DOI: 10.1111/j.1570-7458.2010.01043.x

Spider mites (*Tetranychus urticae*) perform poorly on and disperse from plants exposed to methyl jasmonate

Charles L. Rohwer[1]* & John E. Erwin[2]

[1]*University of Minnesota Southern Research and Outreach Center, 35838 120th St., Waseca, MN 56093, USA, and*
[2]*Department of Horticultural Sciences, University of Minnesota, 305 Alderman Hall, 1970 Folwell Avenue, St. Paul, MN 55108, USA*

Accepted: 21 July 2010

Key words: induced host resistance, *Impatiens wallerana*, *Viola* × *wittrockiana*, *Pelargonium* × *hortorum*, *Solanum lycopersicum*, proteinase inhibitor, Acari, Tetranychidae

Abstract

Jasmonates are plant hormones involved in wound and defense responses against herbivorous arthropods. Methyl jasmonate (MeJA) is used experimentally to induce defense responses in plants. In experiments outlined here we utilized a novel preference assay with unwounded plants that allowed us to study the impact of a MeJA spray on subsequent *Tetranychus urticae* Koch (Acari: Tetranychidae) proliferation and preference. Spraying plants with 100 μM MeJA 1 day before infestation caused mites to disperse within 2 days from treated impatiens [*Impatiens wallerana* Hook f., 'Super Elfin Pink' (Balsaminaceae)], pansy [*Viola* × *wittrockiana* Gams, 'Imperial Beaconsfield' (Violaceae)], and tomato [*Solanum lycopersicum* L., 'Big Boy' (Solanaceae)] plants. In addition, MeJA application reduced mite proliferation rate on impatiens and pansy by 60% (measured 22–34 days after infestation). Proteinase inhibitor (PI) assays suggested that MeJA-induced PIs alone were not responsible for the observed results in pansy and impatiens but may have been a factor in tomato. Implications of these results in the context of MeJA-induced resistance responses and possible directions for future research and application are discussed.

Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a polyphagous pest that can decrease vigor and yield on agricultural crops. In addition, *T. urticae* causes leaf stippling (spots), and in extreme cases, unsightly webbing or defoliation (Powell & Lindquist, 1997), which can decrease the desirability of infested ornamental plants and thereby reduce sales. Eradication or control of two-spotted spider mites requires cultural, chemical, and/or biological control practices (Powell & Lindquist, 1997; Bethke et al., 2004). A unique approach to mite control involves activating plant defenses to reduce mite vigor and/or mite preference for a crop.

Plant resistance to herbivores can be activated by exogenous application of a class of plant hormones called jasmonates, including methyl jasmonate (MeJA) and jasmonic acid (JA). Literature on responses of plant-chewing insects to jasmonate-treated plants is abundant (Rohwer & Erwin, 2008). However, unlike plant-chewing insects, mites are cell content-feeding arachnids. Jasmonate-induced resistance to mite infestation was reported on a number of agricultural crops including, among others, grape [*Vitis vinifera* L. (Vitaceae) (Omer et al., 2000)], strawberry [*Fragaria ananassa* Duch. (Rosaceae) (Warabieda et al., 2005)], lima bean [*Phaseolus lunatus* L. (Fabaceae) (Choh et al., 2004)], and tomato [*Solanum lycopersicum* L. (Solanaceae) (Li et al., 2002; Thaler et al., 2002a,b; Ament et al., 2004)]. Tomato mutants with reduced-jasmonate responses (def-1) were more susceptible to *T. urticae* infestation than untreated plants (Li et al., 2002). Tomato plants wounded by *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) (with enhanced jasmonate-related defenses compared to unwounded plants) reduced *T. urticae* fecundity compared to unwounded plants (Stout et al., 1998). We explored whether *T. urticae* resistance can be induced on uninfested greenhouse crops by applying MeJA. Induced mite resistance could reduce the potential for mite infestation and may ultimately reduce pesticide use.

*Correspondence: Charles L. Rohwer, University of Minnesota Southern Research and Outreach Center, 35838 120th St., Waseca, MN 56093, USA. E-mail: rohw0009@umn.edu

Although limiting mite proliferation with jasmonate-induced plant responses has been studied (Omer et al., 2001; Li et al., 2004), those studies are few, and results could be influenced by the experimental methodology. For instance, in choice studies (in which an herbivore chooses between selected food or oviposition sites), detached-leaf or leaf disc assays have often been used. In one of those studies, *T. urticae* raised on leaf discs had lower fecundity than mites raised on intact plants (Kavousi et al., 2009). Wounding itself can cause JA accumulation and elicits resistance responses similar to those of an exogenous jasmonate application (Wasternack & Parthier, 1997). Therefore, leaf or leaf section removal may confound the results (Wolfson, 1988; Hildmann et al., 1992). Here, we investigated whether foliar MeJA application altered *T. urticae* preference on unwounded, intact plants.

One way that MeJA may elicit plant defense responses is by promoting synthesis of antifeedant PIs (Rohwer & Erwin, 2008). Mite feeding induces PI synthesis in tomato, which is believed to contribute to mite resistance in that crop (Li et al., 2002; Kant et al., 2004). We studied whether our MeJA treatments induced PI activity in impatiens [*Impatiens wallerana* Hook f. (Balsaminaceae)], pansy [*Viola* × *wittrockiana* Gams (Violaceae)], and tomato to determine whether PIs may play a role in the results that we observed.

Exogenous jasmonates often show dose-dependent phytotoxicity (Boughton et al., 2006). Therefore, we were interested in studying whether defense responses could be triggered at sub-phytotoxic rates (<1 mM). Experiments described here explore whether *T. urticae* preference and proliferation is altered on plants sprayed with low-dose MeJA, and explore whether leaf PI content is affected by those applications.

Materials and methods

To study *T. urticae* responses to treating host plants with MeJA, two experiments were conducted. In experiment I, mite preference behavior was studied on four plant species – pansy, impatiens, geranium [*Pelargonium* × *hortorum* Bailey (Geraniaceae)], and tomato – treated with MeJA. In experiment II, *T. urticae* proliferation on pansy and impatiens following MeJA application was studied.

General procedures

Seedlings in 288-cell plug trays (9.7 ml $cell^{-1}$) were obtained from Wagner's Greenhouse (Minneapolis, MN, USA) and transplanted into 9-cell packs separated from a 50-cell (67 ml per cell, 256 cm^2 per group of nine cells) tray for experiment I, or 950- and 650-ml pots in 2006 and 2007, respectively, in experiment II. All plants were grown in Sun Gro Sunshine® LC 8 potting media (SunGro Horticulture, Bellevue, WA, USA) and fertilized at each watering with 10.7 mM N (provided by Peters Excel 15-5-15 Cal-Mag® water-soluble fertilizer; JR Peters, Allentown, PA, USA). Adult female spider mites (identified by the presence of a round abdomen, large body, and eight ambulatory legs) were used for experiments I and II because adult females are the primary dispersers (Smitley & Kennedy, 1985) and were presumably fecund.

Experiment I

To study *T. urticae* evacuation from intact plants treated with MeJA, we devised a system in which a treated plant was placed in the center of eight untreated plants growing in a 3 × 3-cell section (excised from a 50-cell tray), and in contact with at least one of the eight adjacent untreated plants. This arrangement was used so that orientation of the center plant and proximity to a neighboring plant would not affect the ability of the mites to leave the plant. Before placing the treated plant among untreated plants, treated plants were sprayed with a 'control' solution [0.05% acetone and 0.01% Triton X-100 (vol/vol) in water] or MeJA (5 or 100 μM, suspended in the 'control' solution) and allowed to dry for 18–24 h. Treated plants were allowed to dry no closer than 8 m from another treatment. Ten adult female spider mites, reared on *Phaseolus vulgaris* L. (Fabaceae) (described below), were placed on the center (treated) plant 18–24 h after plants were sprayed with MeJA. The experimental unit (a set of nine plants, each plant in a 67-ml cell) was placed on an inverted 12.5-cm square pot resting in a tray of water to prevent mite movement between experimental units, and plants were subirrigated as needed. Approximately 48 h after mite infestation, live mite number on the center (treated) plant and on the eight untreated plants was determined.

Experiment I was replicated 14 times (seven times in 2006 and seven in 2007). Impatiens ('Super Elfin Pink'; PanAmerican Seed, West Chicago, IL, USA), pansy ('Imperial Beaconsfield'; Ball Horticultural, West Chicago, IL, USA), and tomato ('Big Boy'; Burpee, Warminster, PA, USA) were used in 2006 and 2007. Geranium ('Pinto Pink'; Syngenta, Lisle, IL, USA) was only studied in 2006 due to high mite mortality after transferring mites from bean to geranium in 2006. In 2006, transplants were received on 4 September, transplanted on 16 September, and MeJA treatment began on 4 October. In 2007, transplants were received and transplanted on 8 August and MeJA treatment began on 10 September. Some pansy and impatiens had flower buds during treatment. Both these species were similar in size and were shorter in stature than tomato.

In 2006 and 2007, a group of replicates (typically containing each species × treatment combination) was treated with ca. 2.8 ml (pansy, impatiens, and geranium) or 3.8 ml (tomato) of MeJA or 'control' solution, and mites were counted on one set of treatment combinations each day. For example, on each day during the experimental period, we sprayed one impatiens, pansy, geranium, and tomato plant with MeJA and one of each species with the 'control' solution. On the same day, we placed sprayed plants (from the previous day) amongst eight untreated plants, transferred mites to those treated plants, and counted mites placed on plants 2 days before. We sprayed 100 μM MeJA in 2006, and in 2007 we sprayed 5 or 100 μM MeJA. Plants were grown at 20.7 ± 1.6 °C under natural daylight (beginning at sunrise; sunrise varied from 07:04 to 06:19 hours at 45°N latitude) plus supplemental irradiance (150–200 μmol m^{-2} s^{-1}, 16-h photoperiod; approximately 15 MJ m^{-2} day^{-1} global irradiance) to promote growth.

We analyzed the binomial preference data for each mite (did not move from treated plant = 0, moved from treated plant = 1) using a binomial generalized mixed model (Bates et al., 2008) in R (version 2.9.1; R Development Core Team, 2009). We included plant species, MeJA treatment, and the species*treatment interaction as fixed effects in the model, with replicate (1–14) included as a random effect. Each plant was considered a pseudoreplicate because multiple mites were applied to a plant in each replicate (Crawley, 2007). Non-significant interactions allowed us to simplify the model to include main effects only based on AIC reduction (Akaike's information criterion; Crawley, 2007). We analyzed the arcsin-transformed percent of mites recovered in each replicate with ANOVA in R (R Development Core Team, 2009) and used Tukey's-adjusted honestly significant difference (HSD) for mean separation. Year (2006 or 2007), treatment, species, and the treatment*species interaction were used as fixed effects (Crawley, 2007).

To assess possible direct effects of MeJA on *T. urticae*, we performed a similar experiment on MeJA-treated Whatman 3MM paper. We sprayed 5 × 5-cm square paper with 0.05% acetone and 0.01% Triton X-100 (vol/vol) in water (control) or the same solution containing 100 μM MeJA. This paper was treated with ca. 670 mg of solution and allowed to dry. The treated square was then placed in the center of a larger 15 × 15-cm piece of paper 24 h after treatment. The edge of this larger piece of paper was treated with lanolin to prevent mite movement off of the larger paper. Six mites were placed on each small square, and the number of mites on or underneath the smaller square was counted eight times over a 30-min period. The experiment was repeated nine consecutive times on the same day. These data were analyzed with a mixed effects repeated measures model considering MeJA treatment as the between-subjects effect (Crawley, 2007).

Experiment II

We measured *T. urticae* proliferation on pansy and impatiens sprayed with the 'control' solution described in experiment I or with 100 μM MeJA. Plants were sprayed to 'runoff' (wet, but not dripping; ca. 3 ml) and five adult mites were placed on those plants 24 h later. The experiment was first performed in 2006 using 3–4 plants each of pansy and impatiens per treatment, and this was repeated in 2007 using 7–9 plants of each species per treatment. In 2006, mites were originally collected from *Gaura* spp. (Onagraceae) in the plant growth facilities at the University of Minnesota (St. Paul, MN, USA) and reared on *P. vulgaris* 'Blue Lake' in an environmental growth chamber (16-h photoperiod) for a minimum of 3 months prior to the experiment. In the second set of replicates (2007), mites were collected directly from *Ulmus americana* L. (Ulmaceae) or *Populus* × *berolinensis* (K. Koch) Dippel (Salicaceae) growing in the same plant growth facilities.

Growth conditions for the plants and mites in experiment II are detailed in Table 1. All plants were grown in 650- (2007) or 950-ml (2006) pots under thrips-screening (150 μm mesh) and on an inverted 12.5-cm square pot resting in a tray of water (ca. 5 cm deep) to prevent interplant mite movement after plants were infested and to insure lack of infestation from other pests. Mites in all stages, except eggs, were counted 22–25 days after applying them in 2006 and 31–34 days in 2007. Differences in proliferation duration were due to time constraints.

Mite proliferation rate (mites per day) was analyzed using ANOVA in R. Data (number of mites per days since transfer to host) were ln-transformed prior to analysis. Mite source (from the three original host plants), MeJA treatment, plant species, and all interactions were included as fixed effects in the full model. One data point was removed from the analysis to improve residual normality. Model simplification (Crawley, 2007) allowed us to create a minimal model including no interactions (none were significant).

Proteinase inhibitor assay

We measured PIs in plants treated with 0, 100, or 1 000 μM MeJA to elucidate potential PI effects on mites. Plants were grown as described in experiment II. Proteinase inhibitors were analyzed using a method similar to that described by Orians et al. (2000). Units of trypsin inhibition from plant extract were measured by comparing a mixture containing trypsin, plant extract, and azocasein to a mixture containing only trypsin and azocasein. Tris buffer (50 mM, pH 8.0, 100 μl) was added to a 1.5-ml plastic

Table 1 Environmental conditions for the *Tetranychus urticae* performance assay (experiment II). All growth conditions after mite transfer to impatiens or pansy occurred under 16-h photoperiods provided by constant illumination from high-pressure sodium (HPS) lamps (06:00–22:00 hours) to encourage plant growth. Plants were grown under thrips screen to exclude other pests

Before mites transferred to impatiens or pansy			During mite proliferation on impatiens or pansy			
Tetranychus urticae source	Temperature setpoint (°C)	% relative humidity	Start–end date	Supplemental irradiance[1] ($\mu mol.m^{-2}.s^{-1}$)	Measured temperature (°C)	% relative humidity
Phaseolus vulgaris, growth chamber (2006)	23/20 (day/night)	61 ± 15	26 March–20 April	90–160	21.5 ± 3.1 (22.8/20.1, day/night)	41 ± 14
Populus × *berolinensis* or *Ulmus americana*, greenhouse (2007)	21	Not measured	18 May–21 June	50	24.1 ± 6.6 (26.6/19.0, day/night)	49 ± 19

Values are given as mean ± standard deviation.
[1]Supplemental irradiance underneath insect screening at plant canopy height from HPS lamps.

tube containing four 6-mm diameter leaf discs (freshly harvested from 1 to 2 of the most recently fully expanded leaves), along with three 4-mm diameter stainless steel beads. The tissue was vortexed in a Mixer Mill (Retsch MM300; Qiagen, Valencia, CA, USA) for 3 min at 11 kHz or until tissue was completely macerated. Beads were removed from the tubes and samples were centrifuged at 10 000 ***g*** and 4 °C for 10 min. The supernatant was transferred to another tube and re-centrifuged. This supernatant (leaf extract) was then used for PI analysis. To 1.5-ml plastic tubes labeled 'A', we added 70 µl 50 mM Tris (pH 8.0), 25 µl 2% (wt/vol) azocasein in Tris, and 10 µl 1 mM HCl. Tubes labeled 'B' contained the same mixture of Tris and azocasein, with trypsin in 1 mM HCl replacing the pure HCl (0.4 mg ml^{-1} trypsin for pansy and impatiens, 0.2 mg ml^{-1} for tomato). Tubes 'A' and 'B' (assay controls) were repeated three times for each set of plant samples studied to establish a maximum trypsin activity in the absence of plant extract. Tubes labeled 'C' contained 40 µl Tris, 25 µl azocasein, 10 µl HCl, and 30 µl leaf extract. Tubes labeled 'D' contained the same mixture as 'C', but with trypsin replacing the pure HCl. 'C' and 'D' were repeated once for each plant extract to determine trypsin activity in the presence of plant tissue. Trypsin was added last to the PI assay mixtures (on ice), and the mixture was incubated at 30 °C for 15 min for pansy and impatiens and 20 min for tomato. The reaction was stopped by adding 50 µl trichloroacetic acid (10% wt/vol in H_2O) and briefly vortexing. The mixture was then centrifuged at room temperature for 10 min (10 000 ***g***). Supernatant (100 µl) was transferred to a 96-well plate and 100 µl 1 M NaOH was added; this was gently mixed, and absorbance at 450 nm was read with a precision microplate reader (Molecular Devices, Sunnyvale, CA, USA).

Units of trypsin inhibition (Units PI) were defined as inhibition of an increase in OD_{450} of 0.001 min^{-1}, and were calculated as follows, where t = 15 min for impatiens and pansy, and t = 20 min for tomato:

$$\text{Units PI} = \frac{\left[\left(\overline{B} - \overline{A}\right) - (D - C)\right]}{0.001 \times t}.$$

Proteinase inhibitor results were ln-transformed to improve residual normality and analyzed using ANOVA. A significant species effect was observed based on Tukey's HSD ($\alpha = 0.05$). Results were then analyzed separately for each species using ANOVA, and Dunnett's test was used to compare MeJA treatments with the control (Hothorn et al., 2008; R Development Core Team, 2009).

To verify our PI assay, we wounded the most recently fully expanded leaf of tomato, pansy, and impatiens by crushing 50% of the leaf (distal half) with pliers. We then compared PI units of wounded tissue to unwounded tissue. Tomato plants were ca. 1 m tall (grown in the display garden on the University of Minnesota Campus, St. Paul, MN, USA). Flowering impatiens and pansy plants were purchased from a local greenhouse in 10-cm pots (Wagner's Greenhouse, Minneapolis, MN, USA).

Results

Experiment I

Mites were more likely to leave plants treated with 100 µM MeJA compared to control plants ($P<0.001$; Figure 1). The species*treatment interaction was not significant in the global ANOVA. Mites were 2 or 1.3 times more likely to move toward the edge of a group of tomato plants compared to pansy or impatiens, respectively, and 1.6 times more likely to move from impatiens compared to pansy

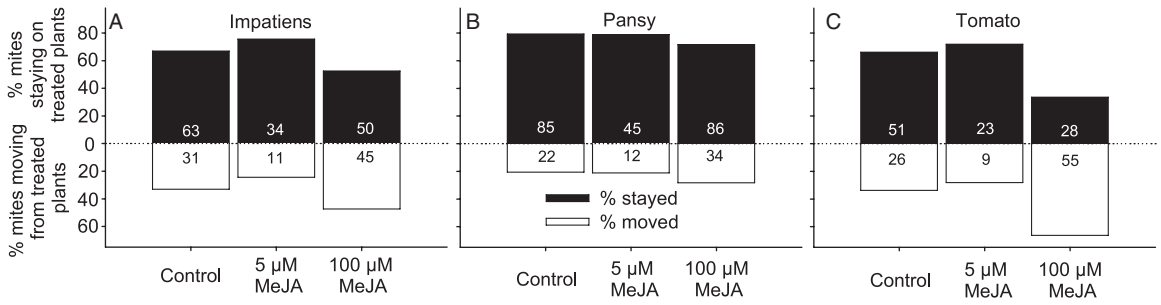

Figure 1 The percent of recovered mites that stayed on (A) treated impatiens, (B) pansy, or (C) tomato (% stayed) or moved off the treated plant onto adjacent untreated plants (% moved). Numbers in columns are total number of mites recovered in each treatment. Treated plants were sprayed with a 'control' solution (0.05% acetone + 0.01% Triton X-100) or sprayed with 5 or 100 µM methyl jasmonate (MeJA) (in acetone + Triton X-100). Ten spider mites were placed on treated plants 24 h after treatment, and mites were counted on treated and adjacent, untreated plants 2 days later. Each treatment combination was repeated 5–14 times in time (see Table 2). More mites left plants treated with 100 µM MeJA than control plants (Contrast: $P<0.001$). The treatment*species interaction was not significant. Geranium data were not included in the analysis due to poor mite survival.

Table 2 Mean (± SD) percentage of mites applied that were recovered alive after feeding for 2 days on four plant species. Plants were sprayed with a control solution (0.05% acetone + 0.01% Triton X-100) or sprayed with 5 or 100 µM methyl jasmonate (in acetone + Triton X-100). Ten spider mites were placed on each replicate of treated plants 24 h after treatment, and mites were counted on treated and adjacent untreated plants 2 days later

	Control		5 µM MeJA		100 µM MeJA		
Species	% recovered	n	% recovered	n	% recovered	n	Mean per species
Geranium	35 ± 25	6	Not studied		34 ± 22	5	35 ± 23a
Impatiens	72 ± 25	13	85 ± 13	5	73 ± 17	13	75 ± 20b
Pansy	82 ± 13	13	81 ± 13	7	92 ± 7	13	86 ± 12b
Tomato	55 ± 19	14	53 ± 16	6	59 ± 16	14	56 ± 17a
Mean per MeJA treatment	65 ± 25a		73 ± 20a		70 ± 23a		

Means within a column (among plant species) or within a row (among MeJA treatments) followed by different letters are significantly different (Tukey's HSD: $P<0.05$).
n, number of replicates. Percent recovery data (out of 10) were arcsin-transformed and subject to ANOVA with MeJA treatment, plant species, and the treatment*species interaction as main effects (n = 5–14).
MeJA, methyl jasmonate.

(Figure 1). Mite survival was lowest on geranium and tomato as indicated by the percent of mites recovered alive after 2 days ($F_{3,103} = 25.6$; Table 2). There was no effect of MeJA application on the percent of mites recovered alive (Table 2). We found no effect of a 5-µM MeJA application on mite preference (Figure 1). We also found no difference in the leaving rate of *T. urticae* between paper treated with MeJA or a 'control' solution (Figure 2). Exposing plants for 24 h to 400 µl MeJA applied to a cotton swab in a vented greenhouse, rather than a MeJA spray, did not affect mite behavior (C Rohwer, unpubl.).

Experiment II

Mites proliferated more slowly on pansy and impatiens treated with MeJA than on control plants ($F_{1,38} = 7.4$; Table 3). Similar to findings in experiment I, there was no species*treatment interaction. Proliferation rate on impatiens was 300% less than that on pansy. Mite proliferation was slower for mites reared on *P. vulgaris* (4.3 mites per day, 2–4 replicates per treatment) compared to the other two sources based on Tukey's HSD (23 mites per day, 3–5 replicates per treatment and source; $F_{2,38} = 46.0$; Table 3).

Proteinase inhibitor assay

We detected a 48 ± 20% increase in PI units in tomato leaves, a 13 ± 5% increase in impatiens, and a 20 ± 2% increase in pansy, 24 h after wounding the leaves with pliers across 50% of the leaf, illustrating the effectiveness of the PI assay ($F_{1,47} = 22.1$). No changes in PIs were observed in impatiens or pansy 24 h after they were treated with 100 µM MeJA. In contrast, tomato leaves treated with 100 or 1 000 µM MeJA showed a significant increase in PI

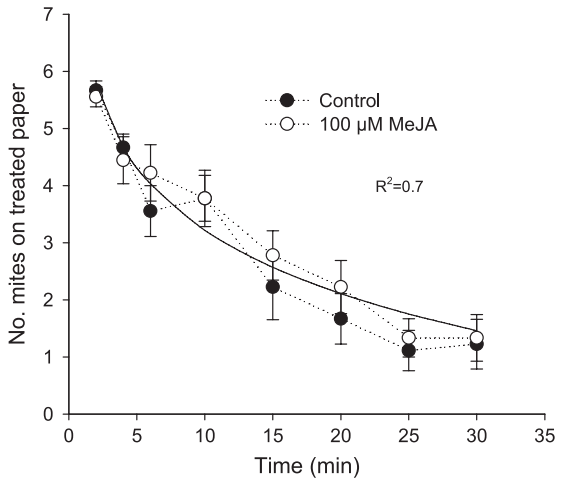

Figure 2 Mean (± SE) number of mites on paper treated 24 h prior to assay with a control solution (0.05% acetone + 0.01% Triton X-100) or a methyl jasmonate (MeJA) solution ('control' + 100 µM MeJA). Repeated measures regression revealed no difference between control and MeJA treatment [y = −1.6(ln x) + 6.9; black line]. Nine replicates per treatment, six mites applied at time = 0.

content ($F_{2,58}$ = 3.9; Figure 3). Overall, tomato had greater PI activity than pansy, though control plants showed similar activity (Figure 3).

Discussion

Mites disperse in greenhouses by walking between plants or by 'roping' (descending from the plant on a silken thread; Kennedy & Smitley, 1985). Our experiment was designed to quantify mites that left, or did not leave, the center (treated) plants by walking, either across the soil surface or between plants directly. Aerial dispersal (Smitley & Kennedy, 1985) was not favored because airspeed at plant level was below the detection threshold (0.3 m s^{-1}) of a hand-held anemometer (Kestrel 3000; Kestrel Meters, Sylvan Lake, MI, USA), and moving between groups of plants via roping was prevented because plants were on inverted pots resting in a tray of water. It is possible that females dispersed from a center (treated) plant to prevent crowding or for other spatial reasons, but this response is assumed to be the same whether or not the center plant was treated with MeJA. In addition, MeJA itself did not affect mite behavior as evidenced by mite movement on treated or untreated filter paper (Figure 2). Therefore, we feel that comparing differences in leaving rate between MeJA-treated and control plants accurately measured the response to MeJA-elicited plant responses. We show here that irrespective of host species, mites evacuated MeJA-treated plants more than untreated plants (Figure 1). Adult female *T. urticae* spent less time feeding and more time moving on tissues previously damaged by mites (Bancroft & Margolies, 1996), and *T. urticae* were more likely to leave *P. vulgaris* plants as the duration of prior damage by conspecifics increased (Bernstein, 1984). After 60 min of a choice test, *T. urticae* preferred leaf disc halves from cut *P. lunatus* stems infiltrated with water over leaf discs from cut stems infiltrated with 0.1 mM JA for 2 days (Gols et al., 2003). Similar results showed a preference for untreated over JA-treated cotton leaf discs (Omer et al., 2001). *Tetranychus urticae* chose jasmonate-insensitive tomato leaflets over wild-type in a detached leaf assay (Li et al., 2004). Our results support the premise that defense-

Table 3 Spider mite proliferation rate (mean number of mites per day ± SD) measured on impatiens and pansy after treating plants, 24 h prior to applying mites, with a control solution (0.05% acetone + 0.01% Triton X-100) or a methyl jasmonate (MeJA) solution (control + 100 µM MeJA)

	Impatiens			Pansy			
MeJA treatment	Pv[y]	Pb[z]	Ua[z]	Pv[y]	Pb[z]	Ua[z]	MeJA treatment mean
Control	2.6 ± 0.9	14.0 ± 6.2	12.6 ± 5.3	7.6 ± 1.7	40.2 ± 23.3	55.3 ± 9.8	22.2 ± 20.8a
100 µM MeJA	1.4 ± 0.4	9.8 ± 3.3	7.6 ± 1.8	4.8 ± 1.5	19.3 ± 9.2	40.0 ± 20.2	13.9 ± 15.5b
Host species mean		8.9 ± 5.8b			27.3 ± 22.4a		

Means within a column (between MeJA treatments), within a row (between plant species), or within rearing source (Pv, Pb, or Ua) followed by different letters are significantly different based on ANOVA F-tests (MeJA or plant species) or Tukey's HSD (rearing source, α = 0.05).

Mites were counted 22–34 days after transferring them to the plants and proliferation rate was ln-transformed prior to ANOVA. Mites were reared on *Phaseolus vulgaris* (Pv), *Populus* × *berolinensis* (Pb), or *Ulmus americana* (Ua). The effects of host species ($F_{1,38}$ = 53.8), MeJA treatment ($F_{1,38}$ = 7.4), and rearing source ($F_{2,38}$ = 46.0) were significant, and no interactions were significant. Replication: n = 10–12 per species × MeJA treatment.

MeJA, methyl jasmonate. Mites were counted 22–34.

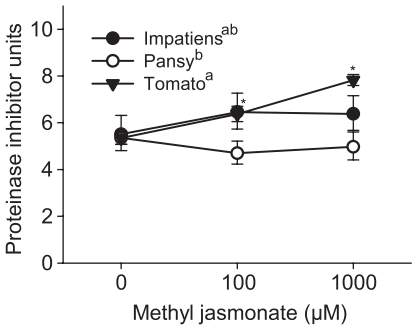

Figure 3 Mean (± SE; n = 6–10) proteinase inhibitor (PI) activity in impatiens, pansy, and tomato measured 24 h after plants were sprayed with methyl jasmonate (MeJA). *PI activity in tomato treated with 100 or 1 000 μM MeJA was significantly greater than in tomato plants treated with 0 MeJA (Dunnett's test: $P<0.05$). Letters next to species name indicate significant difference based on Tukey's HSD ($P<0.05$).

induced plants reduce *T. urticae* preference, although we used intact plants rather than plant parts or leaf discs. Taken together, reported and our results suggest that mites seek feeding sites or plants not treated with or exposed to jasmonates. It is unlikely that MeJA-induced increases in trichome density affected our results over the 3 days between MeJA treatment and final mite counting because jasmonate-induced increases in trichome density are likely limited to leaves forming at the time of treatment (Traw & Bergelson, 2003; Boughton et al., 2005). It is also unlikely that plant size had a direct effect on mite movement, as impatiens and pansy were similar in size and stature, but leaving rate was greater in impatiens compared to pansy.

The number of female *T. urticae* that settle on a host plant is correlated with host plant suitability and with fecundity (Yano et al., 1998). Our results support these findings; female mite proliferation on MeJA-treated plants was (1) lower than proliferation on control plants, and (2) inversely related to mite leaving rate from MeJA-treated plants (Figure 1, Table 3). Encouraging mites to leave a plant, especially when the mites are mobile, may be more beneficial to the plant than killing the mite (Tuomi et al., 1994; van Dam et al., 2000). This suggests that small changes in palatability may defend plants against faster-moving herbivores; *T. urticae* crawling speeds from 5 to 600 cm h^{-1} are reported, depending upon the type of surface on which they are tested (Hussey & Parr, 1963). For comparison, the walking speed of four species of caterpillar was 37–64 cm h^{-1} (Brackenbury, 1999).

We used mites grown on three plant species for experiment II (Table 1). It cannot be determined whether mites reared on *U. americana* or *P.* × *berolinensis* had greater capacity for detoxifying chemical host defenses or had greater plasticity in adjusting to different hosts compared to mites reared on *P. vulgaris* (Table 3; Agrawal et al., 2002). Differences in proliferation rate between these populations could be due to higher air temperature in 2007, when mites were reared on the two woody species (Table 1). We sourced mites from populations infesting the two woody species because the *P. vulgaris* population was not sufficiently large when the plants were ready for treatments.

Differences in mite proliferation among plant species may be related to differences in natural plant defense mechanisms or other factors. *Tetranychus urticae* reared on *P. vulgaris* were sluggish or, in many cases, dead 2 days after transfer to tomato or geranium plants (C Rohwer, pers. obs.; Table 2). When mites forage on tomato, they absorb toxic methyl ketones from glandular trichomes (Chatzivasileiadis et al., 1999). This may explain a previous study showing mites reared on *P. vulgaris* for 8 years, and then transferred to tomato, laid fewer eggs than bean-reared mites transferred to beans (Agrawal et al., 2002). In our experiment, low levels of appropriate detoxifying cytochrome P-450 enzymes in the *P. vulgaris*-reared mite may have caused the inability to thrive, at least without a period of acclimation, on tomato (Agrawal et al., 2002). Geraniums possess glandular trichomes containing toxic anacardic acids (Hesk et al., 1990), which may explain poor overall mite performance on geranium in our study.

Antinutritive compounds and enzymes are important in plant defense against pests. We chose to study PI activity as a mediator of the plant-directed responses observed. We detected no increase in trypsin inhibitor activity in pansy or impatiens treated with 100 μM MeJA, and 100 or 1 000 μM increased PI activity in tomato (Figure 3). Molecular tools to study PI transcripts have not been used in pansy or impatiens, but these or more accurate enzyme assays may be necessary to detect any differences in PIs at low rates of MeJA application. MeJA-induced increase in PI activity was observed in other species (reviewed by Rohwer & Erwin, 2008). For example, 500 μM JA increased chymotrypsin inhibition in tomato (Thaler et al., 2001). Inducible oxidative plant responses associated with defense against *T. urticae*, including enhanced polyphenol oxidase, peroxidase, and lipoxygenase activity (Duffey & Stout, 1996; Stout et al., 1994, 1996; Steinite & Ievinsh, 2002), may have been activated in our plants but these oxidative responses were not quantified here.

We are unaware of specific chemical or structural defenses against phytophagous pests in pansy or impatiens. However, a class of peptides with insecticidal properties (cyclotides) is known to exist in other *Viola* species (Göransson et al., 1999; Jennings et al., 2001; Ireland et al., 2006). Reduced proliferation on impatiens

compared to pansy suggested greater constitutive resistance or the development of mite-induced resistance was more rapid in impatiens. We did not study other specific toxic chemical constituents of the plants in our experiments.

Other studies of MeJA-induced resistance responses often used spray concentrations 5–100 times greater than in our studies to achieve direct resistance responses against herbivores (Thaler et al., 1996; Boughton et al., 2005, 2006), and rates were even as high as 0.1 M applied to *Picea abies* (L.) H. Karst. (Erbilgin et al., 2006). We used 100 μM MeJA for two reasons: we wanted to study a rate lower than that which is known to cause phytotoxic and morphological changes (e.g., Boughton et al., 2006), and sublethal resistance mechanisms in plants may still be effective against herbivores by increasing deterrence. We have yet to elucidate the physiological cause of the *T. urticae* responses to impatiens and pansy shown here, but it will be interesting to discover the degree of defensive compound induction elicited in impatiens and pansy by 100 μM MeJA, and also if higher concentrations of MeJA can cause the same (or a greater) response as we describe.

In this paper, we have described (1) a novel assay that tested the impact of MeJA application on *T. urticae* host plant acceptance using intact plants, (2) increased *T. urticae* leaving rate from three species of MeJA-treated plants, and (3) reduced mite proliferation on MeJA-treated impatiens and pansy. The concentration of MeJA we applied did not result in a detectable increase in PI activity in pansy or impatiens 24 h after treatment, but MeJA-induced PIs may have been a factor affecting mite preference or proliferation on tomato. The utility of MeJA in enhancing plant resistance in greenhouse-grown plants to reduce pesticide application is unclear, but the sum of research and gaps in knowledge on the topic indicates the importance for continued research. Here, we conclude that MeJA-elicited plant defenses caused *T. urticae* to evacuate pansy, impatiens, and tomato and to proliferate less rapidly on pansy and impatiens.

Acknowledgments

The authors thank Wagner's Greenhouse for donation of plant material and the Jerry Cohen & Gary Gardner lab group for useful discussions on PIs. The authors would like to thank the Minnesota Agriculture Experiment Station, the Minnesota Nursery and Landscape Association Foundation, the Fred C. Gloeckner Foundation, Altman Plants, Smith Gardens, Florida Specialty Plants/Oro Farms, USDA-ARS Floriculture and Nursery Research Initiative, the Carl and Virginia Pearlstein Foundation, and the Gordon and Margaret Bailey Endowment for their generous financial support of this project.

References

Agrawal AA, Vala F & Sabelis MW (2002) Induction of preference and performance after acclimation to novel hosts in a phytophagous spider mite: adaptive plasticity? American Naturalist 159: 553–565.

Ament K, Kant MR, Sabelis MW, Haring MA & Schuurink RC (2004) Jasmonic acid is a key regulator of spider mite-induced volatile terpenoid and methyl salicylate emission in tomato. Plant Physiology 135: 2025–2037.

Bancroft JS & Margolies DC (1996) Allocating time between feeding, resting, and moving by the two-spotted spider mite, *Tetranychus urticae* and its predator, *Phytoseiulus persimilis*. Experimental and Applied Acarology 20: 391–404.

Bates D, Maechler M & Dai B (2008) Lme4: Linear Mixed-Effects Models Using S4 Classes. R package version 0.999375-28. Available at: http://lme4.r-forge.r-project.org.

Bernstein C (1984) Prey and predator emigration responses in the acarine system *Tetranychus urticae–Phytoseiulus persimilis*. Oecologia 61: 134–142.

Bethke J, Siapno O & Redak R (2004) The latest miticides. Greenhouse Product News 14: 54–56.

Boughton AJ, Hoover K & Felton GW (2005) Methyl jasmonate application induces increased densities of glandular trichomes on tomato, *Lycopersicon esculentum*. Journal of Chemical Ecology 31: 2211–2216.

Boughton AJ, Hoover K & Felton GW (2006) Impact of chemical elicitor applications on greenhouse tomato plants and population growth of the green peach aphid, *Myzus persicae*. Entomologia Experimentalis et Applicata 120: 175–188.

Brackenbury J (1999) Fast locomotion in caterpillars. Journal of Insect Physiology 45: 525–533.

Chatzivasileiadis EA, Boon JJ & Sabelis MW (1999) Accumulation and turnover of 2-tridecanone in *Tetranychus urticae* and its consequences for resistance of wild and cultivated tomatoes. Experimental and Applied Acarology 23: 1011–1021.

Choh Y, Ozawa R & Takabayashi J (2004) Effects of exogenous jasmonic acid and benzo (1,2,3) thiadiazole-7-carbothioic acid S-methyl ester (BTH), a functional analog of salicylic acid, on the egg production of a herbivorous mite *Tetranychus urticae* (Acari: Tetranychidae). Applied Entomology and Zoology 39: 311–314.

Crawley MJ (2007) The R Book. Wiley, London, UK.

van Dam NM, Hadwich K & Baldwin IT (2000) Induced responses in *Nicotiana attenuata* affect behavior and growth of the specialist herbivore *Manduca sexta*. Oecologia 122: 371–379.

Duffey SS & Stout MJ (1996) Antinutritive and toxic components of plant defense against insects. Archives of Insect Biochemistry and Physiology 32: 3–37.

Erbilgin N, Krokene P, Christiansen E, Zeneli G & Gershenzon J (2006) Exogenous application of methyl jasmonate elicits defenses in Norway spruce (*Picea abies*) and reduces host

colonization by the bark beetle *Ips typographus*. Oecologia 148: 426–436.

Gols R, Roosjen M, Dijkman H & Dicke M (2003) Induction of direct and indirect plant responses by jasmonic acid, low spider mite densities, or a combination of jasmonic acid treatment and spider mite infestation. Journal of Chemical Ecology 29: 2651–2666.

Göransson U, Luijendijk T, Johansson S, Bohlin L & Claeson P (1999) Seven novel macrocyclic polypeptides from *Viola arvensis*. Journal of Natural Products 62: 283–286.

Hesk D, Collins LC, Craig R & Mumma RO (1990) Arthropod-resistant and -susceptible geraniums. Comparison of Chemistry. Naturally Occuring Pest Bioregulators, ACS symposium series 449 (ed. by PA Hedin), pp. 229–250. American Chemical Society, Washington, DC, USA.

Hildmann T, Ebneth M, Peña-Cortés H, Sánchez-Serrano JJ, Willmitzer L & Prat S (1992) General roles of abscisic and jasmonic acids in gene activation as a result of mechanical wounding. Plant Cell 4: 1157–1170.

Hothorn T, Bretz F & Westfall P (2008) Simultaneous inference in general parametric models. Biometrical Journal 50: 346–363.

Hussey NW & Parr WJ (1963) Dispersal of the glasshouse red spider mite. Entomologia Experimentalis et Applicata 6: 207–214.

Ireland DC, Colgrave ML & Craik DJ (2006) A novel suite of cyclotides from *Viola odorata*: sequence variation and the implications for structure, function and stability. Biochemical Journal 400: 1–12.

Jennings C, West J, Waine C, Craik D & Anderson M (2001) Biosynthesis and insecticidal properties of plant cyclotides: the cyclic knotted proteins from *Olenlandia affinis*. Proceedings of the National Academy of Sciences of the USA 98: 10614–10619.

Kant MR, Ament K, Sabelis MW, Haring MA & Schuurink RC (2004) Differential timing of spider mite-induced direct and indirect defenses in tomato plants. Plant Physiology 135: 483–495.

Kavousi A, Chi H, Talebi K, Bandani A, Ashouri A & Naveh VH (2009) Demographic traits of *Tetranychus urticae* (Acari: Tetranychidae) on leaf discs and whole leaves. Journal of Economic Entomology 102: 595–601.

Kennedy GG & Smitley DR (1985) Dispersal. Spider Mites: Their Biology, Natural Enemies, and Control, Vol. 1A. (ed. by W Helle & MW Sabelis), pp. 233–242. Elsevier, Amsterdam, The Netherlands.

Li C, Williams MM, Loh YT, Lee GI & Howe GA (2002) Resistance of cultivated tomato to cell content-feeding herbivores is regulated by the octadecanoid-signaling pathway. Plant Physiology 130: 494–503.

Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J et al. (2004) The tomato homolog of coronatine-insensitive-1 (COI1) is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. Plant Cell 16: 126–143.

Omer AD, Thaler JS, Granett J & Karban R (2000) Jasmonic acid induced resistance in grapevines to a root and leaf feeder. Journal of Economic Entomology 93: 840–845.

Omer AD, Granett J, Karban R & Villa EM (2001) Chemically-induced resistance against multiple pests in cotton. International Journal of Pest Management 47: 49–54.

Orians CM, Pomerleau J & Ricco R (2000) Vascular architecture generates fine scale variation in systemic induction of proteinase inhibitors in tomato. Journal of Chemical Ecology 26: 471–485.

Powell CC & Lindquist RK (1997) Spider mites (Acari-Tetranychidae). Ball Pest & Disease Manual, Vol. 2, pp. 228–233. Ball Publishing, Batavia, IL, USA.

R Development Core Team (2009) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. Available at: http://www.R-project.org

Rohwer CL & Erwin JE (2008) Horticultural applications of jasmonates: a review. Journal of Horticultural Science and Biotechnology 83: 283–304.

Smitley DR & Kennedy GG (1985) Photo-oriented aerial-dispersal behaviour of *Tetranychus urticae* (Acari: Tetranychidae) enhances escape from the leaf surface. Annals of the Entomological Society of America 78: 609–614.

Steinite I & Ievinsh G (2002) Wound-induced responses in leaves of strawberry cultivars differing in susceptibility to spider mite. Journal of Plant Physiology 159: 491–497.

Stout MJ, Workman J & Duffey SS (1994) Differential induction of tomato foliar proteins by arthropod herbivores. Journal of Chemical Ecology 20: 2575–2594.

Stout MJ, Workman KV & Duffey SS (1996) Identity, spatial distribution, and variability of induced chemical responses in tomato plants. Entomologia Experimentalis et Applicata 79: 255–271.

Stout MJ, Workman KV, Bostok RM & Duffey SS (1998) Specificity of induced resistance in the tomato, *Lycopersicon esculentum*. Oecologia 113: 74–81.

Thaler JS, Stout MJ, Karban R & Duffey SS (1996) Exogenous jasmonates simulate insect wounding in tomato plants (*Lycopersicon esculentum*) in the laboratory and field. Journal of Chemical Ecology 22: 1767–1781.

Thaler JS, Stout MJ, Karban R & Duffey SS (2001) Jasmonate-mediated induced plant resistance affects a community of herbivores. Ecological Entomology 26: 312–324.

Thaler JS, Farag MA, Paré PW & Dicke M (2002a) Jasmonate-deficient plants have reduced direct and indirect defences against herbivores. Ecology Letters 5: 764–774.

Thaler JS, Karban R, Ullman DE, Boege K & Bostock RM (2002b) Cross-talk between jasmonate and salicylate plant defense pathways: effects on several plant parasites. Oecologia 131: 227–235.

Traw MB & Bergelson J (2003) Interactive effects of jasmonic acid, salicylic acid, and gibberellin on induction of trichomes in *Arabidopsis*. Plant Physiology 133: 1367–1375.

Tuomi J, Augner M & Nilsson P (1994) A dilemma of plant defences: is it really worth killing the herbivore? Journal of Theoretical Biology 170: 427–430.

Warabieda W, Miszczak A & Olszak RW (2005) The influence of methyl jasmonate (JA-Me) on beta-glucosidase on induction of resistance mechanisms of strawberry against two-spotted spider mite (*Tetranychus urticae* Koch). Communications in Agricultural and Applied Biological Sciences 70: 829–836.

Wasternack C & Parthier B (1997) Jasmonate-signalled plant gene expression. Trends in Plant Science 2: 302–307.

Wolfson JL (1988) Bioassay techniques. An ecological perspective. Journal of Chemical Ecology 14: 1951–1963.

Yano S, Wakabayashi M, Takabayashi J & Takafuji A (1998) Factors determining the host plant range of the phytophagous mite, *Tetranychus urticae* (Acari: Tetranychidae): a method for quantifying host plant acceptance. Experimental and Applied Acarology 22: 595–601.