct defences are effective and rapidly reduce the density of herbivores, then the volatile compounds are no longer accurate indicators of current herbivore distribution. This may result in negative associative learning, where the natural enemies associate the plant volatiles with the absence of prey (Drukker *et al.* 2000).

Using the same signal transduction pathway for induced direct and indirect defence may be beneficial because most induced direct defences are not completely effective against herbivores. Multiple defences may provide better plant protection. Direct plant defences more frequently kill young herbivores, and natural enemies, i.e. indirect defences, frequently kill older herbivores (Cornell *et al.* 1998). Using both kinds of defence may increase the total mortality of herbivores because natural enemies will kill the herbivores that have escaped the plant's direct defences (Sih *et al.* 1998). Multiple defences also have the potential advantage of slowing the evolution of resistance by the herbivore to each defence (Pimentel & Bellotti 1976; but see Gould *et al.* 1991). Moreover, some authors have speculated that it may be especially difficult for herbivores to evolve resistance to both plant defences and natural enemies because their modes of action are radically different (Holt & Hochberg 1997). Future studies may find that the coregulation of direct and indirect plant defences by jasmonic acid has been favoured because of synergistic benefits of the two responses.

ACKNOWLEDGEMENTS

We thank Rieta Gols for help with the olfactometer, Herman Dijkman for the rearing of plants and predators, Ine Derksen-Koppers for kindly providing *S. exigua* eggs, and Marc Johnson for help with the PPO analysis. This manuscript was improved by the comments of Anurag Agrawal, Cesar Rodriguez-Saona, Danush Viswanathan and three anonymous reviewers. The Natural Science and Engineering Research Council Canada, The University of Toronto (JST) and USDA/NRI 35320–9378 (PWP) supported this research.

REFERENCES

Barbosa, P., Gross, P. & Kemper, J. (1991). Influence of plant allelochemicals on the tobacco hornworm and its parasitoid, *Cotesia congregata*. *Ecology*, 72, 1567–1575.

Berenbaum, M.R. & Zangerl, A.R. (1996). Phytochemical diversity: adaptation or random variation? In: *Phytochemical Diversity and Redundancy in Ecological Interactions* (eds Romeo, J.T., Saunders, J.A. & Barbosa, P.). Plenum Press, New York, pp. 1–24.

Boland, W., Hopke, J., Donath, J., Nuske, J. & Bublitz, F. (1995). Jasmonic acid and coronatin induce odor production in plants. *Angew. Chem. Int.*, 34, 1600–1602.

Campbell, B.C. & Duffey, S.S. (1979). Tomatine and parasitic wasps: potential incompatibility of plant antibiosis with biological control. *Science*, 205, 700–702.

Cornell, H.V., Hawkins, B.A. & Hochberg, M.E. (1998). Towards an empirically-based theory of herbivore demography. *Ecol. Entomol.*, 23, 340–349.

Creelman, R.A. & Mullet, J.E. (1997). Biosynthesis and action of jasmonates in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biology*, 48, 355–381.

De Moraes, C.M., Lewis, W.J., Pare, P.W., Alborn, H.T. & Tumlinson, J.H. (1998). Herbivore-infested plants selectively attract parasitoids. *Nature*, 393, 570–573.

Dicke, M. (1999a). Are herbivore induced plants reliable indicators of herbivore identity to foraging carnivorous arthropods? *Entomologia Expis Applicata*, 91, 131–142.

Dicke, M. (1999b). Evolution of induced indirect defense of plants. In: *The Ecology and Evolution of Inducible Defenses* (eds Tollrian, R. & Harvell, C.D.). Princeton University Press, Princeton, New Jersey, pp. 62–88.

Dicke, M., Gols, R., Ludeking, D. & Posthumus, M.A. (1999). Jasmonic acid and herbivory differentially induce carnivore-attracting plant volatiles in lima bean plants. *J. Chem. Ecol.*, 25, 1907–1922.

Dicke, M., Sabelis, M.W., Takabayashi, J., Bruin, J. & Posthumus, M.A. (1990b). Plant strategies of manipulating predator–prey interactions through allelochemicals: prospects for application in pest control. *J. Chem. Ecol.*, 16, 3091–3118.

Dicke, M., Takabayashi, J., Posthumus, M.A., Schutte, C. & Krips, O.E. (1998). Plant–phytoseiid interactions mediated by herbivore-induced plant volatiles: variation in production of cues and in responses of predatory mites. *Exp. Appl. Acarol.*, 22, 311–333.

Dicke, M., van Beek, T.A., Posthumus, M.A., Ben Dom, N., van Bokhoven, H. & De Groot, A.E. (1990a). Isolation and identification of volatile kairomone that affects acarine predator–prey interactions: involvement of host plant in its production. *J. Chem. Ecol.*, 16, 381–396.

Drukker, B., Bruin, J. & Sabelis, M.W. (2000). Anthocorid predators learn to associate herbivore-induced plant volatiles with presence or absence of prey. *Physiol. Entomol.*, 25, 260–265.

Drukker, B., Scutareanu, P. & Sabelis, M.W. (1995). Do anthocorid predators respond to synomones from *Psylla*-infested pear trees under field conditions? *Entomologia Expis Applicata*, 77, 193–203.

Du, Y., Poppy, G.M., Powell, W., Pickett, A., Wadhams, L.J. & Woodcock, C.M. (1998). Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. *J. Chem. Ecol.*, 24, 1355–1368.

Dyer, L.A., Dodson, C.D., Beihoffer, J. & Letourneau, D.K. (2001). Trade-offs in antiherbivore defenses in *Piper cenocladum*: ant mutualists versus plant secondary metabolites. *J. Chem. Ecol.*, 27, 581–592.

Farmer, E.E. & Ryan, C.A. (1992). Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. *The Plant Cell*, 4, 129–134.

Felton, G.W., Donato, K., Del Vecchio, R.J. & Duffey, S.S. (1989). Activation of plant foliar oxidases by insect feeding reduces nutritive quality of foliage for noctuid herbivores. *J. Chem. Ecol.*, 15, 2667–2694.

Fritzsche-Hoballah, M.E. & Turlings, T.C.J. (2001). Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. *Evol. Ecol. Res.*, 3, 553–565.

Gols, R., Posthumus, M.A. & Dicke, M. (1999). Jasmonic acid induces the production of gerbera volatiles that attract the biological control agent *Phytoseiulus persimilis*. *Entomologia Expis Applicata*, 93, 77–86.

Gomez, J.M. & Zamora, R. (1994). Top-down effects in a tritrophic system: parasitoids enhance plant fitness. *Ecology*, 75, 1023–1030.

Gould, F., Kennedy, G.G. & Johnson, M.T. (1991). Effects of natural enemies on the rate of herbivore adaptation to resistant host plants. *Entomol. Exp. Appl.*, 58, 1–14.

van Haren, R.J.F., Steenhuis, M.M., Sabelis, M.W. & De Ponti, O.M.B. (1987). Tomato stem trichomes and dispersal success of *Phytoseiulus persimilis* relative to its prey *Tetranychus urticae*. *Exp. Appl. Acarol.*, 3, 115–121.

Havill, N.P. & Raffa, K.F. (2000). Compound effects of induced plant responses on insect herbivores and parasitoids: implications for tritrophic interactions. *Ecol. Entomol.*, 25, 171–179.

Holt, R.D. & Hochberg, M.E. (1997). When is biological control evolutionarily stable (or is it)? *Ecology*, 78, 1673–1683.

Hopke, J., Donath, J., Blechert, S. & Boland, W. (1994). Herbivore-induced volatiles: the emission of acyclic homoterpenes from leaves of *Phaseolus lunatus* and *Zea mays* can be triggered by a B-glucosidase and jasmonic acid. *FEBS Lett.*, 352, 146–150.

Howe, G.A., Lightner, J., Browse, J. & Ryan, C.A. (1996). An octadecanoid pathway mutant (JL5) of tomato is compromised in signalling for defense against insect attack. *Plant Cell*, 8, 2067–2077.

Kahl, J., Siemens, D., Aerts, R., Gäbler, R., Kühnemann, F., Preston, C. *et al.* (2000). Herbivore-induced ethylene supresses a direct defense but not a putative indirect defense against an adapted herbivore. *Planta*, 210, 336–342.

Kauffman, W.C. & Kennedy, G.G. (1989). Toxicity of allelochemicals from wild insect-resistant tomato *Lycopersicon hirsutum* f. glabrum to *Campoetis sonorensis*, a parasitoid of *Heliothis zea*. *J. Chem. Ecol.*, 15, 2051–2060.

Kessler, A. & Baldwin, I.T. (2001). Defensive function of herbivore-induced plant volatile emissions in nature. *Science*, 291, 2141–2144.

Koch, T., Krumm, T., Jung, V., Engelberth, J. & Boland, W. (1999). Differential induction of plant volatile biosynthesis in the lima bean by early and late intermediates of the octadecanoid-signaling pathway. *Plant Physiol.*, 121, 153–162.

Lightner, J., Pearce, G., Ryan, C.A. & Browse, J. (1993). Isolation of signaling mutants of tomato (*Lycopersicon esculentum*). *Mol. General Genet.*, 241, 595–601.

van Loon, J.J.A., de Boer, J.G. & Dicke, M. (2000). Parasitoid-plant mutualism: parasitoid attack of herbivore increases plant reproduction. *Entomologia Expis Applicata*, 97, 219–227.

McConn, M., Creelman, R.A., Bell, E., Mullet, J.E. & Browse, J. (1997). Jasmonate is essential for insect defense in Arabidopsis. *Proc. Natl Acad. Sci. USA*, 94, 5473–5477.

Ozawa, R., Arimura, G., Takabayashi, J., Shimoda, T. & Nishioka, T. (2000). Involvement of jasmonate- and salicylate-related signaling pathway for the production of specific herbivore-induced volatiles in plants. *Plant Cell Physiol.*, 41, 391–398.

Pimentel, D. & Bellotti, A.C. (1976). Parasite host population systems and genetic stability. *The Am. Naturalist*, 110, 877–888.

Schneider, M., Schweitzer, P., Meuwly, P. & Metraux, J.P. (1996). Systemic acquired resistance in plants. *Int. Rev. Cytol.*, 168, 303–339.

Shimoda, T. & Dicke, M. (1999). Volatile stimuli related to feeding activity of nonprey caterpillars, *Spodoptera exigua*, affect olfactory response of the predatory mite *Phytoseiulus persimilis*. *J. Chem. Ecol.*, 25, 1585–1595.

Shimoda, T. & Dicke, M. (2000). Attraction of a predator to chemical information related to nonprey: when can it be adaptive? *Behav. Ecol.*, 11, 606–613.

Sih, A., Englund, G. & Wooster, D. (1998). Emergent impacts of multiple predators on prey. *Trends Ecol. Evol.*, 13, 350–355.

Staswick, P. & Lehman C. (1999). Jasmonic acid-signaled responses in plants. In: *Inducible Plant Defenses Against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture* (eds Agrawal, A.A., Tuzun, S. & Bent, E.). American Phytopathological Society, St Paul, pp. 117–136.

Steward, J.L. & Keeler, K.H. (1988). Are there trade-offs among antiherbivore defenses in *Ipomoea* (Convolvulaceae). *Oikos*, 53, 79–86.

Stout, M.J., Workman, K.V., Bostock, R.M. & Duffey, S.S. (1998). Stimulation and attenuation of induced resistance by elicitors and inhibitors of chemical induction in tomato (*Lycopersicon esculentum*) foliage. *Entomologia Expis Applicata*, 86, 267–279.

Takabayashi, J. & Dicke, M. (1992). Response of predatory mites with different rearing histories to volatiles of uninfested plants. *Entomologia Expis Applicata*, 64, 187–193.

Takabayashi, J. & Dicke, M. (1993). Volatile allelochemicals that mediate interactions in a tritrophic system consisting of predatory mites, spider mites and plants. In: *Mutualism and Community Organization: Behavioural, Theoretical, and Food-Web Approaches* (eds Kawanabe, H., Cohen, J.E. & Iwasaki, K.). Oxford University Press, Oxford, pp. 280–295.

Thaler, J.S. (1999). Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature*, 399, 686–688.

Thaler, J.S. (2002). Effect of jasmonate-induced plant responses on the natural enemies of herbivores. *J. Anim. Ecol.*, 71, 141–150.

Thaler, J.S., Stout, M.J., Karban, R. & Duffey, S.S. (1996). Exogenous jasmonates simulate insect wounding in tomato plants (*Lycopersicon esculentum*) in the laboratory and field. *J. Chem. Ecol.*, 22, 1767–1781.

Thipyapong, P. & Steffens, J.C. (1997). Tomato polyphenol oxidase: differential expression of the polyphenol oxidase F promoter to injuries and wound signals. *Plant Physiol.*, 115, 409–418.

Turlings, T.C.J., Tumlinson, J.H. & Lewis, W.J. (1990). Exploitation of herbivore-induced plant odors by host seeking parasitic wasps. *Science*, 250, 1251–1253.

Editor, M. E. Hochberg
Manuscript received 11 June 2002
First decision made 19 July 2002
Manuscript accepted 21 August 2002