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Responses of *Helicoverpa armigera* to Tomato Plants Previously Infected by ToMV or Damaged by *H. armigera*

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Abstract We report the comparative inducing effects of a phytopathogen and a herbivorous arthropod on the performance of an herbivore. Tomato, *Lycopersicon esculentum* Mill., was used as the test plant, and tomato mosaic virus (ToMV) and corn earworm, *Helicoverpa armigera* Hübner, were used as the phytopathogen and herbivore, respectively. There were decreases in the efficiency of conversion of ingested food and efficiency of conversion of digested food when *H. armigera* was reared on tomato plants that had been previously inoculated with ToMV. However, virus inoculation did not affect feeding or oviposition preferences by *H. armigera*. In contrast, approximate digestibility, total consumption, relative growth rate, and relative consumption rate were lower for fourth-instar *H. armigera* that fed on plants previously damaged by the same herbivore. Feeding and oviposition were both deterred for *H. armigera* that fed on previously damaged plants. The duration of development of *H. armigera* was also prolonged under this treatment. Infection by ToMV and feeding damage by *H. armigera* increased the host plant's peroxidase and polyphenol oxidase activity, respectively, suggesting that the performance of *H. armigera* may be affected by the induced phytochemistry of the host plant. Overall, this study indicated that, in general, insect damage has a stronger effect than ToMV infection on plant chemistry and, subsequently, on the performance of *H. armigera*.

Keywords Corn earworm · *Helicoverpa armigera* · Induced plant chemistry · Peroxidase · Plant–phytopathogen–herbivore interaction · Polyphenol oxidase · Tomato mosaic virus (ToMV)

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Introduction

Almost every plant species is used as a food source by a variety of phytopathogens and herbivores, and these exploiters seldom exist in isolation from each other. When considering their abundance and biodiversity (Hawksworth 1991), it is obvious that the concurrent or sequential occurrence of phytopathogens and herbivores on a host plant is common, and that interactions between phytopathogens and herbivores can be expected. Thus, plants often deal with diverse enemies (Moran 1998; Genoud and Metraux 1999; Maleck and Dietrich 1999; Paul et al. 2000; Kruess 2002; Rostás and Hilker 2002, 2003). Owing to their sedentary life traits, plants have evolved specific ways to cope with their multiple enemies. They synthesize a broad range of constitutive and induced phytochemicals that may directly affect the inducers themselves or indirectly affect subsequent intruding herbivores or phytopathogens (Moran 1998; Rostás et al. 2002; Wittstock and Gershenzon 2002; Johnson et al. 2003).

Interactions among diverse plant enemies can be direct or indirect, mutualistic, detrimental or neutral, and these effects may be exerted by induced plant chemistry (Hatcher et al. 1995; Moran 1998; Rostás and Hilker 2002, 2003; Johnson et al. 2003; Stout et al. 2006). Both phytopathogens and herbivores are biotic stress factors that induce changes in the plant metabolism, such as changes in patterns of nutrient allocation and induction of defense-related phytochemistry (Ayres 1992; Baldwin and Preston 1999; Hammerschmidt 1999; Rostás and Hilker 2002; Stout et al. 2006). Thus, phytopathogens and herbivores interact indirectly by influencing the suitability of their shared host plant.

Several studies have indicated that cross-resistance between herbivores and phytopathogens occurs, e.g., prior attacks by phytopathogenic fungi increase resistance against subsequent attacks by herbivores (Karban et al.

1987; Hatcher et al. 1994, 1995; Hatcher 1995; Siemens and Mitchell-Olds 1996; Kruess 2002; Rostás and Hilker 2002; Rostás et al. 2003; Stout et al. 2006), and previous herbivore-damaged plants become less suitable for fungi (Karban et al. 1987; Hatcher et al. 1994; Hatcher and Paul 2000; Rostás et al. 2003). Most studies of plant–phytopathogen–herbivore interactions, however, have been focused on the effects of phytopathogenic fungi (Moran 1998; Kruess 2002; Rostás and Hilker 2002; Rostás et al. 2002, 2003; Johnson et al. 2003), and relatively little is known about the effects of phytopathogenic viruses on plants and on lepidopteran insects (Stout et al. 1999; Mayer et al. 2002; McKenzie et al. 2002). In addition to the plant–phytopathogen–herbivore interactions, many studies have also revealed a plant–herbivore–herbivore interaction (Karban and Myers 1989; Stout and Duffey 1996; Karban and Baldwin 1997; Denno et al. 2000). Different herbivores of a host plant may be separated spatially or temporally, but they may interact with each other through mediation of the host plant. Most plant–herbivore–herbivore research has been concerned with the effect of plant-induced responses on herbivores (Karban and Baldwin 1997; Felton and Eichenseer 1999; Underwood 1999; Bezemer and van Dam 2005). However, diverse effects have been found for plant-mediated interaction, and this may be because of complicating interactions among the defense-related signalling pathways and the resulting induction of plant secondary compounds (Stout et al. 2006).

Corn earworm, *Helicoverpa armigera* (Hübner), has a worldwide distribution and is a highly polyphagous agricultural pest. The host plant spectrum of *H. armigera* includes important agricultural crops such as tomato, cotton, maize, chickpea, sorghum, cereals, and soybean (Fitt 1989; Cunningham et al. 1999; Gupta et al. 2003; Diongue et al. 2004). Feeding on foliage or fruiting structures by insatiable larvae usually leads to substantial economic losses (Reed and Pawar 1982). Tomato mosaic virus (ToMV) causes mosaic disease of tomato and other important crop plants (Green et al. 1987; Breman 1989; Duarte et al. 2001). The ToMV-infected tomato plants display light and dark green mottled areas of the leaves, and fruits may be reduced in size and number with irregular ripening (Green et al. 1987). In tomato plantations, both *H. armigera* and ToMV may occur simultaneously. We investigated the interactions among tomato, ToMV, and corn earworm (*H. armigera*). Specifically, this study focused primarily on plant-mediated indirect interactions between ToMV and *H. armigera*, and on the indirect interactions between insect-induced responses and *H. armigera* performance. In addition, we assessed changes in plant chemistry because of induction by herbivore damage or virus infection that might be relevant to the performance of *H. armigera*.

Methods and Materials

Plants Tomato plants (*L. esculentum* Mill. cv Tainan-Yasu No. 6) were grown from seeds in a greenhouse (27–30°C). Supplemental light was provided 16 hr/d in addition to natural daylight. Before sowing, seeds were soaked first in 5% bleach for 30 min and rinsed with distilled water three times to eliminate contamination. Seeds were potted in standard potting soil, watered daily, and fertilized once a week with commercial synthetic 25–5–20 (N–P–K) fertilizer (1/1,000 Hyponex®4). Plants were transplanted to 17.8 cm diameter pots when they had three fully expanded leaves (20 to 28 d after sowing).

H. armigera and ToMV H. armigera were obtained as eggs from the Taiwan Agricultural Chemicals and Toxic Substances Research Institute, Council of Agriculture. Larvae were reared on an artificial diet in a growth chamber (27°C, 12L/12D photoperiod). ToMV was obtained from the laboratory of Dr. F. J. Jan (Department of Plant Pathology, National Chung Hsing University, Taiwan). As the standard method, ToMV was suspended in 10 mM sodium phosphate (pH 7.0) and inoculated onto the true leaf of *Chenopodium quinoa*. For the experimental inoculation treatments, the virus suspension was obtained by grinding the infected *C. quinoa* leaves with a sodium phosphate buffer. To prevent loss of viral activity, all treatments were conducted in a cold room (4°C), and the virus suspension was used within 8 hr after suspension.

Plant-mediated interactions between ToMV and H. armigera To evaluate the effects of systemic infection by ToMV on the resistance of tomato foliage to *H. armigera*, four-leaf tomato plants were assigned in equal numbers to two treatment groups (approximately 60 plants per treatment). Plants in the first group were subjected to a localized infection by ToMV, and those in the second group were the control group. Inoculations were confined to the terminal leaflet of the third leaf. Leaflets were sprayed first with corundum powder; then, they were inoculated by gently rubbing the upper surfaces of the leaflet with a pestle saturated with a virus suspension (in phosphate buffer, pH 7.0). Plants in the control group received the same inoculation treatment, except that a phosphate buffer was used instead of the virus suspension. Three days after the inoculation, leaflets of the fourth leaf (not the treated leaf) were used to evaluate the effects, to assess the chemical changes caused, and to determine the suitability of the treatment for *H. armigera*. Chemical analyses and the bioassays used to assess the suitability of the inoculation treatment for *H. armigera* are described below.

A larval feeding preference bioassay utilized newly molted, fourth-instar *H. armigera*. One leaflet from the fourth leaf was collected from both treated and control tomato plants. Areas of these leaflets were measured first with a portable area meter (Li-3000A, Li-Cor, Lincoln, NE, USA). The leaf petiole of each leaflet was inserted into a water pik to maintain leaf turgor, and leaflets from both treatments were placed in a Petri dish (140×15 mm). Seven 4th instars were placed in the center of each Petri dish and allowed to randomly select and feed on foliage for 6 hr. After they had fed, larvae were removed and the leaf areas of both leaflets were measured again. Feeding percentage was calculated as: Feeding preference (%)=[(leaf area consumed, either treated or control leaflet)/(leaf area consumed of treated leaflet+leaf area consumed of control leaflet)]×100%. Six replicates were performed.

We also evaluated the effect of foliage quality of treated plants on the oviposition choice of adult *H. armigera*. Pupae were separated by sex, and 3 d after eclosion, 10 moths (five of each sex) were placed into a glass cylinder (90 mm long×55 mm diameter) for mating. One day after mating, these 10 moths were transferred to a nylon mesh cage (60×60×60 cm), and two plants, one representing treatment (virus infection) and the other control, were provided for the moths to deposit eggs. Seventy-two hours later, plants were removed from cages, eggs were counted, and the oviposition preference was calculated as: Oviposition preference (%)=[(egg number from either treated or control leaflet)/(egg number from treated leaflet+egg number from control leaflet)]×100%. Six replicates were performed.

Short-term feeding trials were conducted to evaluate the effect of foliage quality on growth rate, food consumption rates, and food processing efficiencies of fourth instars of *H. armigera*. Three days after the inoculation, leaflets of the fourth leaf (untreated leaf) were used. Fifty newly hatched larvae were grown on artificial diet in a Percival growth chamber (12L/12D photoperiod) at a constant 27°C until molting to fourth instars. Each assay consisted of a newly molted and weighed larva placed into a rearing cup (250 ml) that contained a leaf from a plant that had received one of the two different treatments (N=15 replicates per plant treatment). Leaves (above the fourth leaf) were changed every 1–2 d or as necessary during the bioassay. Upon molting to fifth instar, larvae were frozen, oven-dried at 50°C for 1 wk, and reweighed. Nutritional indices were calculated to evaluate insect growth, consumption, and food utilization efficiency (Haynes and Millar 1998; Schoonhoven et al. 1998). These indices were calculated from standard formulas for approximate digestibility (AD), efficiency of conversion of digested food (ECD), and efficiency of conversion of ingested food (ECI) as described by Waldbauer (1968) and Haynes and Millar (1998). The initial rather than the average weights of the larvae were used to calculate the relative growth rate (RGR) and relative consumption rate (RCR) (Farrar et al. 1989). Analysis of covariance (ANCOVA) has been suggested to be more appropriate than the use of ratio variables for the analysis of nutritional indices (Raubenheimer and Simpson 1992, 2003; Raubenheimer 1995; Packard and Boardman 1999; Thompson et al. 2005). Therefore, we performed an ANCOVA (PROC GLM; SAS Institute 1999) on the absolute growth rate (AGR) (weight gained per day) and absolute consumption rate (ACR) (food consumed per day) by using initial weights as covariates. We reported the results from both the standard (ratio) and ANCOVA approaches for a reason: any errors introduced by variation in initial larval weights are inconsequential when the range of initial weights is small, as was the case in this study. Initial dry weights of the test insects were estimated based on a wet-to-dry weight conversion factor determined from five newly molted fourth instars. Similarly, initial dry weights of leaves fed to insects were estimated by dry weight conversion using foliage collected from each plant group at the time of the bioassay. Means and standard errors were calculated for duration, RGR, AGR, RCR, ACR, total consumption (TC), AD, ECD, and ECI for insects fed on foliage from differently treated plants. During the bioassay, additional leaf material from the test plants was collected to measure their chemical content.

Plant-mediated interactions between insect damage and H. armigera To evaluate the effects of systemic induction by *H. armigera* feeding on resistance of tomato foliage to *H. armigera*, four-leaf tomato plants (28-d-old) were assigned in equal numbers to the two treatment groups (approximately 60 plants per treatment). Plants in the first group were subjected to localized feeding by *H. armigera*, and those in the second were the control group. Feeding was confined to the third leaf. One newly molted fourth-instar larva was restricted by a mesh bag and fed on the third leaf. Twenty-four hours after feeding (about half of the leaf area was removed), each larva was removed from the plant. Plants in the control group received the same bag treatment except no larva was used. Three days after feeding, the leaflets of the fourth leaf (untreated leaf) were used to evaluate the effects of the treatment on the suitability of the leaf in preference and feeding trials for *H. armigera* and to assess any chemical changes caused by the treatments. The bioassays used to assess the suitability of leaflets for *H. armigera* (preference and feeding trials) and leaf sampling were similar to those used previously (see "Plant-mediated interactions between ToMV and *H. armigera*").

Chemical analyses Concurrent with the feeding bioassay, foliar samples were collected for chemical analysis. Systemic leaves (i.e., not the virus- or insect-treated leaves, but the fourth leaf and leaves growing above the fourth leaf) from treated tomato plants (six plants), and similarly aged leaves from control plants (six plants) were harvested (0, 3, 7, 14, and 21 d after treatment). Leaves were sampled from different plants (not previously sampled) at the various sampling time intervals to insure independence of sampling. The fourth leaf was used for measuring the total protein concentration and the activities of polyphenol oxidase and peroxidase. The fifth and above leaves were flash frozen in liquid nitrogen, freeze-dried, ground, and stored in a freezer for water and nonstructural carbohydrate analyses at a later date.

Spectrophotometric assays of the activities of polyphenol oxidase and peroxidase were performed with an extract of the fourth leaf (Moran 1998; Stout et al. 1999). Leaf extract was prepared with a tissue grinder (Drill Press Stand Model 212; Dermel, Racine, WI, USA) to homogenize the whole leaf in pH 7 phosphate buffer containing 7% (*w*/*v*) polyvinylpolypyrrolidone. A volume (1.5 ml) of homogenate was removed and placed into a 1.7-ml centrifuge tube. Then, 100 µl of a 10% solution of Triton X-100 were added by mixing with the homogenate. This homogenate was centrifuged at 6,000×*g* for 15 min. The resulting supernatant was used for enzyme activity determination. Total protein was assessed with bovine serum albumin as the standard (Bradford 1976). Polyphenol oxidase and peroxidase activity were measured as in Stout et al. (1999) for the rate of formation of melanin-like material from the phenolic substrates. For polyphenol oxidase assays, 10 to 100 µl of enzyme extract were added to 500 µl of 10 mM catechol in pH 8 potassium phosphate buffer (0.1 M), and the change in absorbance of the mixture at 470 nm was recorded for 30 sec. The method for measuring peroxidase activity was similar, but the substrate for peroxidase activities consisted of 5 mM guaiacol with 0.02 mM H_2O_2 added as a cofactor. Polyphenol oxidase and peroxidase activities were reported as ΔOD_{470} min^{-1}mg fresh $weight^{-1}$ (Ryan et al. 1982).

Foliar water and total nonstructural carbohydrate contents were also quantified for each foliar sample. Differences between wet and dry weights of leaf samples were used to determine water contents. An enzymatic method was used to measure the total nonstructural carbohydrates (TNC) of each sample. Extracts of TNC (starch plus soluble carbohydrates/sugars) were incubated with amyloglucosidase to completely hydrolyze starch before assaying for reducing sugars (Madsen 1997; Liao 2003).

Statistical analyses For all bioassays, means and standard errors (SE) were calculated for the insect performance parameters (feeding and oviposition preference, growth rate, consumption rate, and food utilization efficiencies) and plant chemistry. The Student's *t* test (PROC TTEST; SAS Institute 1999) was used to compare insect performance among virus-inoculated, previously damaged (by insects), and control host plants.

Results

Plant-mediated interactions between ToMV and H. armigera Infection of tomato plants by ToMV had no effect on larval feeding and adult oviposition preference (Fig. 1). Larvae consumed almost equal amounts of foliage from both the virus-infected and the control leaves. Adult moths laid slightly more eggs on the control plants (58%) than on the virus-infected plants (42%), but this difference was not significant.

Performance (duration, growth rates, and consumption rates) of fourth-instar *H. armigera* was similar between the virus-infected and the control plants (Table 1). Analysis of covariance (rate variables) and analysis of variance (ratio variables) gave identical results for growth (AGR, RGR) and consumption (ACR, RCR) parameters (Table 1). However, larvae that fed on virus-infected plants had significantly reduced food conversion efficiencies (ECD and ECI).

Plant-mediated interactions between insect damage and H. armigera Previous feeding on tomato plants by *H. armigera* resulted in a dramatic change in preference of the fed-upon plants to subsequent feeding by *H. armigera*. Fourth-instar larvae consumed more than nine times the amount of foliage of control plants than they did of treated plants (Fig. 1). Female moths laid significantly more eggs on control plants than they did on treated plants (Fig. 1).

Performance (duration, growth rates, and consumption rates) of the fourth instars varied substantially between treated and control plants (Table 1). Analysis of covariance (rate variables) and analysis of variance (ratio variables) also gave similar results for growth (AGR, RGR) and consumption (ACR, RCR) parameters (Table 1). In contrast to the virus-infected study, growth rates (RGR) and consumption rates (RCR) varied significantly between control and treatment plants. Both growth rates (RGR) and consumption rates (RCR) were higher for insects fed on foliage of the control tomatoes (Table 1). Larval duration (DUR), however, was longer for insects that fed on insect-damaged plants. In addition, *H. armigera* larvae had higher digestibility (AD) on control than on treated host plants. The efficiencies of conversion of ingested and digested food (ECD and ECI), however, were not significantly

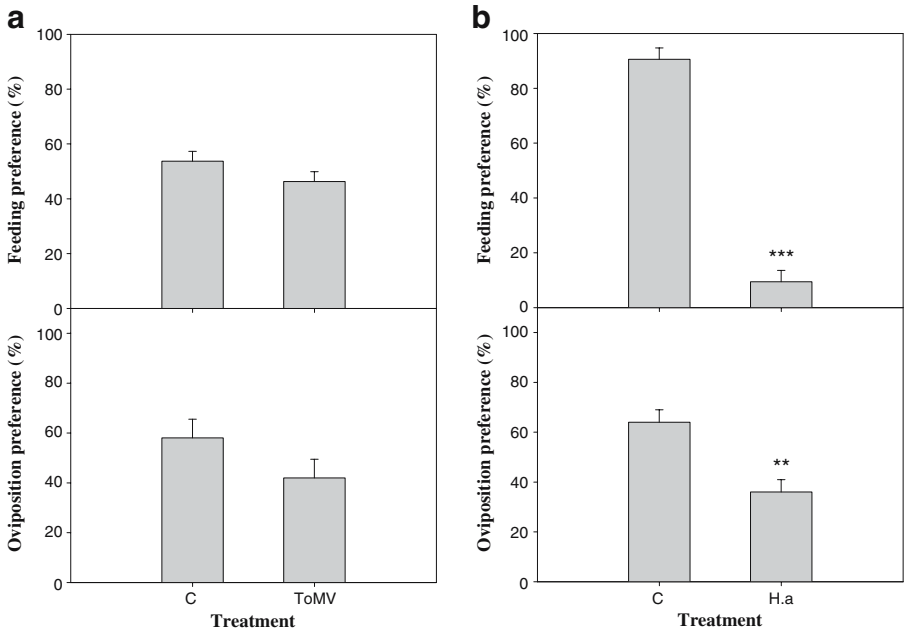

Fig. 1 Larval feeding and adult oviposition preferences (mean±SE, N=6 per treatment) of *H. armigera* in differently treated tomato leaves. **a** Foliage of tomato was inoculated with ToMV. **b** Foliage of tomato was fed on previously by *H. armigera*. *C* control, *ToMV* leaf of tomato plants that were inoculated previously with ToMV, *H. a* leaf of tomato plants that were fed on previously by *H. armigera* larvae. *Asterisks* denote significant differences (**P<0.01, ***P<0.001; Student's t test for independent samples)

different between control and treated plants. In summary, when fed on the previously fed-upon foliage, *H. armigera* larvae consumed less foliage (reduced RCR), had lower digestibility (reduced AD), and grew more slowly (reduced RGR and increased DUR).

Foliage chemistry Based on the bioassays, biochemical analyses were conducted to assess the relationship between the effect of the treatments on pest resistance and the expression of specific enzyme activities or compounds that are probably resistance mechanisms. Peroxidase activity increased slightly with leaf age in all experiments. Peroxidase activity, however, was significantly induced in tomato plants inoculated with ToMV in the systemic leaves by the seventh day after infection (Fig. 2a), and was 30% higher than in control plants. In contrast, peroxidase activity was not significantly different between the previously fed-upon plants and the controls (Fig. 2b).

Polyphenol oxidase activity also increased with leaf age of tomatoes, but the activity was not significantly different between the control and virus-infected plants (Fig. 2a). In contrast, in the insect-feeding treatment (Fig. 2b), polyphenol oxidase activity was significantly higher (30%) than in control plants on the third day after the feeding treatment.

Our results also indicated that foliar water and total protein contents were similar between the treatments and their controls (Fig. 3). Foliar water contents remained between 85% and 90% throughout the experiments. In

Table 1 Performance of corn earworm, *H. armigera*, reared on tomato leaves from plants that were infected with ToMV or infested previously with *H. armigera*

	AD (%)	ECD (%)	ECI (%)	DUR (d)	TC (mg)	RGR (mg/mg/d)	AGR (mg/d)	RCR (mg/mg/d)	ACR (mg/d)
Infected with ToMV									
C	66.35±1.90	23.80±1.27	15.73±0.80	3.33±0.13	65.16±3.55	0.53±0.03	3.08±0.20	3.43±0.19	19.61±0.81
ToMV	63.79±0.81	20.06±1.12	12.79±0.72	3.42±0.16	66.38±3.96	0.46±0.04	2.57±0.34	3.57±0.19	19.71±1.63
P[a]	0.2342	0.0477	0.0183	0.6644	0.8228	0.1128	0.1994	0.6051	0.955
Infested previously with *H. armigera*[b]									
C	60.49±3.95	33.26±3.43	19.19±1.01	3.12±0.06	49.82±2.76	0.53±0.03	3.04±0.20	2.79±0.09	15.97±0.87
H. a	44.43±2.43	42.51±4.58	18.36±1.65	3.67±0.20	39.60±2.36	0.38±0.05	2.05±0.25	2.10±0.21	11.19±1.08
P[a]	0.0029	0.132	0.6216	0.022	0.0116	0.0169	0.0014	0.0103	<0.0001

AD: approximate digestibility, *ECD*: efficiency of conversion of digest food, *ECI*: efficiency of conversion of ingested food, *DUR*: duration, *TC*: total consumption, *RGR*: relative growth rate, *AGR*: absolute growth rate, *RCR*: relative consumption rate, *ACR*: absolute consumption rate, *C*: control, *ToMV*: leaf from plant inoculated with ToMV, *H. a*: leaf from plant fed on by *H. armigera* larvae

[a] AD, ECD, and ECI values are transformed to arcsine values for analysis by t test. Values for AGR and ACR were analyzed by ANCOVA.

[b] Short-term feeding trial (mean±SE, N=15 plants, one insect per plant).

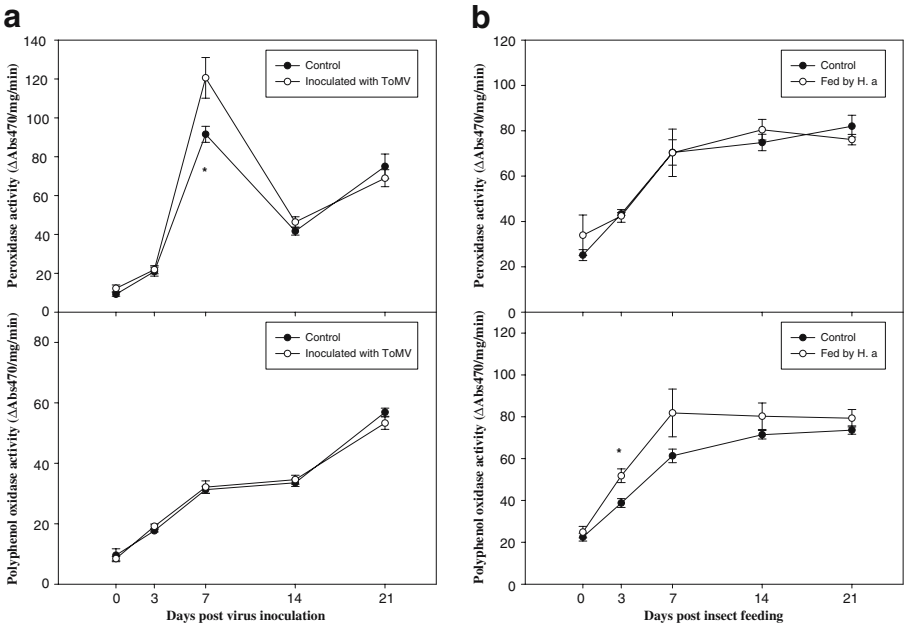

Fig. 2 Response of tomato leaf peroxidase and polyphenol oxidase activities (mean±SE, N=6 plants per treatment) to ToMV/*H. armigera* infestation. **a** Foliar peroxidase and polyphenol oxidase activities in tomato inoculated with ToMV over time. **b** Foliar peroxidase and polyphenol oxidase activities in tomato previously fed on by *H. armigera* over time. *Asterisks* denote significant differences (*P<0.05; Student's t test for independent samples)

addition, the foliar total nonstructural carbohydrate contents were similar between the virus-infected and control treatments during the experiments (Fig. 3). However, in the insect-damage treatment, the total nonstructural carbohydrate content decreased significantly on the third day after the feeding treatment (Fig. 3).

Discussion

We showed that herbivory and viral infections have varied effects on performance and food processing efficiencies of *H. armigera*. Herbivory and virus infection may also affect host plant biochemistry, and changes in host oxidase activity because of induction might affect subsequent performance of *H. armigera*.

Previous investigations have indicated that plant-mediated interactions may occur between phytopathogens and herbivores or between various herbivorous species (Moran 1998; Stout et al. 1999; Thaler et al. 1999; Kruess 2002; Mayer et al. 2002; Rostás and Hilker 2002; Rostás et al. 2002; Johnson et al. 2003). Many studies were focused on the effect of prior attacks by phytopathogens on herbivores or vice versa (Karban et al. 1987; Hatcher et al. 1994, 1995; Siemens and Mitchell-Olds 1996; Hatcher and Paul 2000; Kruess 2002; Rostás and Hilker 2002; Stout et al. 2006), or on plant–herbivore–herbivore interaction (Karban and Myers 1989; Stout and Duffey 1996; Karban and Baldwin 1997; Denno et al. 2000). Among the plant–phytopathogen–herbivore interaction studies, most were focused on the cross-effects between fungal infection and insects (Hatcher 1995; Stout et al. 1999, 2006; Rostás and Hilker 2002; Rostás et al. 2003), and only a few looked at the cross-effects between virus infection and lepidopteran insects.

In our study, we compared concurrently the relative inducing effects of phytopathogenic virus and feeding by an arthropod herbivore on herbivore performance. The larvae of *H. armigera* prefer to feed on undamaged foliage rather than on herbivore-attacked foliage, and the adults also exhibit a decreased preference for leaflets of herbivore-damaged plants. Previous studies have indicated that after a prior attack by herbivores, plants can be induced to increase their polyphenol oxidase activity, which decreases the nutrient value of the foliage and reduces the feeding preference of the insects (Felton et al. 1992; Stout and Duffey 1996; Bostock et al. 2001). Our results show that *H. armigera* prefers to feed or oviposit on the control (undamaged) foliage, which also contains the lower polyphenol oxidase activity. In contrast to the herbivore-induced results, no difference was found in the preference behavior of *H. armigera* between control (undamaged) foliage and virus-infected foliage. Although some workers have suggested that adult beetles will avoid feeding and will avoid ovipositing on fungus-infested leaves (Kruess 2002; Rostás and Hilker 2002; Rostás et al. 2002), conflicting results have also been reported (Moran 1998). The cause for these varied results is unclear. Recent literature that deals with herbivore and pathogen effects has indicated that the effects of induced resistance are often not specific to any particular attacker, and the signal transduction pathways involved in induction after attack by herbivores or pathogens may overlap (Paul et al. 2000). Hypothetically, the resistance response induced by one attacker might be effective against a range of other potential attackers. However, in our system, the induced resistances

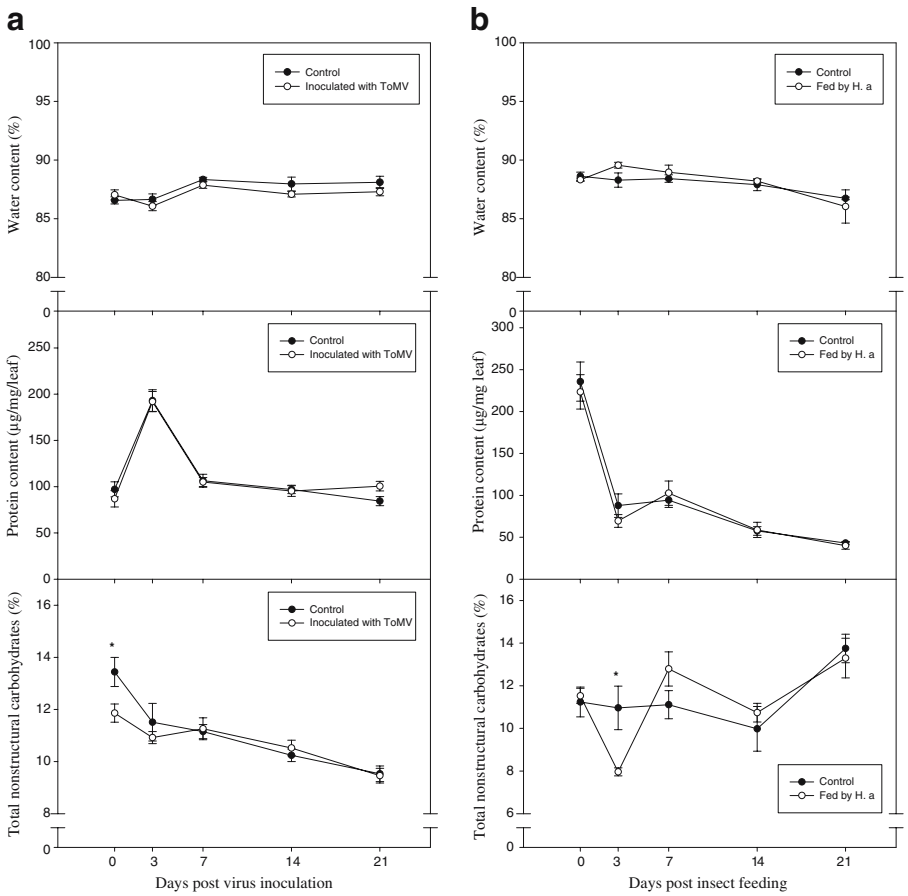

Fig. 3 Response of foliar water, protein, and total nonstructural carbohydrates (mean±SE, N=6 plants per treatment) to ToMV/*H. armigera* infestation. **a** Foliar water, protein, and total nonstructural carbohydrates contents in tomato inoculated with ToMV over time. **b** Foliar water, protein, and total nonstructural carbohydrates contents in tomato previously fed on by *H. armigera* over time. *Asterisks* denote significant differences (*P<0.05; Student's t test for independent samples)

show some degree of specificity, and resistance induced by a phytopathogenic virus may not be effective against a herbivore. In addition, studies of the role of cross-talk in tripartite interactions that involve jasmonic acid (JA) and salicylic acid (SA) pathways have demonstrated that a potential negative effect may occur between these induction-related pathways. This may lead to a reduction in resistance to the second attacker relative to plants not subject to initial attack (Stout et al. 2006).

In the short-term feeding trials, fourth-instar *H. armigera* performed differently between treatments and controls. Fourth instars grew more slowly and consumed less food on herbivore-damaged foliage than on control (undamaged) foliage. Stout and Duffey (1996) also indicated that beet armyworm, *Spodoptera exigua*, larvae grew slower and consumed less leaf tissue from corn earworm (*Helicoverpa zea*)-damaged plants than from control plants. This may be because of the decrease in the nutritional value of herbivore-damaged foliage. As previously mentioned, after having been damaged by insect feeding, plants increase their polyphenol oxidase activity and reduce their foliar carbohydrate concentrations. This increase in oxidase activity and decrease in carbohydrate content might decrease the nutritive value and thus reduce performance of subsequent feeding insects (Felton et al. 1989, 1992; Stout and Duffey 1996; Simpson and Raubenheimer 2001; Bostock et al. 2001; Lee et al. 2002). Similarly, our results of food utilization efficiencies show that larvae fed on herbivore-induced foliage have significantly lower absorption efficiency (AD). However, food conversion efficiency was not different. On the other hand, larval growth and consumption rates were not significantly different between virus-induced and control (undamaged) foliage. We found that larvae fed on virus-infected foliage had slightly lower food processing efficiencies (ECD and ECI) than those on control foliage, but the consumption and growth rates were not different. Other studies as well as ours have revealed an increase in peroxidase (POD) after insect or artificial damage in cucumber, corn, tomato, and other plants (Miller and Kelley 1989; Svalheim and Robertsen 1990; Dowd and Norton 1995; Bi et al. 1997; Moran 1998; Stout et al. 1998). Some studies have indicated that peroxidase induction is concurrent with the induction of systemic acquired resistance (SAR) against subsequent infection by the phytopathogenic fungus *Colletotrichum orbiculare* (Hammerschmidt et al. 1976; Moran 1998) or other phytopathogens. However, this increased peroxidase activity may also play a role in a plant's defense against insects, although the specific function of this enzyme in defense is unclear. We found that the elevated level of peroxidase activity in tomato after virus inoculation

may have a slight effect on an insect's food processing efficiencies, and this may be because of the toxicity of oxidized metabolites or free radicals (Duffey and Felton 1991).

In summary, this study compared the relative inducing effects of a phytopathogenic virus and herbivores on plants and on their subsequent herbivory. The result indicated that damage by insects had a stronger effect on plant chemistry and on performance of *H. armigera* than did virus infection. In addition, phytochemical analysis indicated that ToMV infection and damage by *H. armigera* can increase a host plant's level of peroxidase and polyphenol oxidase activity, respectively. In addition, *H. armigera* feeding can also reduce a host plant's carbohydrate concentration shortly after damage. A synthesis of these effects suggests that the increase in the level of polyphenol oxidase activity and the decrease in carbohydrate concentration may have some negative effects on the behavior and growth performance of *H. armigera*. Finally, although the literature concerning herbivores and pathogens has frequently shown that the effects of induced resistance are often not specific to any particular attacker (Paul et al. 2000), we found that the induced resistance did show a certain degree of specificity and that the resistance induced by a phytopathogen may not have a strong effect against subsequent herbivore attack.

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References

AYRES, P. G. 1992. Pests and Pathogens: Plant Responses to Foliar Attack. Bios Scientific, Oxford.

BALDWIN, I. T., and PRESTON, C. A. 1999. The eco-physiological complexity of plant responses to insect herbivores. *Planta* 208:137–145.

BEZEMER, T. M., and DAM, N. M. 2005. Linking aboveground and belowground interactions via induced plant defenses. *Trends Ecol. Evol* 20:617–624.

BI, J. L., MURPHY, J. B., and FELTON, G. W. 1997. Antinutritive and oxidative components as mechanisms of induced resistance in corn to *Helicoverpa zea*. *J. Chem. Ecol* 23:97–117.

BOSTOCK, R. M., KARBAN, R., THALER, J. S., WEYMAN, P. D., and GILCHRIST, D. 2001. Signal interactions in induced resistance to pathogens and insect herbivores. *Eur. J. Plant Pathol* 107:103–111.

BRADFORD, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem* 72:284–285.

BREMAN, L. L. 1989. Tomato mosaic virus. *Plant Pathol. Circ.* 322:1–2.

CUNNINGHAM, J. P., ZALUCKI, M. P., and WEST, S. A. 1999. Learning in *Heliothis armigera*: a new look at the behaviour and control of a polyphagous pest. *Bull. Entomol. Res* 89:210–207.

DENNO, R. F., PETERSON, M. A., GRATTON, C., CHENG, J., LANGELLOTTO, G. A., HUBERTY, A. F., and FINKE, D. L. 2000. Feeding-induced changes in plant quality mediate interspecific competition between sap-feeding herbivores. *Ecology* 81:1814–1827.

DIONGUE, A., TALEKAR, N. S., CHANG, Y. F., and LAI, P. Y. 2004. Ovipositional responses of the tomato fruitworm (*Helicoverpa armigera*) to the presence of eggs and to different stages of the tomato plant. *Plant Prot. Bull* 46:303–314.

DOWD, P. F., and NORTON, R. A. 1995. Browning-associated mechanisms of resistance to insects in corn callus tissue. *J. Chem. Ecol* 21:583–600.

DUARTE, K. M. R., GOMES, L. H., GESZTESI, J. L., LOPES, J. D., and TAVARES, C. A. 2001. Monoclonal antibodies to identify tomato mosaic tobamovirus (ToMV). *Brazilian Journal of Microbiology* 32:240–242.

DUFFEY, S. S., and FELTON, G. W. 1991. Enzymatic antinutritive defenses of the tomato plant against insects, pp. 166–197, in P. A. Hedin (ed.). Naturally Occurring Pest BioregulatorsAmerican Chemistry Society, Washington, DC, USA.

FARRAR, R. R. Jr, BARBOUR, J. D., and KENNEDY, G. G. 1989. Quantifying food consumption and growth in insects. *Ann. Entomol. Soc. Am* 82:593–598.

FELTON, G. W., and EICHENSEER, H. 1999. Herbivore saliva and its effects on plant defense against herbivores and pathogens, pp. 19–36, in A. A. Agrawal, S. Tuzun, and E. Bent (eds.). Induced Plant Defenses against Pathogens and HerbivoresAPS, St. Paul, MN, USA.

FELTON, G. W., DONATO, K. K., VECCHIO, R. J., and 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.

FELTON, G. W., DONATO, K. K., BROADWAY, R. M., and DUFFEY, S. S. 1992. Impact of oxidized plant phenolics on the nutritional quality of dietary protein to a noctuid herbivore, *Spodoptera exigua*. *J. Insect Physiol* 38:277–285.

FITT, G. P. 1989. The ecology of *Heliothis* in relation to agro-ecosystems. *Annu. Rev. Entomol* 34:17–52.

GENOUD, T., and METRAUX, J. P. 1999. Crosstalk in plant cell signaling: structure function of the genetic network. *Trends Plant Sci* 4:503–507.

GREEN, S. K., HWANG, L. L., and YOU, Y. J. 1987. Epidemiology of tomato mosaic virus in Taiwan and identification of strains. *Journal of Plant Diseases and Protection* 4::86–387.

GUPTA, R., NARAYANA, B. V. L., REDDY, P. K., and RAO, G. V. R. 2003. Understanding *Helicoverpa armigera* pest population dynamics related to chicken crop using neural networks. pp. 723–726, in Proceedings of the Third IEEE International Conference on Data Mining, Nov.19–22, Melbourne, FL, USA.

HAMMERSCHMIDT, R. 1999. Induced disease resistance: how do induced plant stop pathogens? *Physiol. Mol. Plant Pathol* 55:77–84.

HAMMERSCHMIDT, R., ACRES, S., and KUC, J. 1976. Protection of cucumber against *Colletotrichum lagenarium* and *Cladosporium cucumerinum*. *Phytopathology* 66:790–793.

HATCHER, P. E. 1995. Three-way interactions between plant pathogenic fungi, herbivorous insects and their host plants. *Biol. Rev* 70:639–694.

HATCHER, P. E., and PAUL, N. D. 2000. Beetle grazing reduces natural infection of *Rumex obtusifolius* by fungal pathogens. *New Phytol* 146:325–333.

HATCHER, P. E., PAUL, N. D., AYRES, P. G., and WHITTAKER, J. B. 1994. Interactions between *Rumex* spp., herbivores and a rust fungus: *Gastrophysa viridula* grazing reduces subsequent infection by *Uromyces rumicis*. *Funct. Ecol* 8:265–272.

HATCHER, P. E., AYRES, P. G., and PAUL, N. D. 1995. The effect of natural and simulated insect herbivory, and leaf age, on the process of infection of *Rumex crispus* L. and *R. obtusifolius* L. by *Uromyces rumicis* (Schum.) Wint. *New Phytol* 130:239–249.

HAWKSWORTH, D. L. 1991. The fungal dimension of biodiversity: magnitude, significance, and conservation. *Mycol. Res* 95:641–655.

HAYNES, K. F., and MILLAR, J. G. 1998. Methods in Chemical Ecology, Vol. 2. Chapman and Hall, New York.

JOHNSON, S. N., DOUGLAS, A. E., WOODWARD, S., and HARTLEY, S. E. 2003. Microbial impacts on plant–herbivore interactions: the indirect effects of a birch pathogen on a birch aphid. *Oecologia* 134:388–396.

KARBAN, R., and MYERS, J. H. 1989. Induced plant responses to herbivory. *Annu. Rev. Ecol. Syst* 20:331–348.

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

KARBAN, R., ADAMCHAK, R., and SCHNATHORST, W. C. 1987. Induced resistance and interspecific competition between spider mites and a vascular wilt fungus. *Science* 235:678–679.

KRUESS, A. 2002. Indirect interaction between a fungal plant pathogen and a herbivorous beetle of the weed *Cirsium arvense*. *Oecologia* 130:563–569.

LEE, K. P., BEHMER, S. T., SIMPSON, S. J., and RAUBENHEIMER, D. 2002. A geometric analysis of nutrient regulation in the generalist caterpillar *Spodoptera littoralis* (Boisduval). *J. Insect Physiol* 48:655–665.

LIAO, L. H. 2003. Nutritional adaptation of galling insects investigated by globular galls of *Trioza shuiliensis* (Yang) on *Machilus japonica* var. *kusanoi* (Hyata) Liao. MS thesis. Department of Entomology, National Chung Hsing University, Taiwan.

MADSEN, J. D. 1997. Seasonal biomass and carbohydrate allocation in a southern population of *Eurasian watermilfoil*. *J. Aquat. Plant Manage* 35:15–21.

MALECK, K., and DIETRICH, R. A. 1999. Defense on multiple fronts: how do plants cope with diverse enemies? *Trends Plant Sci* 4:215–219.

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.

MCKENZIE, C. L., SHATTERS, R. G. Jr., DOOSTDAR, H., LEE, S. D., INBAR, M., and MAYER, R. T. 2002. Effect of geminivirus infection and *Bemisia* infection on accumulation of pathogenesis-related proteins in tomato. *Arch. Insect Biochem. Physiol* 49:203–214.

MILLER, A. R., and KELLEY, T. J. 1989. Mechanical stress induces peroxidase activity in cucumber fruit. *HortScience* 24:650–652.

MORAN, P. J. 1998. Plant-mediated interactions between insects and a fungal plant pathogen and the role of plant chemical responses to infection. *Oecologia* 115:523–530.

PACKARD, G. C., and BOARDMAN, T. J. 1999. The use of percentages and size-specific indices to normalize physiological data for variation in body size: wasted time, wasted effort? *Comp. Biochem. Physiol* 122:37–44.

PAUL, N. D., HATCHER, P. E., and TAYLOR, J. E. 2000. Coping with multiple enemies: an integration of molecular and ecological perspectives. *Trends Plant Sci* 5:220–225.

RAUBENHEIMER, D. 1995. Problems with ratio analysis in nutritional studies. *Funct. Ecol* 9:21–29.

RAUBENHEIMER, D., and SIMPSON, S. J. 1992. Analysis of covariance: an alternative to nutritional indices. *Entomol. Exp. Appl* 62:221–231.

RAUBENHEIMER, D., and SIMPSON, S. J. 2003. Nutrient balancing in grasshoppers: behavioural and physiological correlates of dietary breadth. *J. Exp. Biol* 206:1669–1681.

REED, W., and PAWAR, C. S. 1982. Heliothis: a global problem, pp. 9–14, in W. Reed and V. Kumble (eds.). Proceedings of the International Workshop on Heliothis Management. Pantanchera, India, ICRISAT.

ROSTÁS, M., and HILKER, M. 2002. Asymmetric plant-mediated cross-effects between a herbivorous insect and a phytopathogenic fungus. *Agric. For. Entomol* 4:223–231.

ROSTÁS, M., and HILKER, M. 2003. Indirect interactions between a phytopathogenic and an entomopathogenic fungus. *Naturwissenschaften* 90:63–67.

ROSTÁS, M., BENNETT, R., and HILKER, M. 2002. Comparative physiological responses in Chinese cabbage induced by herbivory and fungal infection. *J. Chem. Ecol* 28:2449–2463.

ROSTÁS, M., SIMON, M., and HILKER, M. 2003. Ecological cross-effects of induced plant responses toward herbivores and phytopathogenic fungi. *Basic Appl. Ecol* 4:43–62.

RYAN, C. A., GREGORY, P., and TINGEY, W. 1982. Phenolic oxidase activities in glandular trichomes of *Solanum berthaultii*. *Phytochemistry* 21:1885–1887.

SAS Institute 1999. User's Manual, Version 8.0. SAS Institute, Cary, NC.

SCHOONHOVEN, L. M., JERMY, T., and LOON, J. J. A. 1998. Insect–Plant Biology. Chapman and Hall, New York.

SIEMENS, D. H., and MITCHELL-OLDS, T. 1996. Glucosinolates and herbivory by specialists (Coleoptera: Chrysomelidae, Lepidoptera: Plutellidae): Consequences of concentration and induced resistance. *Environ. Entomol* 25:2344–2353.

SIMPSON, S. J., and RAUBENHEIMER, D. 2001. The geometric analysis of nutrient–allelochemical interactions: a case study using locusts. *Ecology* 82:422–439.

STOUT, M. J., and DUFFEY, S. S. 1996. Characterization of induced resistance in tomato plants. *Entomol. Exp. Appl* 79:273–283.

STOUT, M. J., FIDANTSEF, A. L., and BOSTOCK, R. M. 1999. Signal interactions in pathogen and insect attack: systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. *Physiol. Mol. Plant Pathol* 54:115–130.

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

STOUT, M. J., THALER, J. S., and THOMMA, B. 2006. Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. *Annu. Rev. Entomol* 51:1663–1689.

SVALHEIM, O., and ROBERTSEN, B. 1990. Induction of cucumber hypocotyls by wounding and fungal infection. *Physiol. Plant* 78:261–267.

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* 25:1597–1608.

THOMPSON, S. N., REDAK, R. A., and WANG, L. W. 2005. Nutrition interacts with parasitism to influence growth and physiology of the insect *Manduca sexta* L. *J. Exp. Biol* 208:611–623.

UNDERWOOD, N. 1999. The influence of induced plant resistance on herbivore population dynamics, pp. 211–229, in A. A. Agrawal, S. Tuzun, and E. Bent (eds.). Induced Plant Defenses against Pathogens and HerbivoresAPS, St. Paul, MN, USA.

WALDBAUER, G. P. 1968. The consumption and utilization of food by insect. *Adv. Insect Physiol* 5:229–288.

WITTSTOCK, U., and GERSHENZON, J. 2002. Constitutive plant toxins and their role in defense against herbivores and pathogens. *Curr. Opin. Plant Biol* 5:300–307.