Journal of Chemical Ecology, Vol. 30, No. 12, December 2004 (© 2004)

ACQUIRED AND R-GENE-MEDIATED RESISTANCE AGAINST THE POTATO APHID IN TOMATO

W. C. COOPER,[1,2] L. JIA,[1] and F. L. GOGGIN[1,*]

[1]*Department of Entomology, University of Arkansas Agriculture Building, Fayetteville, Arkansas, USA*

(Received June 3, 2004; accepted September 3, 2004)

Abstract—We examined the effects of three forms of host plant resistance in tomato, *Lycopersicon esculentum*, on the potato aphid, *Macrosiphum euphorbiae*. *Mi-1.2*, a resistance gene (R-gene) in tomato that deters aphid feeding, reduced the population growth of both potato aphid isolates tested, although it appeared to have a greater impact on isolate WU11 than on isolate WU12. The results suggest that there may be quantitative differences in virulence between these two aphid isolates. We also examined two distinct forms of acquired resistance in tomato, jasmonic acid (JA)-dependent and salicylic acid (SA)-dependent induced defenses. Exogenous foliar application of JA triggered expression of a JA-inducible proteinase inhibitor in tomato cultivars with and without *Mi-1.2*, although the effects of treatment on aphid performance differed between these cultivars. JA-treatment reduced aphid population growth on a susceptible tomato cultivar that lacks *Mi-1.2*, but did not significantly enhance or inhibit aphid control on a near-isogenic resistant tomato cultivar that carries this gene. Foliar application of an SA analog, benzothiadiazole (BTH), was used to induce SA-dependent defenses. BTH treatment reduced the population growth of both aphid isolates on a susceptible tomato cultivar, and also enhanced aphid control on a resistant cultivar. The results indicate that both SA- and JA-dependent acquired resistance in tomato have a direct negative effect on a phloem-feeding insect. Furthermore, this study demonstrates that acquired resistance and R-gene-mediated resistance can interact for enhanced suppression of insect herbivores.

Key Words—Insect resistance, induced resistance, systemic acquired resistance, *Mi*, *Meu1*, jasmonic acid, salicylic acid, benzothiadiazole, Homoptera, Aphididae.

[2]Current address: Department of Entomology, University of Kentucky, S-225 Agricultural Science Center North, Lexington, Kentucky 40546, USA.
[*]To whom correspondence should be addressed. E-mail: fgoggin@uark.edu

INTRODUCTION

Studies of host plant resistance typically have focused on two broad categories of plant defense acquired and innate resistance. Acquired resistance mechanisms, including systemic acquired resistance to pathogens and induced resistance to insects, are dependent upon systemic defenses that are induced by an initial pest infestation, and that render plants less susceptible to subsequent attack (Karban and Baldwin, 1997; Metraux et al., 2002). Furthermore, initial induction of these defenses by one pest can induce resistance to other attackers (Ryan et al., 1986; Stout et al., 1998a). In contrast, innate resistance targets specific pest species, and prevents or limits their initial establishment on the plant. Innate resistance, in many cases, depends upon single, dominant resistance genes, often termed R-genes (Hammond-Kossack and Jones, 1996). R-genes are thought to mediate gene-for-gene interactions with pests; in other words, a particular R-gene confers resistance against specific biotypes of pest that carry a corresponding avirulence (Avr) gene (Flor, 1955). The most extensively studied gene-for-gene interactions mediate resistance against plant pathogens, but R-genes are also thought to play a role in resistance against herbivores, including aphids, Hessian flies, gall midges, and nematodes (Puterka and Peters, 1989; Roberts, 1995; Zantoko, 1997; Milligan et al., 1998; Rossi et al., 1998; Stuart et al., 1998; Sardesai et al., 2001; Brotman et al., 2002).

Important signaling conflicts occur between different forms of acquired resistance. Induction of systemic acquired resistance to pathogens (SAR) can be inhibited by jasmonic acid, a signaling compound involved in induced resistance to chewing insects (Sano and Ohashi, 1995; Thaler et al., 1999). Likewise, salicylic acid, a key signal in SAR, can inhibit synthesis of jasmonic acid and suppress induction of induced resistance to insects (Pena-Cortes et al., 1993; Doares et al., 1995; Thaler et al., 1999). In contrast, little is known about the potential for signaling conflicts or synergisms between acquired and innate resistance, because these two broad categories of resistance have typically been studied separately. Certain studies, however, indicate that there is overlap between the physiological mechanisms that underlie acquired and innate resistance. For example, in addition to its role in SAR, salicylic acid is also required for the function of some but not all R-genes (Delaney et al., 1994; Glazebrook et al., 1996; Jirage et al., 1999; Brading et al., 2000; Kachroo et al., 2000; Rairdan and Delaney, 2002; Takahashi et al., 2002; Branch et al., 2004). Overexpression of certain R-genes also results in heightened SA levels and activation of SAR (Oldroyd and Staskawicz, 1998; Xiao et al., 2001). These findings suggest that SA-dependent systemic defenses and certain R-genes could potentially interact in a synergistic manner. For R-genes that are dependent upon SA for function, it is also possible that these genes might be incompatible with JA-dependent defenses.

The objectives of this study were to examine the effects of both acquired and innate resistance on an insect pest of tomato, and to determine if induction of acquired resistance would enhance or inhibit the effects of innate resistance. The two forms of acquired resistance examined in this study are SA- and JA-dependent systemic defenses. Relatively little is known about the impact of either of these defensive pathways on piercing–sucking insects, and so one goal was to evaluate their effects on the potato aphid, *Macrosiphum euphorbiae* Thomas. Our second goal was to examine potential interactions between these forms of acquired resistance and *Mi-1.2*, a source of innate aphid resistance in tomato, *Lycopersicon esculentum* Mill. *Mi-1.2* is a R-gene that confers resistance to certain isolates of the potato aphid, root-knot nematodes (*Meloidogyne* spp.), and the sweet potato whitefly (*Bemisia tabaci*) (Milligan et al., 1998; Rossi et al., 1998; Nombela et al., 2003). The physiological basis for *Mi*-mediated resistance is not yet well understood, but recent evidence indicates that SA is involved (Branch et al., 2004). *Mi*-mediated nematode resistance is inhibited by salicylate hydroxylase, a bacterial enzyme that degrades SA; conversely, nematode resistance can be restored to plants that express salicylate hydroxylase by treating the plants with a synthetic SA analog, benzothiadiazole (Branch et al., 2004). In addition, induction of *Mi*-mediated aphid resistance is correlated with the expression of marker genes associated with SA induction (de Ilarduya et al., 2003). Given that SA may play a role in *Mi*-mediated aphid resistance, one objective of this study was to determine if prior induction of SA-dependent defenses could enhance aphid resistance in a tomato cultivar that carries *Mi-1.2*. Furthermore, we wished to determine if induction of JA-dependent defenses would enhance or inhibit *Mi*-mediated aphid resistance.

METHODS AND MATERIALS

Plant Materials. Two near-isogenic tomato cultivars with and without *Mi-1.2* were used for our bioassays: Moneymaker (*Mi*−), and Motelle (*Mi*+). All plants were grown in 3.8-l pots of LC1 Sunshine potting mix (Sungro Horticulture, Bellevue, WA) under stable greenhouse conditions ($\sim$ 24°C–27°C; 16:8 L:D photoperiod). Plants were watered daily with a dilute nutrient solution containing 1000 mg/l $CaNO_3$ (Hydro Agri North America, Tampa, FL), 500 mg/l $MgSO_4$ (Giles Chemical Corp, Waynesville, NC), and 500-mg/l Hydroponic 4-18-38 Growmore fertilizer (Growmore, Gardena, CA).

Insect Cultures. Two potato aphid isolates, which we designated WU11 and WU12, were utilized. Each was a clonal population established from a single female. Isolate WU11 originated from the laboratory colony of Dr. Yvan Rahbe, which was originally maintained on eggplant (*Solanum melongena*) (Goggin et al.,

2001). Aphid isolate WU12 was obtained from the laboratory colony of Dr. Stuart Seah, and was originally maintained on potato (*Solanum tuberosum*) (Goggin et al., 2004). Both isolates were maintained on susceptible tomato seedlings (cv. UC82) for more than 2 yr prior to these experiments, to insure that both were well adapted to tomato. Aphids were maintained in Conviron growth chambers (Controlled Environments, Inc., Winnipeg, Canada) under optimal conditions for aphid development (20°C, 16:8 L:D photoperiod).

Benzothiadiazole Application. SA-dependent defenses were induced in tomato by applying a foliar treatment of benzothiadiazole (BTH), a synthetic analog of SA. Exogenous treatments of SA and BTH have both been shown to induce acquired resistance to pathogens and induction of associated pathogenesis-related proteins (Friedrich et al., 1996; Gorlach et al., 1996; Lawton et al., 1996). We chose to use BTH rather than SA as a defense elicitor because it lacks the phytotoxic effects associated with SA, and has stronger systemic effects than exogenous SA (Friedrich et al., 1996).

BTH (Syngenta Crop Protection, Greensboro, NC) was dissolved in acetone at a rate of 28 g/l and dispersed in water to achieve a 1.2 mM BTH solution (Friedrich et al., 1996). For the control treatments, an equal quantity of acetone (without BTH) was dispersed in water. Approximately 6 wk after germination, at which stage *Mi*-mediated resistance is active in the foliage, tomato plants were sprayed with BTH solution or control solution applied at a rate of 1 ml per leaf using an atomizer (~12 ml/plant). The eighth leaf from the cotyledon of each plant was protected from treatment with a plastic bag, which was removed when all other leaves were dry. Aphid bioassays (described below) were performed using this untreated leaf, so that we could measure the effects of acquired resistance on aphids independent of any effects that BTH residue might have on the insects.

Jasmonic Acid Application. JA-dependent defenses were induced in tomato by applying a foliar treatment of exogenous JA, as previously described by Thaler et al. (1999). Jasmonic acid (Sigma Chemicals, St Louis, MO) was dissolved in acetone at a rate of 1 g/ml and dispersed in water to achieve a 1.5 mM JA solution (Thaler, 1999). An equal quantity of acetone was dispersed in water (without JA) for control treatment. JA and control treatments were applied 6 wk after germination as described for BTH. One leaf per plant was protected from treatment for use in aphid bioassays.

Aphid Bioassays. In trial 1, the effects of BTH on population growth of aphid isolates WU11 and WU12 were measured by using two independent bioassays. In each assay, aphid performance was measured on Moneymaker (*Mi*−) or Motelle (*Mi*+) sprayed with either BTH or control solution. Forty-eight hr after treatment, the terminal leaflet of the eighth leaf from the cotyledon was inoculated with 15 aphids, which were confined to the leaflet with a sleeve cage (12 plants/treatment for the WU11 assay; 8 plants/treatment for the WU12 assay). Plants were inoculated with a combination of fourth-instar juveniles and young

adult aphids, and the ratio of adults to juveniles was standardized from plant to plant. Six days after inoculation, aphid performance was evaluated by counting the total number of aphids/cage. Bioassays were performed in a Conviron growth chamber (20°C; 16:8 L:D photoperiod).

In trial 2, the effects of BTH treatment on WU11 performance on Motelle (*Mi*+) were examined further. Plants were treated with BTH or control solution as described above, and inoculated with 15 fourth-instars confined to a single sleeve cage per plant (9 plants/treatment). Six days after inoculation, aphid performance was evaluated by counting the number of aphids that survived to adulthood and the number of offspring they produced. Adults and juveniles were recorded separately in order to detect any subtle effects on aphid survivorship or fecundity that might not be apparent from total aphid numbers.

The effects of JA on WU11 and WU12 were tested in the same manner as described above for trial 1 of the BTH study (13 plants/treatment for the WU11 assay; 9 plants/treatment for the WU12 assay). Plants sprayed with JA vs. control treatments were maintained in separate growth chambers to insure that volatiles from plants treated with JA would not affect control plants.

For each assay, foliar application and genotype were compared as independent fixed factors by full factorial two-way ANOVA using JMP version 5.01 (SAS, Cary, NC). Treatment combinations were analyzed by Tukey's HSD statistics and paired *t*-tests using JMP version 5.01.

Analysis of Gene Expression. To determine if JA-dependent defenses are induced equally in different tomato cultivars, expression of *Proteinase Inhibitor II* (*Pin2*) was measured on Motelle and Moneymaker tomato using reverse transcriptase-polymerase chain reaction (RT-PCR). Motelle and Moneymaker plants were treated with a foliar application of synthetic JA or control solution as described above. Forty-eight hr after treatment, ~1 g of leaf tissue was collected from one untreated leaf per plant (2 reps/treatment) and flash frozen with liquid nitrogen. RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA) and DNase-treated with DNA-*free* reagents (Ambion, Austin, TX) according to manufacturers' protocols. Aliquots of RNA were quantified using a spectrophotometer and run on an agarose/formaldehyde gel to check quality. cDNA was synthesized from 0.4 μg of total RNA using a RetroScript Reverse Transcriptase kit and oligo-DT primers (Ambion, Austin, TX). To amplify *Pin2* cDNA, the following primers were used: *Pin2* forward, 5′-CCG TTC ACA AGG AAA ATC GT-3′, and reverse, 5′-ATT TTG GGC AAT CCA GAA GA-3′. The size of the amplified fragment was 186 bp. In separate PCR reactions, expression of the housekeeping gene ubiquitin III (*Ubi3*) was measured using the following primers: *Ubi3* forward 5′-GTG TGG GCT CAC CTA CGT TT-3′, and reverse, 5′-ACA ATC CCA AGG GTT GTC AC-3′. The size of the amplified fragment was 162 bp. PCR with *Ubi3* primers was also performed on total RNA from each sample to confirm that the samples did not contain genomic DNA (RT − controls). PCR was performed by using an MJ

Research PTC-200 thermal cycler (MJ Research, San Francisco, CA). Conditions for all PCR reactions were 4 min at 95°C, 24 cycles (94°C, 30 sec; 57°C, 30 sec; 72°C 30 sec) followed by 5 min at 72°C. PCR products were visualized by gel electrophoresis with a 2% agarose gel.

RESULTS

Effects of BTH and Mi-1.2 on Aphids. In trial 1, WU11 aphid numbers after 6 days of feeding differed among treatment groups (Figure 1) ($F = 26.14$; $df = 3$; $P < 0.001$). There was no significant interaction between plant genotype and foliar treatment ($F = 1.32$; $df = 1$; $P = 0.265$). Aphid numbers were lower on Motelle ($Mi+$) plants compared to Moneymaker ($Mi-$) ($F = 69.7$; $df = 1$; $P < 0.001$), which confirms previous findings that WU11 is impacted by *Mi-1.2* (Goggin et al., 2001). Aphid numbers were also reduced by BTH application

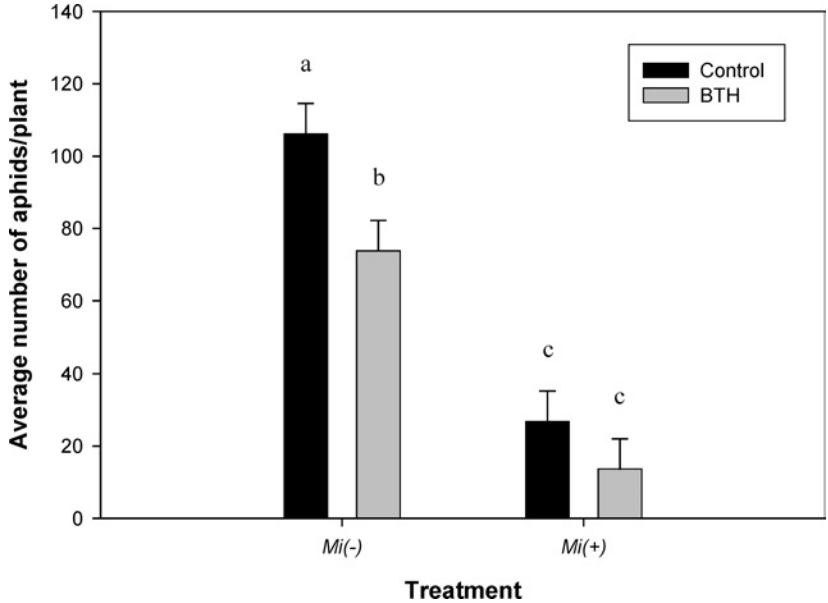

FIG. 1. *Effects of BTH Treatment and Mi-1.2 on Aphid Isolate WU11.* Near-isogenic tomato plants with and without *Mi-1.2* were sprayed with a 1.2 mM solution of BTH, or with a control solution. Forty-eight-hr after treatment, one leaflet per plant was inoculated with 15 WU11 aphids each. Total aphid numbers were measured 6 days after inoculation. Error bars represent the standard deviations. Values with differing letters are significantly different at the $\alpha = 0.05$ confidence interval according to Tukey HSD statistics.

TABLE 1. EFFECTS OF BTH TREATMENT ON APHID ISOLATE WU11 PERFORMANCE ON A RESISTANT (*Mi*+) TOMATO CULTIVAR[a]

	Adults/Plant ± SE	Juveniles/Plant ± SE	Total/Plant ± SE
Control	5 ± 3 A	18 ± 6 A	23 ± 7 A
BTH	4 ± 2 A	10 ± 7 B	14 ± 8 B

[a] Within each column, values labeled with different letters are significantly different at $\alpha = 0.05$ according to a paired t-test.

($F = 7.42$; $df = 1$; $P = 0.013$). BTH treatment dramatically reduced aphid numbers on the susceptible cultivar Moneymaker ($t = -2.74$; $df = 1$; $P = 0.013$). On the resistant cultivar, aphid numbers were lower on BTH-treated plants than on control plants, but this difference was not significant according to a paired t-test ($t = -1.114$; $df = 1$; $P = 0.278$). In trial 2 (Table 1), BTH treatment reduced total WU11 aphid numbers on the resistant cultivar Motelle ($F = 5.852$; $df = 1$; $P = 0.030$). This effect appeared to be due to a reduction in aphid reproduction on plants treated with BTH. While the number of surviving adults did not differ between treatments ($F = 0.221$; $df = 1$; $P = 0.64$), the number of juveniles on BTH-treated plants was lower compared to controls ($F = 7.215$; $df = 1$; $P = 0.018$).

WU12 aphid numbers after six days of feeding differed among treatments (Figure 2) ($F = 1.44$; $df = 3$; $P < 0.001$). There was no significant interaction between *Mi*-mediated resistance and BTH application ($F = 2.08$; $df = 1$; $P = 0.161$). Aphid numbers were lower on Motelle vs. Moneymaker plants ($F = 2.89$; $df = 1$; $P = 0.008$), which confirms previous findings that *Mi-1.2* is active against aphid isolate WU12 (Goggin et al., 2004). Aphid numbers were also lower on plants treated with BTH compared to control plants ($F = 4.93$; $df = 1$; $P < 0.001$). BTH treatment reduced aphid numbers on both Moneymaker and Motelle ($t = -4.588$; $df = 1$; $P < 0.001$ and $t = -2.426$; $df = 1$; $P = 0.022$, respectively).

Although both aphid isolates were impacted by *Mi-1.2*, this resistance gene appeared to have a stronger effect on WU11 than on WU12. WU11 numbers on control Motelle plants (untreated with JA) were 74.7% lower than WU11 numbers on control Moneymaker plants (Figure 1); in contrast, WU12 numbers on Motelle control plants were only 33% lower than WU12 numbers on Moneymaker control plants (Figure 2).

Effects of JA and Mi-1.2 on Aphids. WU11 aphid numbers differed among treatments after 6 days of feeding (Figure 3) ($F = 268.92$; $df = 3$; $P < 0.001$). There was a significant interaction between plant genotype and foliar application ($F = 7.57$; $df = 1$; $P = 0.008$), because the effects of JA treatment differed between genotypes. Therefore, the effects of JA and plant genotype were analyzed

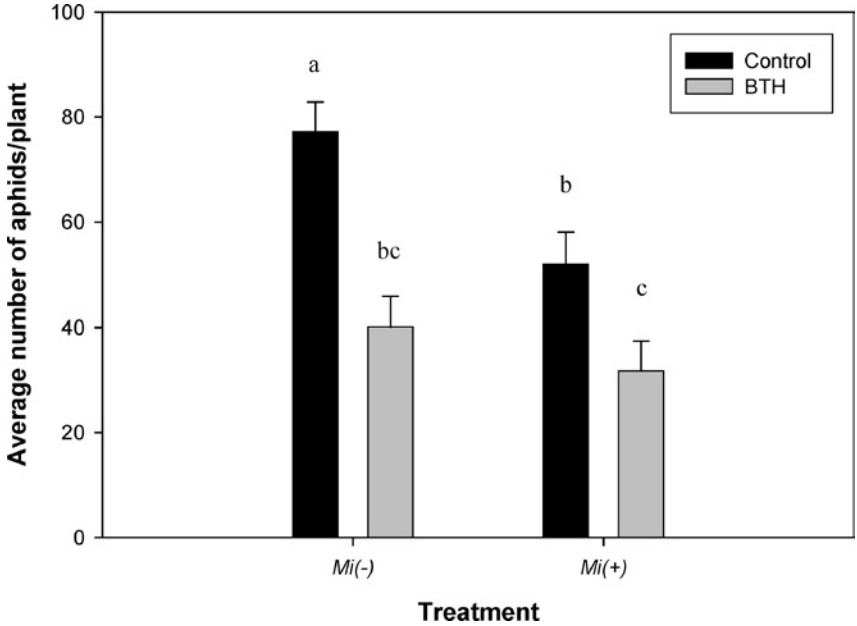

FIG. 2. *Effects of BTH Treatment and Mi-1.2 on Aphid Isolate WU12*. Near isogenic tomato plants with and without *Mi-1.2* were sprayed with a 1.2 mM solution of BTH, or with a control solution. Forty-eight hr after treatment, one leaflet per plant was inoculated with 15 WU12 aphids each. Total aphid numbers were measured 6 days after inoculation. Error bars represent the standard deviations. Values with differing letters are significantly different at the $\alpha = 0.05$ confidence interval according to Tukey HSD statistics.

separately. Comparisons between Moneymaker and Motelle plants sprayed with the control treatment revealed that *Mi-1.2* had a strong negative effect on aphid numbers ($t = 21.88$; $df = 1$; $P < 0.001$). JA treatment reduced aphid numbers on the susceptible cultivar Moneymaker ($t = 3.41$; $df = 1$; $P = 0.001$), but did not influence aphid numbers on the resistant cultivar Motelle ($t = 0.419$; $df = 1$; $P = 0.632$).

WU12 aphid numbers after 6 days of feeding differed among treatments (Figure 4) ($F = 5.24$; $df = 3$; $P > F = 0.049$). There was no interaction between genotype and JA application ($F = 2.093$; $df = 1$; $P = 0.158$). The overall effect of the plant genotype was not significant at $\alpha = 0.05$ confidence interval ($F = 3.24$; $df = 1$; $P = 0.082$), although aphid numbers were lower on Motelle vs. Moneymaker control plants ($t = 3.33$; $df = 1$; $P = 0.002$). Aphid numbers were reduced on plants sprayed with JA compared to control plants ($F = 11.14$; $df = 1$; $P = 0.002$), although JA did not enhance aphid control on the resistant cultivar Motelle ($t = 1.36$; $df = 1$; $P = 0.185$).

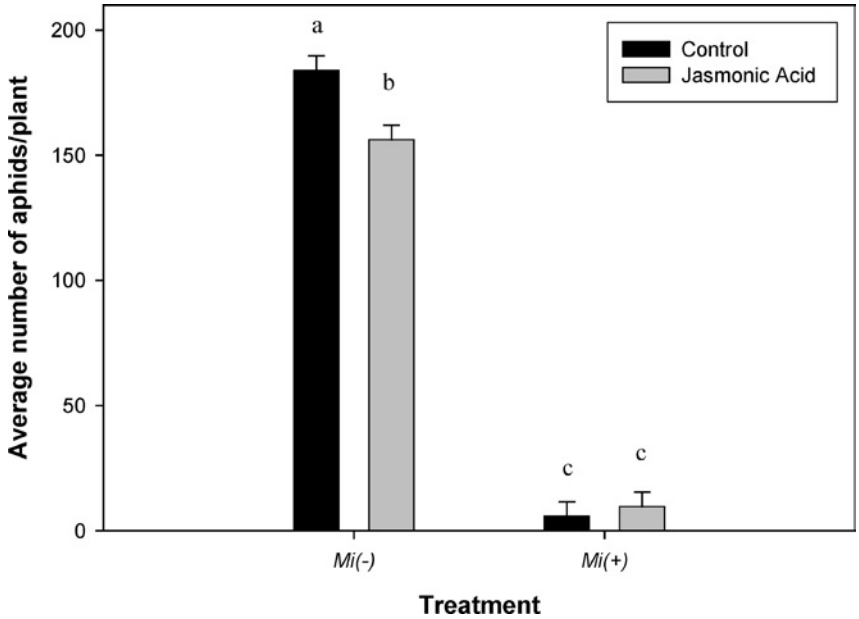

FIG. 3. *Effects of JA Treatment and Mi-1.2 on Aphid Isolate WU11*. Near isogenic tomato plants with and without *Mi-1.2* were sprayed with a 1.5 mM solution of JA, or with a control solution. Forty-eight hr after treatment, one leaflet per plant was inoculated with 15 WU11 aphids each. Total aphid numbers were measured 6 days after inoculation. Error bars represent the standard deviations. Values with differing letters are significantly different at the $\alpha = 0.05$ confidence interval according to Tukey HSD statistics.

Similar to our bioassays with BTH, our assays with JA indicated that aphid isolates WU11 and WU12 appear to differ in their response to *Mi*-mediated resistance. When we compare aphid numbers on untreated Moneymaker vs. untreated Motelle plants within each assay, we find that WU11 numbers were reduced by >99% on Motelle (Figure 3), whereas WU12 numbers were reduced by only 24.2%.

Induction of Pin2 Expression. Because we were unable to detect a significant biological effect of JA application on Motelle plants, we performed RT-PCR to confirm that foliar application of JA could induce JA-dependent defenses in this genotype. Expression of the *Pin2* gene has been shown to be a reliable marker of induction of JA-dependent defenses (Graham et al., 1985; Stout et al., 1998b), and so we compared *Pin2* expression in Moneymaker and Motelle plants treated with JA or control solution. *Pin2* transcripts were detected in comparable abundance in Moneymaker and Motelle plants sprayed with JA, and were absent in plants treated with control solution (Figure 5). These results indicate that foliar

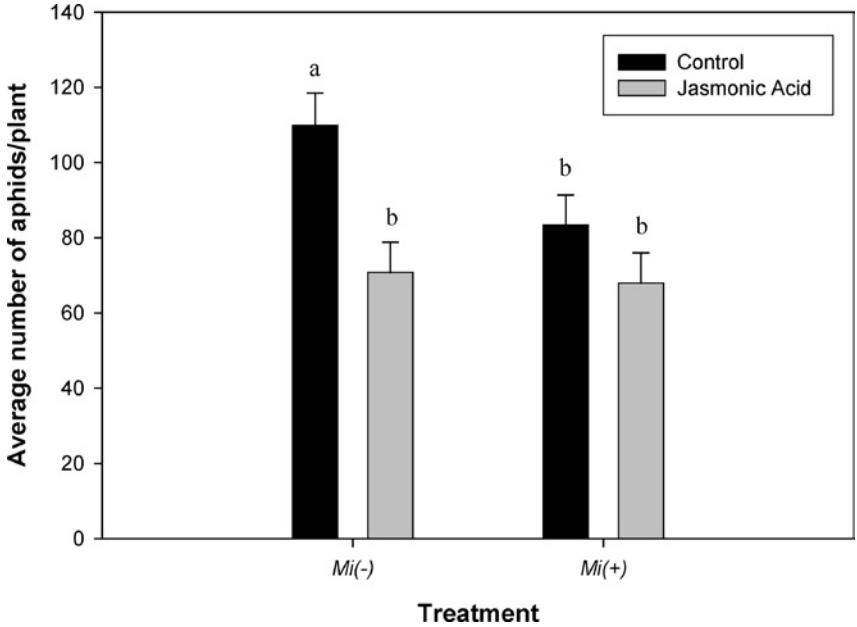

FIG. 4. *Effects of JA Treatment and Mi-1.2 on Aphid Isolate WU12*. Near-isogenic tomato plants with and without *Mi-1.2* were sprayed with a 1.5 mM solution of JA, or with a control solution. Forty-eight hr after treatment, one leaflet per plant was inoculated with 15 WU12 aphids each. Total aphid numbers were measured 6 days after inoculation. Error bars represent the standard deviations. Values with differing letters are significantly different at the $\alpha = 0.05$ confidence interval according to Tukey HSD statistics.

application of JA induced proteinase inhibitor expression in both genotypes tested. The housekeeping gene ubiquitin III was uniformly expressed in all samples, confirming that these samples contained comparable amounts of mRNA (Figure 5). No amplification products were detected when our RNA samples were used as a template for PCR (Figure 5). This indicates that our RNA samples were free from contaminating genomic DNA.

DISCUSSION

This study demonstrates that both SA- and JA-dependent defenses reduce potato aphid populations on a susceptible (*Mi*−) tomato cultivar. The results also indicate that artificial induction of SA-dependent defenses can enhance aphid control on a resistant (*Mi*+) cultivar. In contrast, induction of JA-dependent defenses did not appear to enhance or inhibit *Mi*-mediated aphid resistance.

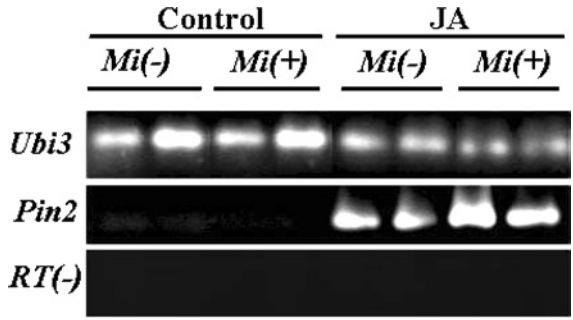

FIG. 5. *Induction of Pin2 Expression*. RT-PCR was used to compare expression of proteinase inhibitor II (*Pin2*), which is associated with induction of JA-dependent defenses, in Moneymaker (*Mi*−) and Motelle (*Mi*+) plants treated with control solution or 1.5 mM JA. Expression of the housekeeping gene ubiquitin III (*Ubi3*) was analyzed to verify that all samples contained comparable amounts of mRNA. PCR with ubiquitin primers was also performed on RNA aliquots (RT − samples) to confirm that the RNA samples were not contaminated with genomic DNA.

These findings add to a growing body of evidence that SA may play a role in induced resistance to piercing-sucking insects. Although induction of SA and related compounds is typically associated with plant defenses against pathogens, recent studies indicate that aphid infestation triggers SA accumulation in barley and wheat, and that psyllids elicit methyl salicylate production in pear (Havlickova et al., 1998; Mohase and van der Westhuizen, 2002; Chaman et al., 2003; Scutareanu et al., 2003). Aphid and whitefly feeding on a variety of plant species also has been shown to induce pathogenesis-related (PR) proteins associated with SA induction, such as glucanases, chitinases, and peroxidases (Krishnaveni et al., 1999; Forslund et al., 2000; Mayer et al., 2002; Moran et al., 2002; de Ilarduya and Delany, 2003; Zhu-Salzman et al., 2004). By comparing cultivars that vary in their susceptibility to aphids, some of these studies have demonstrated a positive correlation between levels of aphid resistance and levels of SA or PR protein induction (Forslund et al., 2000; Chaman et al., 2003; de Ilarduya et al., 2003). To our knowledge, however, only one prior study has directly demonstrated that SA-dependent defenses impact aphid fitness. In this case, a modest reduction in green peach aphid numbers was observed on *Arabidopsis* foliage treated with benzothiadiazole (BTH), a functional analog of SA (Moran and Thompson, 2001). Our study demonstrates that BTH also induces systemic defenses that dramatically reduce potato aphid population growth on a susceptible tomato cultivar (Figures 1 and 2; Table 1). Furthermore, BTH treatment can enhance R-gene mediated aphid resistance in tomato (Table 1; Figure 2). This enhanced aphid control may be due to an additive effect of BTH-induced defenses and *Mi*-mediated resistance. Alternatively, the defense response mediated

by *Mi-1.2* may be faster or stronger in plants that have previously been conditioned by BTH treatment.

Interestingly, the impact of *Mi*-mediated resistance appeared to differ between aphid isolates WU11 and WU12. This finding is intriguing because it suggests that virulence could potentially be a quantitative rather than a qualitative trait. WU11 and WU12 both establish significantly higher numbers on susceptible vs. resistant plants; therefore, they are both classified as avirulent (Goggin et al., 2001, 2004). Nonetheless, *Mi-1.2* appears to have a greater impact on the population growth of WU11 than on WU12. This suggests that the outcome of the plant-insect interaction is influenced by factors other than the simple presence or absence of a single avirulence factor. The extreme sensitivity of WU11 to *Mi*-mediated resistance may also explain why BTH treatment did not always enhance control of WU11 on resistant plants (Figure 1). *Mi-1.2* suppresses WU11 population growth so effectively that the impact of further control measures such as BTH is often not statistically significant.

This study also investigated the effects of JA-dependent defenses on the potato aphid. Most previous studies of JA have focused on its role in induced resistance against caterpillars and other chewing insects. Artificial induction of JA, however, is also known to affect aphids (Thaler et al., 1999; Omer et al., 2001; Ellis et al., 2002; Zhu-Salzman et al., 2004). In tomato, for example, foliar application of JA aphid reduces infestations under field conditions (Thaler et al., 1999). This reduction may have been due to an effect of JA or JA-dependent defenses on aphid migration, aphid population growth, or rates of predation and parasitism. In this study, we demonstrate that systemic defenses induced by JA in susceptible tomato plants have a negative impact on aphid population growth in the absence of natural enemies or aphid migration (Figures 3 and 4). In contrast, JA treatment did not dramatically enhance aphid control on a resistant tomato cultivar that carries *Mi-1.2* (Figures 3 and 4). This finding is also supported by the results of a life table study, in which we found that artificial induction of JA-dependent defenses reduced the longevity and lifetime fecundity of potato aphids on susceptible, but not on resistant, tomato plants (Cooper and Goggin, unpublished data).

It is not yet clear why JA treatment does not enhance aphid control on resistant varieties. Exogenous JA induced strong expression of the *Pin2* gene in both the resistant and susceptible cultivar, which suggests that treatment induces JA-dependent defenses in both genotypes (Figure 5). Potentially, the effects of *Mi-1.2* may prevent these JA-dependent defenses from having a dramatic impact on aphids. *Mi*-mediated resistance dramatically reduces aphid feeding (Kaloshian et al., 1997), whereas JA-dependent defenses appear to have primarily antibiotic effects on aphids (Cooper and Goggin, unpublished data). By reducing aphid feeding, *Mi-1.2* could thereby reduce aphids' exposure to toxic or antinutritive defenses induced by JA. In other words, a difference in the mode of action of these two forms of resistance could prevent them from having additive effects. While JA

treatment did not enhance *Mi*-mediated resistance, it is important to note that it also did not render plants more susceptible to aphids. This is significant because signaling conflicts can in some cases occur between SA- and JA-dependent defensive pathways, and SA is thought to play an important role in *Mi*-mediated resistance. Potentially, plants that carry *Mi-1.2* could be treated with JA to control other pests such as caterpillars, without compromising *Mi*-mediated aphid resistance.

Plant activators that elicit SA- or JA-dependent defenses are used in commercial tomato production to control pathogens and other pests, and these activators represent an environmentally safe alternative to pesticides. The results from this study suggest that artificial induction of these defensive pathways could also protect susceptible tomato cultivars against potato aphid infestation. This finding is of practical significance because the potato aphid is a major pest of tomato (Walgenbrach, 1997), and many widely used commercial tomato varieties are susceptible to this pest. In addition, this study demonstrates that induction of SA-dependent defenses could enhance aphid control on resistant tomato varieties that carry *Mi-1.2*. Aphid isolates that can overcome *Mi*-mediated resistance have been identified in the US (Goggin et al., 2001), and BTH or other elicitors of SAR could potentially help control these virulent biotypes. We hope that this work contributes to the utilization of host plant defenses for pest management, as well as to our understanding of the interactions between acquired and innate resistance.

Acknowledgments—The authors thank Drs. Ron McNew and Andronikos Mauromoustakos for assistance with statistical analysis, and Stephanie Hebert, John Geurber, and Alan Kline for technical advice and assistance with this project. We also thank Syngenta Crop Protection for supplying benzothiadiazole for this study. This research was supported by funding from the Arkansas Agricultural Experimental Station and the California Tomato Commission, as well as by USDA Southern Agricultural Research and Education program (SARE) student grant No. GS03–027.

REFERENCES

BRADING, P. A., HAMMOND-KOSACK, K. E., PARR, A., and JONES, J. D. G. 2000. Salicylic acid is not required for *Cf-2*- and *Cf-9*-dependent resistance of tomato to *Cladosporium fulvum*. *Plant J.* 23:305–318.

BRANCH, C., HWANG, C. F., NAVARRE, D. A., and WILLIAMSON, V. M. 2004. Salicylic acid is part of the *Mi*-1-mediated defense response to root-knot nematode in tomato. *Mol. Plant-Microbe Interact.* 17:351–356.

BROTMAN, Y., SILBERSTEIN, L., KOVALSKI, I., PERIN, C., DOGIMONT, C., PITRAT, M., KLINGLER, J., THOMPSON, G. A., and PERL-TREVES, R. 2002. Resistance gene homologues in melon are linked to genetic loci conferring disease and pest resistance. *Theor. Appl. Genet.* 104:1055–1063.

CHAMAN, M. E., COPAJA, S. V., and ARGANDONA, V. H. 2003. Relationships between salicylic acid content, phenylalanine ammonia-lyase (PAL) activity, and resistance of barley to aphid infestation. *J. Agric. Food Chem.* 51:2227–2231.

DE ILARDUYA, O. M., XIE, Q. G., and KALOSHIAN, I. 2003. Aphid-induced defense responses in *Mi-1*-mediated compatible and incompatible tomato interactions. *Mol. Plant-Microbe Interact.* 16:699–708.

DELANEY, T. P., UKNES, S., VERNOOIJ, B., FRIEDRICH, L., WEYMANN, K., NEGROTTO, D., GAFFNEY, T., GUTRELLA, M., KESSMANN, H., WARD, E., and RYALS, J. 1994. A central role of salicylic acid in plant-disease resistance. *Science* 266:1247–1250.

DOARES, S. H., NARVAEZ-VASQUEZ, J., CONCONI, A., and RYAN, C. A. 1995. Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. *Plant Physiol*. 108.

ELLIS, C., KARAFYLLIDIS, L., and TURNER, J. G. 2002. Constitutive activation of jasmonate signaling in an *Arabidopsis* mutant correlates with enhanced resistance to *Erysiphe cichoracearum*, *Pseudomonas syringae*, and *Myzus persicae*. *Mol. Plant Microbe Interact*. 15:1025–1030.

FLOR, H. H. 1955. Host–parasite interaction in flax rust—it's genetics and other implications. *Phytopathology* 45:680–685.

FORSLUND, K., PETTERSSON, J., BRYNGELSSON, T., and JONSSON, L. 2000. Aphid infestation induces PR-proteins differently in barley susceptible or resistant to the bird cherry-oat aphid (*Rhopalosiphum padi*). *Physiol. Plantarum* 110.

FRIEDRICH, L., LAWTON, K., WILHELM, R., MASNER, P., SPECKER, N., RELLA, M. G., MEIER, B., DINCHER, S., STAUB, T., UKNES, S., METRAUX, J. P., KESSMANN, H., and RYALS, J. 1996. A benzothiadiazole derivative induces systemic acquired resistance in tobacco. *Plant J*. 10:61–70.

GLAZEBROOK, J., ROGERS, E. E., and AUSUBEL, F. M. 1996. Isolation of *Arabidopsis* mutants with enhanced disease susceptibility by direct screening. *Genetics* 143:973–982.

GOGGIN, F. L., WILLIAMSON, V. M., and ULLMAN, D. E. 2001. Variability in the response of *Macrosiphum euphorbiae* and *Myzus persicae* (Hemiptera : Aphididae) to the tomato resistance gene *Mi*. *Environ. Entomol*. 30:101–106.

GOGGIN, F. L., GOWRI, S., WILLIAMSON, V. M., and ULLMAN, D. E., 2004. Developmental regulation of *Mi*-mediated aphid resistance is independent of *Mi*-1.2 transcript levels. *Mol. Plant Microbe Interact*. 17:532–536.

GORLACH, J., VOLRATH, S., KNAUF-BEITER, G., HENGY, G., BECKHOVE, U., KOGEL, K. H., OOSTENDORP, M., STAUB, T., WARD, E., KESSMANN, H., and RYALS, J. 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Plant Cell*. 8:629–643.

GRAHAM, J. S., PEARCE, G., MERRYWEATHER, J., TITANI, K., ERICSSON, L. H., and RYAN, C. A. 1985. Wound-induced proteinase inhibitors from tomato leaves; the cDNA-deduced primary structure of pre-inhibitor II. *J. Biol. Chem*. 260:6561–6564.

HAMMOND-KOSSACK, K. E. and JONES, J. D. G. 1996. Resistance gene-dependent plant defense responses. *Plant Cell* 8:1773–1791.

HAVLICKOVA, H., CVIKROVA, M., EDER, J., and HRUBCOVA, M. 1998. Alternations in the levels of phenolics and peroxidase activities induced by *Rhopalosiphum padi* (L.) in two winter wheat cultivars. *Z. Pflanzenk. Pflanzen.* 105:140–148.

JIRAGE, D., TOOTLE, T. L., REUBER, T. L., FROST, L. N., FEYS, B. J., PARKER, J. E., AUSUBEL, F. M., and GLAZEBROOK, J. 1999. *Arabidopsis thaliana Pad4* encodes a lipase-like gene that is important for salicylic acid signaling. *Proc. Natl Acad. Sci. USA* 96:13583–13588.

KACHROO, P., YOSHIOKA, K., SHAH, J., DOONER, H. K., and KLESSIG, D. F. 2000. Resistance to turnip crinkle virus in *Arabidopsis* is regulated by two host genes and is salicylic acid dependent but *Npr1*, ethylene, and jasmonate independent. *Plant Cell* 12:677–690.

KALOSHIAN, I., KINSEY, M. G., ULLMAN, D. E., and WILLIAMSON, V. M. 1997. The impact of *Meu1*-mediated resistance in tomato on longevity, fecundity and behavior of the potato aphid, *Macrosiphum euphorbiae*. *Entomol. Exp. Appl.* 83:181–187.

KARBAN, R. and BALDWIN, I. T. 1997. Induced Response to Herbivory. University of Chicago Press, Chicago, IL.

KRISHNAVENI, S., LIANG, G. H., MUTHUKRISHNAN, S., and MANICKAM, A. 1999. Purification and partial characterization of chitinases from sorghum seeds. *Plant Sci.* 144:1–7.

LAWTON, K., FRIEDRICH, L., HUNT, M., WEYMANN, K., DELANEY, T. P., KESSMANN, H., STAUB, T., and RYALS, J. 1996. Benzothiadiazole induces disease resistance in *Arabidopsis* by activation of the systemic acquired resistance signal transduction pathway. *Plant J.* 10:71–82.

MAYER, R. T., INBAR, M., MCKENZIE, C. L., SHATTERS, R., BOROWICZ, V., ALBRECHT, U., POWELL, C. A., and DOOSTDAR, H. 2002. Multitrophic interactions of the silverleaf whitefly, host plants, competing herbivores, and phytopathogens. *Arch. Insect Biochem. Physiol.* 51:151–169.

METRAUX, J. P., NAWRATH, C., and GENOUD, T. 2002. Systemic acquired resistance. *Euphytica* 124:237–243.

MILLIGAN, S. B., BODEAU, J., YAGHOOBI, J., KALOSHIAN, I., ZABEL, P., and WILLIAMSON, V. M. 1998. The root knot nematode resistance gene *Mi* from tomato is a member of the leucine zipper, nucleotide binding, leucine rich repeat family of plant genes. *Plant Cell* 10:1307–1319.

MOHASE, L. and VAN DER WESTHUIZEN, A. J. 2002. Salicylic acid is involved in resistance responses in the Russian wheat aphid-wheat interaction. *J. Plant Physiol.* 159:585–590.

MORAN, P. J. and THOMPSON, G. A. 2001. Molecular responses to aphid feeding in *Arabidopsis* in relation to plant defense pathways. *Plant Physiol.* 125:1074–1085.

MORAN, P. J., CHENG, Y. F., CASSELL, J. L., and THOMPSON, G. A. 2002. Gene expression profiling of *Arabidopsis thaliana* in compatible plant-aphid interactions. *Arch. Insect Biochem. Physiol.* 51:182–203.

NOMBELA, G., WILLIAMSON, V. M., and MUNIZ, M. 2003. The root-knot nematode resistance gene *Mi-1.2* of tomato is responsible for resistance against the whitefly *Bemisia tabaci*. *Mol. Plant Microbe Interact.* 16:645–649.

OLDROYD, G. E. D. and STASKAWICZ, B. J. 1998. Genetically engineered broad-spectrum disease resistance in tomato. *Proc. Natl Acad. Sci. USA* 95:10300–10305.

OMER, A. D., GRANETT, J., KARBAN, R., and VILLA, E. M. 2001. Chemically-induced resistance against multiple pests in cotton. *Int. J. Pest Manage.* 47:49–54.

PENA-CORTES, H., ALBRECHT, T., WEILER, E. W., and WILLLMITZER, L. 1993. Aspirin prevents wound-induced gene expression in tomato leaves by blocking jasmonic acid biosynthesis. *Planta* 191.

PUTERKA, G. J. and PETERS, D. C. 1989. Inheritance of greenbug,*Schizaphis graminum* (Rondani), virulence to *Gb2* and *Gb3* resistance genes in wheat. *Genome* 32:109–114.

RAIRDAN, G. J. and DELANEY, T. P. 2002. Role of salicylic acid and *Nim1*/*npr1* in race-specific resistance in *Arabidopsis*. *Genetics* 161:803–811.

ROBERTS, P. A. 1995. Conceptual and practical aspects of variability in root-knot nematodes related to host-plant resistance. *Annu. Rev. Phytopathol.* 33:199–221.

ROSSI, M., GOGGIN, F. L., MILLIGAN, S. B., KALOSHIAN, I., ULLMAN, D. E., and WILLIAMSON, V. M. 1998. The nematode resistance gene *Mi* of tomato confers resistance against the potato aphid. *Proc. Natl Acad. Sci. USA* 95:9750–9754.

RYAN, C. A., BISHOP, P. D., GRAHAM, J. S., BROADWAY, R. M., and DUFFEY, S. S. 1986. Plant and fungal cell wall fragments activate expression of proteinase-inhibitor genes for plant defense. *J. Chem. Ecol.* 12:1025–1036.

SANO, H. and OHASHI, Y. 1995. Involvement of small GTP-binding proteins in defense signal transduction pathways of higher plants. *Proc. Natl Acad. Sci. USA* 92:4138–4144.

SARDESAI, N., RAJYASHRI, K. R., BEHURA, S. K., NAIR, S., and MOHAN, M. 2001. Genetic, physiological and molecular interactions of rice and its major Dipteran pest, gall midge. *Plant Cell Tissue Org.* 64:115–131.

SCUTAREANU, P., BRUIN, J., POSTHUMUS, M. A., and DRUKKER, B. 2003. Constitutive and herbivore induced volatiles in pear, alder, and hawthorn trees. *Chemoecology* 13:63–74.

STOUT, M. J., WORKMAN, K. V., BOSTOCK, R. M., and DUFFEY, S. S. 1998a. Specificity of induced resistance in tomato, *Lycopersicon esculentum*. *Oecologia* 113:74–81.

STOUT, M. J., WORKMAN, K. V., BOSTOCK, R. M., and DUFFEY, S. S. 1998b. Stimulation and attenuation of induced resistance by elicitors and inhibitors of chemical induction in tomato (*Lycopersicon esculentum*) foliage. *Entomol. Exp. Appl.* 86:267–279.

STUART, J. J., SCHULTE, S. J., HALL, P. S., and MAYER, K. M. 1998. Genetic mapping of Hessian fly avirulence gene *Vh6* using bulked segregant analysis. *Genome* 41:702–708.

TAKAHASHI, H., MILLER, J., NOZAKI, Y., SUKAMTO, TAKEDA, M., SHAH, J., HASE, S., IKEGAMI, M., EHARA, Y., and DINESH-KUMAR, S. P. 2002. *Rcy1*, an *Arabidopsis thaliana Rpp8*/*hrt* family resistance gene, conferring resistance to cucumber mosaic virus requires salicylic acid, ethylene and a novel signal transduction mechanism. *Plant J.* 32:655–667.

THALER, J. S. 1999. Induced resistance in agricultural crops: Effects of jasmonic acid on herbivory and yield in tomato plants. *Environ. Entomol.* 28:30–37.

THALER, J. S., FIDANTSEF, A. L., DUFFEY, S. S., and BOSTOCK, R. M. 1999. Trade-offs in plant defense against pathogens and herbivores: A field demonstration of chemical elicitors of induced resistance. *J. Chem. Ecol.* 25:1597–1609.

WALGENBRACH, J. F. 1997. Effect of potato aphid (Homoptera: Aphididae) on yield, quality, and economics of staked-tomato production.*J. Econ. Entomol.* 90:996–1004.

XIAO, F., TANG, X., and ZHOU, J. 2001. Expression of *35S::Pto* globally activates defense-related genes in tomato plants. *Plant Physiol.* 126:1637–1645.

ZANTOKO L. S. R. H. 1997. Genetics of virulence in the Hessian fly to resistance gene *H*13in wheat. *J. Hered.* 88:120–123.

ZHU-SALZMAN, K., SALZMAN, R. A., AHN, J., and KOIWA, H. 2004. Transcription regulation of sorghum defense determinants against phloem-feeding aphid. *Plant Physiol.* 134:420–431.