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THE ROLE OF PLANT RAPIDLY INDUCED RESPONSES IN ASYMMETRIC INTERSPECIFIC INTERACTIONS AMONG INSECT HERBIVORES[1]

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Abstract—The role of induced responses of tomato, *Lycopersicon esculentum*, in interspecific interactions between two polyphagous herbivores, the silverleaf whitefly, *Bemisia argentifolii* (WF), and the vegetable leafminer, *Liriomyza trifolii* (LM), was characterized in laboratory and field experiments. Feeding by LMs and WFs induced local and systemic production of putative defensive proteins, i.e., chitinases, peroxidases, β-1,3-glucanases, and lysozymes. The magnitude of the induction for each defensive protein varied between species. Unlike WFs, LMs caused a 33% local reduction in total foliar protein content. In a whole-plant choice experiment, adult LM feeding, oviposition, and larval survival were reduced by 47.7%, 30.7%, and 26.5%, respectively, for the WF-infested host compared with the controls. Early WF infestations also had negative systemic (plant-mediated) effects on LMs. Adult LMs preferred leaves from control plants to leaves of plants that had been previously infested with WFs; no reciprocal effect of LMs on WFs were found. Feeding by *Helicoverpa zea* larvae, which has been shown previously to affect LM performance, had no effect on WF survival and development. LM natural population dynamics were monitored on WF-preinfested and control plants in a field experiment. WF-infested plants were less suitable for LM development with an overall 41% reduction in LM population density. These results demonstrate asymmetric direct and plant-mediated interspecific interactions between generalist herbivores feeding simultaneously on the

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same host. Possible mechanisms by which WFs overcome plant defenses are suggested. This ability may also contribute to WF success that makes them a major pest worldwide. The study supports the idea that over an evolutionary time scale, herbivores sharing the same host plant will automatically compete.

Key Words—Herbivory, induced response, interspecific interactions, leafminers, pathogenesis related proteins, plant defense, tomato, whiteflies.

INTRODUCTION

The importance of interspecific competition affecting populations of insect herbivores has drawn much attention and debate among ecologists (e.g., Strong et al., 1984; Denno et al., 1995). Janzen (1973) suggested that, considering the evolutionary time scale, insect herbivores sharing the same host plant (resource) will automatically compete. Thus, in addition to direct interference and exploitative interactions among sympatric herbivores, interactions should include insects that do not interact directly due to spatial and/or temporal separation, i.e., indirect effects mediated by the host plant (Strauss, 1991; Wootton, 1994). There are few mechanisms by which different herbivorous insects may indirectly affect each other via the host plant. Changes in plant primary and secondary metabolites induced by an insect may alter nutritional quality and palatability, increase toxicity, and alter anatomy, phenology, and physiology of the host plant. These changes may affect other herbivores (Haukioja, 1990; Masters et al., 1993; Tallamy and Raupp, 1991; Inbar et al., 1995). Price et al. (1980) proposed that indirect competition among herbivores may act via the third trophic level, the natural enemies. Natural enemies may be attracted to chemical and physical signals generated in damaged plants following insect herbivory.

Induced responses of host plants refer to biochemical, physiological, and developmental changes in plants following stimuli originating from abiotic and biotic factors. It is now well established that some of the components of herbivore-induced responses are defensive and can negatively affect insect herbivore populations (Karban and Myers, 1989; Tallamy and Raupp, 1991; Karban and Baldwin, 1997; Fowler and Lawton, 1985). It is important to note that host-plant-induced responses are not necessarily negative (induced resistance) and could actually result in induced susceptibility for herbivory (Danell et al., 1985; Karban and Baldwin, 1997; Martinsen et al., 1998). Timing of plant responses and insect phenology are important in that they may determine herbivore fate. Preformed-induced responses are immediate but are locally restricted around the damaged areas. A rapidly induced response occurs within hours or days and may be localized around the damaged tissue or may be systemic in undamaged tissue. Delayed induced responses are changes in plants in the following season or year(s) (Baldwin, 1994). Here we shall refer to the induced response in infested

leaves as a local induced response; induced responses in noninfested leaves on infested plants will be referred to as a systemic response.

Many plant responses, especially to insect herbivory, are rather general (i.e., not species specific) and, therefore, may have a negative effect on the inducers themselves as well as on other herbivores (Baldwin, 1994; Karban and Myers, 1989; Stout and Duffey, 1996; Agrawal, 1998). We have examined how host-plant-induced responses to one herbivore change the host-plant suitability for another cofeeding insect herbivore while the inducer itself remains apparently unaffected by those changes.

The sympatric feeding of the silverleaf whitefly (WF), *Bemisia argentifolii* Bellows and Perring (Homoptera: Aleyrodidae), and the leafminer (LM), *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), on tomatoes, *Lycopersicon esculentum*, provides us with an excellent opportunity to address the role of induced host-plant responses in interspecific interactions among generalist herbivores. Both WFs and LMs are polyphagous and occasionally feed simultaneously on tomatoes. Feeding by LMs and WFs induces a variety of defensive phytochemicals including pathogenesis-related (PR) proteins (Stout et al., 1994; Mayer et al., 1996). PR proteins such as peroxidase, glucanase, and chitinase are induced in pathological or related situations including herbivory and are thought to have defensive roles in the plant (van Loon et al., 1994). Induced responses in tomatoes may effectively reduce insect herbivore performance (Stout et al., 1994; Stout and Duffey, 1996; Inbar et al., 1998). Several biological characteristics of the LM and WF limit the possibilities of direct interference between them and, consequently, emphasize the role of the host plant as a mediator of interspecific interactions. Adult and immature WF stages are phloem feeders that feed predominantly on the abaxial side of host-plant leaves. In contrast, adult LMs feed and oviposit almost entirely on the adaxial surface of the leaves. LM larvae mine the adaxial side of the leaves, feeding on the palisade and spongy mesophyll (Parrella, 1987). Larval mobility of both species is limited, reducing the chance of direct interference. WF nymphs are stationary, with limited movement during the first instar, the crawler. LM larval mobility is restricted to the mines. As far as we know, these species do not share common natural enemies.

Here we have addressed the following questions:

1. Do WFs and LMs affect each other when feeding on the same host plant?
2. If WFs and LMs do affect each other, is the effect mediated by the plant (localized and/or systemic)?
3. If interspecific interactions are found, do they correlate with the induction of plant biochemical responses (e.g., PR proteins) and foliar protein levels?
4. Do these interspecific interactions alter herbivore population dynamics in the field?

METHODS AND MATERIALS

Host Plants and Insect Culture. Tomato (*Lycopersicon esculentum* cv. Agriset) plants were 4–5 weeks old and grown in 5.7-cm pots with Metro Mix 500 growing medium (Grace, Sierra, California). All plants were initially treated with a fungicide (0.4 g/liter; Bayleton, Bayer Corp., Kansas City, Missouri) and fertilized weekly. WFs were from a colony of *B. argentifolii* that was maintained in a greenhouse on collards and tomatoes. Lack of external symptoms on the plants indicated that the colony was apparently free of pathogenic viruses. The LMs used in this study were from a colony initiated in 1983 and maintained on cowpea, *Vigna sinensis* (Stickm.).

Preinfestation Protocol. When experiments required early insect infestation, plants were placed in the LM or WF colonies for three days. Subsequently, all adult insects were removed from the plants by shaking and aspiration; no attempt was made to remove the immature stages. LM preinfestation resulted in 0.43 ± 0.08 mines/cm^2. WF preinfestation resulted in 6.1 ± 1.9 nymphs/cm^2. After removal of adult insects, preinfested plants were transferred to a different greenhouse together with the control (insect-free) plants. Immature stages were allowed to develop on the preinfested plants for 14 days (LM, reached the pupal stage), and 20 days (WF, reached the fourth instar). This was sufficient time for these insects to induce host-plant biochemical responses (Stout et al., 1994; Mayer et al., 1996), and it eliminated the problem of having adults (from the preinfested generation) in the experimental systems. By the end of the preinfestation period, each plant had six fully expanded, infested leaves (with mines or WF nymphs) and an additional one to three unexpanded leaves. Control plants had similar numbers of leaves that were insect-free.

Effect of WFs on LM Preference and Performance. Twenty-four randomly assigned pairs of control and WF-preinfested plants were each placed in buckets (15.2-liter capacity; one pair/bucket) sealed with cellophane. Five male and five female newly emerged adult LMs were introduced into each bucket with an aspirator. Adult LMs were allowed to feed and oviposit for 24 hr and then removed with an aspirator. Each plant was placed separately in a plastic dish to allow convenient collection of the developing LM larvae that fell off the leaves 12 days later (to pupate). Adult LM host preference was measured by counting the number of oviposition and feeding punctures per leaf and per square centimeter. The oldest leaf was designated leaf 1, the second from the bottom as leaf 2, etc. Collected puparia were considered as individuals that successfully completed their development. Thus, larval survival rates were calculated by dividing the number of puparia by the number of egg punctures. The data were analyzed by the paired comparison t test on square root transformed data.

Effect of LM Preinfestation of WFs Oviposition Preference. Control and LM-preinfested plants ($N = 20$ each) were placed in the greenhouse with the WF

colony on a 1-m-high bench in a complete randomized design. After three days, WF eggs were counted on two leaf disks (2 cm^2) sampled from the terminal trifoliate of all leaves (1–9) by using a stereomicroscope. Two-way ANOVA was used to test the effect of LM preinfestations on WF host preference, with preinfestation and leaf position as main effects.

Systemic Effect of Early WF Infestation on Sequential LM Preference and Performance. Gauze sleeves were sealed around the top two leaves (7 and 8) and the apical bud. Half the plants were preinfested with WFs. After removal of the adult preinfesting WFs, the sleeves were removed, and the control and experimental plants were placed together for an additional 20 days. Subsequently, all six bottom leaves were removed with a razor blade to prevent any direct interspecific interactions. The control plants were treated similarly; all plants had the three top WF-free, fully expanded leaves (7, 8, and unfolded 9). Leaf excising might weaken herbivore systemic effects, but it was deemed necessary to eliminate any direct interactions including pheromonal agents. Recent studies demonstrated that artificial damage (such as leaf clipping) causes less induction compared with that produced by herbivory, probably because of the reduced actual leaf area damaged and the effects of insect saliva (Alborn et al., 1997; Agrawal, 1998, and references therein). Furthermore, excising tomato leaflets did not overwhelm the induced responses to herbivores (Stout and Duffey, 1996). Control and WF-infested plants were randomly divided into 24 pairs and challenged with adult LMs in buckets as described earlier (see WF effect on LM at whole plant level).

Effects of Induced Systemic Response by Corn Earworm on WF Performance. Stout and Duffey (1996) demonstrated that induced responses of tomato foliage following feeding damage by larvae of corn earworm, *Helicoverpa zea* Boddie, have a negative effect on subsequent herbivores (LMs and the beet armyworm, *Spodoptera exigua*). The most pronounced effects were documented when systemic induction was tested at the leaf level, i.e., different leaflets within the same leaf (Stout et al., 1994; Stout and Duffey, 1996). Since we did not find any effect of LMs on WFs in the whole plant experiment (see Results), we repeated part of Stout and Duffey's (1996) experiment to determine whether or not WF fitness would be affected by a strong induced response that results from *H. zea* feeding. A single second instar of *H. zea* (obtained from the USDA, ARS, CMAVE, Gainesville, Florida) was restricted to the terminal leaflet of leaf 3 of 4-week-old plants with five leaves ($N = 20$) in clip cages (2.9 cm diameter). Larvae were allowed to feed for 24 hr and then removed. The control plants ($N = 20$) had empty clip cages placed identically for 24 hr. After an additional 48 hr, the terminal leaflet was excised with a razor blade. Ten adult WFs were allowed to oviposit for 1 hr (and then removed) to similar clip cages on the ventral side of a leaflet located two leaflets down from the terminal leaflet. WF performance was determined after four weeks. Opened nymphal cases (used as an indicator of adult WF emergence) and red-eyed nymphs (fourth instar) were counted.

Phytochemical Analyses. Another batch of controls and LM- and WF-preinfested plants that were treated similarly ($N = 15$ each) were used to detect local and systemic induction of PR proteins. The top two leaves (7 and 8) were covered with gauze sleeves to prevent LM feeding and oviposition; control plants were treated likewise. Leaves 4 and 7 of each plant were used for biochemical analyses as represented for local and systemic responses.

Fresh samples were weighed prior to protein extraction. Leaf samples were crushed with an electric roller grinder (Ravenel Specialties Co., Seneca, South Carolina). The extracts were washed from the rollers with 20 ml of 0.1 M sodium phosphate (pH 7.4) into tubes containing 0.6 g of hydrated polyvinylpolypyrrolidone (Sigma Chemical Co., St. Louis, Missouri). The tubes were capped and mixed for 30 min at 4°C. The samples were centrifuged at 20,000*g* for 15 min. The supernatant was filtered through a layer of Miracloth (Calbiochem, La Jolla, California) into dialysis tubing (Spectrum, Laguna Hills, California) with a molecular weight cutoff of 6000–8000 Da. The samples were dialyzed overnight in distilled water and subsequently lyophilized. The dried samples were resuspended in 3 ml of water and centrifuged for 10 min at 10,000*g*. The resulting supernatant was used for analyses. Total protein, chitinase, peroxidase, β-1,3-glucanase, and lysozyme levels were measured as described previously (Mayer et al., 1996). These proteins were selected for their potential defensive role against herbivores.

We calculated ANOVA in a split plot design with preinfestation as the main plot and leaf position on the plant as the subplot to test the local and systemic induction of PR proteins by LMs and WFs. Although this design sacrifices precision in estimating the main plot effects, it improves comparison of the subplot (systemic) effects and provides a tool to examine the interaction between the main effects. Since systemic and local responses are different (see Results and Stout et al., 1996), a mean separation (LSD) test was conducted for each protein and each leaf at positions 4 and 7 (Little and Hills, 1978). Each enzyme for LMs and WFs was tested separately in the SAS CD statistical package (SAS Institute, 1988). All data on enzyme activities were transformed by $\log_{10}(x + 1)$ before analysis.

Field Experiment. Control and WF-preinfested plants were transplanted to field plots at the University of Florida, Indian River Research and Education Center, Fort Pierce, Florida. Raised beds 15 cm high × 1.06 m wide were spaced at 2.13-m bed centers. Plants were set 0.6 m apart in the center of each bed. Beds were covered with black polyethylene mulch and watered with subsurface irrigation. Plots, each with 10 plants, were replicated seven times in a completely randomized design. The plants were not treated with any pesticides at any time. Preinfested plants contained WF nymphs and eggs when transplanted (see preinfestation protocol). WF and LM natural infestations and movements between plants were not interfered with after transplanting.

The terminal trifoliate of the seventh leaf from the top (a different leaf was sampled every week) of all plants was sampled weekly to determine insect population densities. The number of mines on the adaxial surface of the terminal leaflets represented LM density (Schuster and Beck, 1992). The number of WF nymphs on the entire abaxial surface of the same leaflet was used to monitor WF density. Leaflet area was measured after counting with a leaf area meter (LI 3000, Lambda Instruments Corp., Lincoln, Nebraska). Data were analyzed by a repeated measurement ANOVA with date and preinfestation as the main effects. All sets of data of LM and WF eggs, nymphs, and adults were square-root-transformed before analysis. This trial examines the overall effect in the field and does not distinguish between direct and plant-mediated mechanisms.

RESULTS

Effect of Early WF Infestations on LM Preference and Performance. Adult LM females preferred the control plants to WF-preinfested plants (Table 1). WF preinfestations resulted in a 30.6% reduction in the number of eggs laid per plant, which was almost twofold lower on an area comparison. WF preinfestations also reduced the number of LM feeding events and the number of leaves and leaflets used by LMs for feeding and oviposition compared with the control plants (Table 1). The survival of LM larvae on the control plants was 26.5% higher than on WF-preinfested plants (Table 1). Almost no WF nymphs were observed on the youngest unfolded leaves (7–9) of the preinfested plants. Regardless of the presence of WFs, LM female activities (Figure 1) were concentrated in the middle-position leaves of the plants. Peak feeding activity of adult LMs on the control plants was on leaf 4, while on the WF-preinfested plants these activities peaked

TABLE 1. EFFECT OF WF PREINFESTATIONS ON ADULT LM PREFERENCES AND LARVAL PERFORMANCE[a]

Variable	Control plants	WF-infested plants	Paired t
Eggs/plant	18.6 ± 2.7	12.9 ± 4.4	2.32*
Eggs/cm^2	0.52 ± 0.07	0.36 ± 0.09	2.01*
Feedings/plant	58.8 ± 14.4	30.7 ± 14.1	2.34*
Leaves with eggs	2.9 ± 0.3	2.3 ± 0.4	1.7 NS
Leaflets with eggs	7.0 ± 0.9	4.4 ± 1.2	2.6*
Leaves used for feeding (N)	3.04 ± 0.32	2.0 ± 0.25	3.2**
Leaflets used for feeding (N)	8.1 ± 1.2	3.9 ± 0.6	3.8**
Larval survival (%)	33.9 ± 4	34.9 ± 4.1	2.41*

[a]Values are the means ± SE, $N = 24$ (pairs). NS = not significant; *$P < 0.05$, **$P < 0.01$.

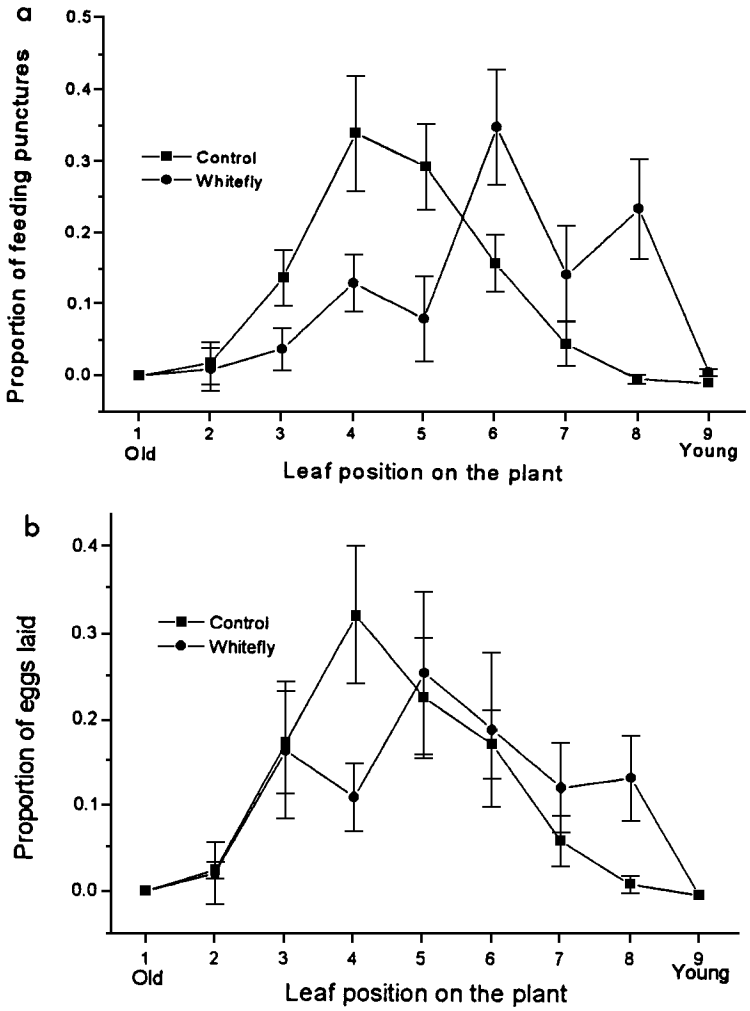

FIG. 1. Distribution of LMs among leaves of WF-preinfested and control plants in the whole plant experiment: (a) proportion of leafminer feeding, and (b) oviposition punctures. Total numbers of LM preference are given in Table 1. Values are means ± SE.

more distally on leaf 6 (Figure 1a). The distribution of oviposition was similar on both host plants (Figure 1b).

Effect of LM Preinfestation on WFs Oviposition Preference. Since LM activity is concentrated in the middle, fully expanded leaves of the plant, most of the preinfested mines were located on leaves 3–5. Two-way ANOVA indi-

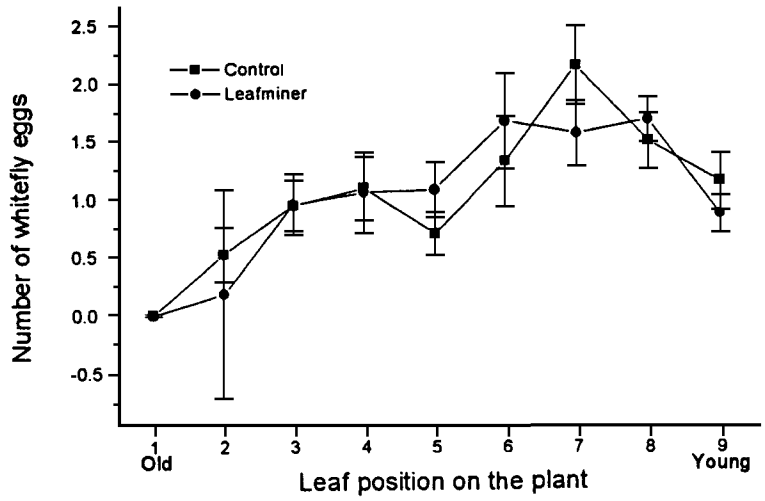

FIG. 2. Effect of LM preinfestation on WF oviposition preference. Young leaves (7–9) of the LM-preinfested plants were mine-free. Values are mean numbers of eggs per square centimeter ± Se.

cated that LM preinfestations did not affect WF adult oviposition ($F_{1,342} = 0.45$, $P = 0.53$, Figure 2). WFs preferred to oviposit on younger (6–8) rather than on older leaves, regardless of LM preinfestation ($F_{8,342} = 14.34$, $P \ll 0.01$, Figure 2). Consequently, there were no LM × leaf position interactive effects on WF preference ($F_{8,342} = 1.24$, $P = 0.27$). Within the LM-preinfested plants, we found no correlation between the number of mines and the number of WF eggs ($r = 0.12$, $P = 0.66$; data not shown). These results indicate that WF host selection was not influenced by LM preinfestation.

Systemic Effect of Early WF Infestation on Sequential LM Preference and Performance. Significant reductions in LM oviposition (24%) and feeding (27%) incidents were found on leaf 7 on the WF-preinfested plants (Table 2). Although we found a 25% reduction of LM survival on leaf 7 of the WF-preinfested plants, the data were not significant. The systemic effect on WFs on LMs was found to be limited to the proximal noninfested leaf only. No significant effects were detected on leaf 8 located two leaves above the WF-preinfested leaves.

Effects of Induced Systemic Response by Corn Earworm Caterpillars on WF Performance. The mean number of WF eggs laid on the control (13.1 ± 1.9) and *H. zea*-fed leaves (12.3 ± 2.95) was similar (Mann-Whitney U test, $Z = 0.907$, $P = 0.36$). On both the experimental and control plants, more than 98% of the eggs hatched. WF nymph development was not affected by systemic responses caused by prior feeding of *H. zea* (Figure 3).

TABLE 2. SYSTEMIC EFFECTS OF WF PREINFESTATION ON LM PREFERENCE AND PERFORMANCE[a]

	Eggs/cm^2	Feed/cm^2	Larval survival
Leaf 7			
Control	0.37 ± 0.04	0.77 ± 0.06	0.29 ± 0.03
WF	0.28 ± 0.04	0.56 ± 0.04	0.16 ± 0.04
Paired t	2.23*	2.32*	0.98 NS
Leaf 8			
Control	0.31 ± 0.04	0.56 ± 0.03	0.13 ± 0.04
WF	0.26 ± 0.04	0.51 ± 0.05	0.17 ± 0.07
Paired t	1.08 NS	0.89 NS	0.33 NS

[a]Since the last leaf along the shoot axis that was preinfested was leaf 6, any effects detected on leaves 7 and 8 were systemic. Values are the means ± SE and the results of the paired t test (N = 24 pairs). NS = not significant, $^*P < 0.05$.

Phytochemical Analyses. Overall, infestation of both species induced higher levels of PR proteins. Irrespective of the species of herbivore, induced and constitutive levels of PR proteins were higher in old leaves (leaf 4) compared with young ones (Tables 3 and 4).

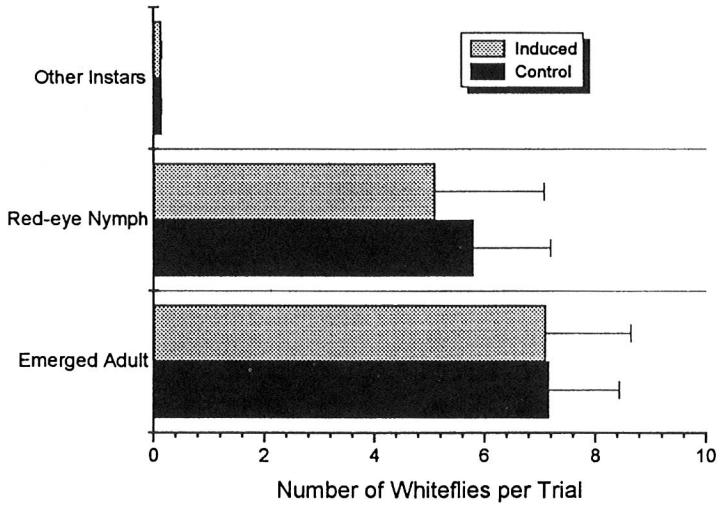

FIG. 3. Effect of induced plant systemic response to *H. zea* larval feeding on WF survival and development. Red-eye nymphs (pupa) are the last stage (fourth instar) in WF development. Empty nymphal cases indicate that an individual successfully completed its development and emerged as an adult. Other instars are individuals that remain as eggs or as instars 1–3. Values are means ± SE.

TABLE 3. EFFECTS OF WF PREINFESTATION ON LOCAL AND SYSTEMIC INDUCTION OF PR PROTEINS IN TOMATOES[a]

Treatment	Total protein (mg/g tissue)	ΔA_{510}/min/g tissue			β-1,3-glucanase (mmol/min/g tissue)
		Peroxidase	Lysozyme	Chitinase	
Local (leaf 4)					
Control	0.90 ± 0.06a	21.27 ± 1.49a	328.83 ± 34.41a	3.89 ± 0.19a	0.95 ± 0.17a
WFs	1.11 ± 0.14a	22.92 ± 1.98a	425.36 ± 46.78b	4.31 ± 0.16b	2.22 ± 0.16b
Systemic (leaf 7)					
Control	1.54 ± 0.13a	9.87 ± 1.96a	81.80 ± 26.62a	1.94 ± 0.25a	0.21 ± 0.03a
WFs	1.27 ± 0.11b	19.92 ± 1.39b	295.92 ± 24.12b	4.19 ± 0.22b	0.81 ± 0.11a
			F value		
Split plot ANOVA, source of variation					
Treatment (WF)	0.18 NS	14.81***	32.94***	31.21***	57.51***
Leaf position	28.41***	25.21***	45.42***	39.46***	91.44***
Treatment × position	66.75***	26.30***	18.58***	43.29***	0.71 NS

[a]Leaf 4 (in the treated plants) was preinfested with WFs and thus represents local induction. Leaf 7 was not exposed to WFs at any time and thus reflects systemic induction. Values are the means ± SE (N = 15). The results of the split-plot ANOVA test (F values) for each enzyme are given at the bottom. Similar letters within pairs indicate nonsignificant mean separation (LSD). NS = not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

TABLE 4. EFFECTS OF LM PREINFESTATION ON LOCAL AND SYSTEMIC INDUCTION OF PR PROTEINS IN TOMATOES[a]

Treatment	Protein (mg/g tissue)	ΔA_{510}/min/g tissue			β-1,3-glucanase (mmol/min/g tissue)
		Peroxidase	Lysozyme	Chitinase	
Local (leaf 4)					
Control	3.01 ± 0.31a	33.93 ± 9.59a	107.23 ± 108.5a	3.69 ± 1.33a	0.21 ± 0.21a
WFs	1.98 ± 0.68b	67.25 ± 2.77b	422.36 ± 633.4b	4.62 ± 1.83a	0.41 ± 0.48a
Systemic (leaf 7)					
Control	0.45 ± 0.12a	9.78 ± 3.16a	87.8 ± 43.38a	1.49 ± 0.39a	0.56 ± 0.16a
WFs	0.58 ± 0.27a	8.51 ± 8.36a	65.6 ± 42.83a	2.70 ± 1.03b	1.06 ± 0.47b
	F value				
Split plot ANOVA, source of variation					
Treatment (LM)	12.01***	2.31 NS	4.75*	6.56*	10.77**
Leaf position	174.15***	68.27***	6.85*	26.08***	37.93***
Treatment × position	13.34***	11.51***	8.8**	NS	0.51 NS

[a]Leaf 4 (in the treated plants) was preinfested with LMs and thus represents local induction. Leaf 7 was not exposed to LMs at any time and thus reflects systemic induction. Values are the means ± SE (N = 15). The results of the split-plot ANOVA test (F values) for each enzyme are given at the bottom. Similar letters within pairs indicate nonsignificant mean separation (LSD). NS = not significant, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Local and Systemic Response of Tomatoes to WFs. WFs feeding on tomato leaves induced higher levels of PR proteins locally and systemically on noninfested young leaves (Table 3). The levels of β-1,3-glucanase, chitinase, peroxidase, and lysozyme were increased 1.5- to 3-fold in the infested leaves (leaf 4) compared with the control leaves. Local induction of peroxidase was not statistically significant. All PR proteins that were measured after WF feeding increased systemically in young noninfested leaves (i.e., leaf 7); however, the systemic induction β-1,3-glucanase was not significant. Total protein levels were not significantly changed either locally or systemically (Table 3).

Local and Systemic Response of Tomatoes to LMs. As was found with WFs, LM feeding locally induced higher levels of the PR proteins, although only peroxidase and lysozyme were significant (Table 4). Only β-1,3-glucanase and chitinase were induced systemically in the young uninfested leaves (increased by approximately 50%). LM larvae that consumed the leaf mesophyll locally reduced foliar protein content by 37%, but the systemic effect was not significant.

Field Experiments. Differences in WF densities between control and WF-preinfested plants persisted for three weeks after transplanting and declined rapidly in week 4 ($F_{1,96} = 131.1$, $P < 0.01$; Figure 4a). At four weeks, leaves 7 from the top no longer reflected the preinfestation period since they had expanded entirely after that period. From week 4 to the end of the experiment, WF populations on both control and experimental plants were low, with fewer than 0.1 nymphs/cm^2. Natural field infestations of LMs occurred quickly. Small mines were found one week after transplanting. LM density on control plants peaked two weeks after transplanting. Significantly lower LM densities were observed for weeks 2–6 after transplanting in the WF-preinfested plants compared to controls ($F_{1,96} = 23.62$, $P < 0.01$). No significant differences were found in LM densities between the control and WF-preinfested plants after week 6 (Figure 4b). From week 4, the new leaves currently sampled (leaf 7 from top) had similar (and negligible) WF densities, while preinfested plants still had large WF populations on their basal old leaves.

DISCUSSION

Strong asymmetric relationships (amensalistic) were detected between WFs and LMs when they were feeding simultaneously on the same host plant. The effects of WFs on LMs were both direct (interference or exploitation interactions) and systemic. However, the systemic effect was restricted only to leaves located proximally to WF infestations. WF and LM feeding induces higher levels of PR proteins in tomato, although they differ in the magnitude of the local and systemic response for each enzyme. High WF infestations in the field resulted in the reduction of LM populations during young plant stages (two to six weeks after transplanting).

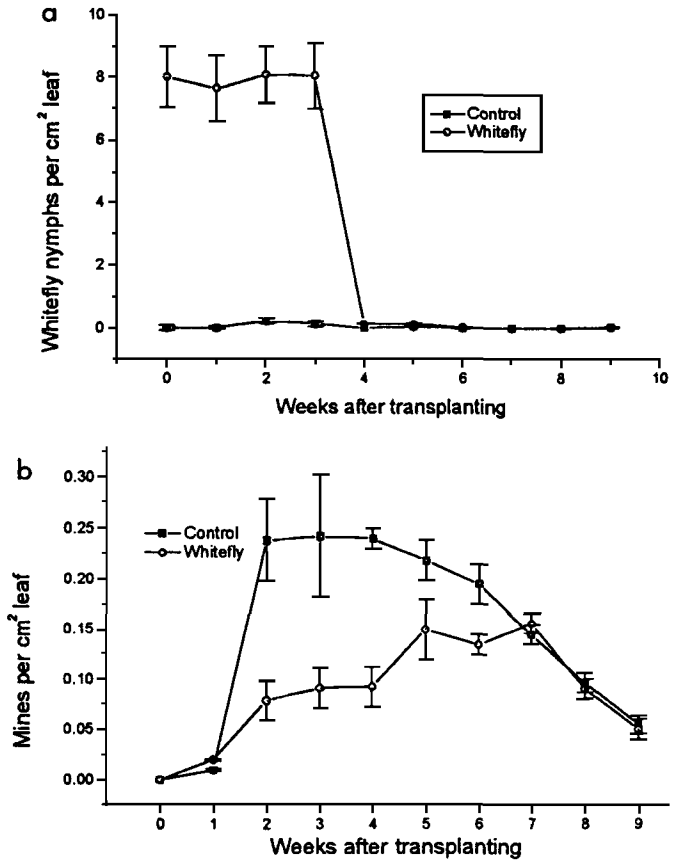

FIG. 4. (a) Field populations of WF nymphs on WF-preinfested and control plants. Values are means ± SE. (b) Effect of WF preinfestation on LM population dynamics in the field. Values are means ± SE.

Since induced plant responses to herbivory are thought to be general (Baldwin, 1994; Karban et al., 1987), most studies that examined systemic (rapid) induced responses reported negative effects on the inducing individual(s) or other conspecific and allospecific herbivores that feed simultaneously or shortly after (Hougen-Eitzman and Karban, 1995; Karban and Myers, 1989; Zangerl, 1990; Kogan and Fischer, 1991; Jones et al., 1993; Stout and Duffey, 1996). Therefore, the ability to use local and systemic induced responses to reduce competition between generalist insects feeding simultaneously on the same plant is probably constrained by the general negative effect of the host-plant responses. These effects can be avoided when the induced response is delayed, relative to the life-

span of the insect causing the induction. In this case, qualitative and quantitative changes take place after the insect has completed its development. Indeed, some of the most compelling evidence of induced-response-mediated asymmetrical interactions comes from species that are temporally separated (Faeth, 1986; Harrison and Karban, 1986; Haukioja, 1990). WFs appear to be able to overcome rapidly induced responses in tomatoes. This ability was observed regardless of source of induction, LMs or *H. zea* and it is in sharp contrast to other studies that reported that insect herbivores are severely affected by tomato-induced responses (Edwards et al., 1986; Duffey and Felton, 1991; Stout and Duffey, 1996).

Both LMs and WFs can induce local and systemic responses that differ in strength. Similarly, Stout et al. (1994) found that the ability of LMs to induce tomato responses was quantitatively mild compared with russet mites or *H. zea*. Differences in insect-specific abilities to induce host-plant responses have been found in several systems (Hartley and Lawton, 1991; Felton et al., 1994; Stout et al., 1994). We suggest that in our system the asymmetric relationship between WFs and LMs may stem from their differential ability to overcome plant defenses rather than their differential ability to induce specific responses. This conclusion is based on several pieces of evidence. First, the induced response triggered by *H. zea* feeding in tomatoes that was found to be harmful to LMs and leaf-chewing caterpillars (Stout and Duffey, 1996) did not affect WF survival and development. Second, young tomato leaves are also highly protected by constitutive chemicals, resulting in diminished herbivory on terminal leaves (Wilkens et al., 1996). In contrast, WFs are unaffected by these constitutive defenses and prefer young unfolded tomato leaves (Liu and Stansly, 1995). Finally, exogenous application of elicitors to tomatoes induced responses that lowered LM populations, but failed to affect WFs (Inbar et al., 1998). Nevertheless, differences in tomato-induced responses did vary between species, and we did not measure other possible defensive compounds (e.g., proteinase inhibitors and polyphenol oxidase). Thus, the role of specific tomato responses to each insect may also determine the outcome of the observed interaction.

How do WFs overcome tomato-induced responses? One explanation could be provided by the physiological traits used to detoxify plant defensive compounds. Additional explanations may be related to the insect feeding habits. WFs are phloem feeders (Cohen et al., 1996), while LM larvae feed in the palisade mesophyll (Parrella, 1987). Other insects that were found to be affected by induced responses in tomatoes are mainly leaf-chewing lepidopterans. It has long been suggested that phloem feeders are less exposed to plant chemical defense systems. Many plant toxins and other defensive compounds, including proteinase inhibitors in tomatoes (Walker-Simmons and Ryan, 1977), are thought to be stored in intracytoplasmic vacuoles in parenchyma and epidermal cells (McKey, 1979; Mullin, 1986; Berenbaum, 1991; Rosenheim et al., 1996). Vascular tissues should not be considered defenseless, but it is possible that fewer defensive com-

pounds and possibly their lower concentrations in the vascular system are less effective against phloem (and xylem) feeders (Rosenheim et al., 1996). Recently, Denno et al. (1995) recognized the importance of feeding guilds in insect herbivore competition. They found that leafminers are less successful when feeding on previously induced plants. However, they did not find clear evidence that sap feeders in general prevail in interguild interactions.

Tomato resistance is based on a complex of defensive systems that includes PR proteins, proteinase inhibitors, polyphenol oxidases, and phytoalexins (Duffey and Stout, 1996). The levels of several PR proteins that probably affect insect fitness were induced by WFs both locally and systemically. Chitinase degrades chitin and can consequently damage chitin-based structures such as the peritrophic membrane, which provides a physical barrier to ingested pathogens and other substances that pose a hazard to the insect. Chitinase activity may interfere with insect development, feeding, and growth; facilitate microbial infection; and finally cause death (e.g., Wang et al., 1996). Peroxidases are involved in production and polymerization of phenolics, lignification, and hypersensitive responses, thus affecting food digestibility and protein availability to herbivores (Bowles, 1990; Duffey and Stout, 1996). Peroxidase and chitinase genes have been introduced into several crops in an attempt to create transgenic insect-resistant plants (Carozzi and Koziel, 1997). Lysozymes and β-1,3-glucanases are defensive enzymes that protect plants against bacterial pathogens. Ingested lysozymes may affect insect symbiotic flora and interfere with insect digestion. Because WFs are phloem feeders, they may create a nutritional sink that diverts nutrients from neighboring leaves (Inbar et al., 1995) resulting in a reduction in LM performance. WFs also systemically reduce leaf photosynthetic efficiency (Inbar, Doostdar, and Mayer, unpublished data). However, total foliar proteins, which are important to LM development (Minkenberg and Ottenheim, 1990) were not significantly altered by WFs and, therefore, were not considered to be a major factor in the interactions observed.

Local effects of WFs on LMs were stronger than the systemic effects, which were temporally and spatially limited. The possible systemic effect of WFs lasted approximately two weeks and then disappeared in the field experiment, although the direct effects in the field have not been controlled. Similarly, in the greenhouse, WF systemic effects on LMs were pronounced near WF-infested leaves. Natural WF infestations cover most of a plant's leaves. Eggs are laid on the young upper leaves, and immature stages (nymphs and pupa) are distributed on middle and lower leaves (Liu and Stansly, 1995; Schuster, 1998). LMs, on the other hand, prefer mature middle leaves and do not shift to the young WF-free leaves (Minkenberg and Ottenheim, 1990). This is probably because of constitutive plant defense systems in young tomato leaves (e.g., soluble phenolics) (Wilkens et al., 1996) that had stronger effects on LM preference than the tomato-induced responses to WFs. Thus, the effects of WF natural infesta-

tions on LMs will be more detrimental and will involve direct and indirect (local and systemic) mechanisms, as were found in the whole plant experiment where LMs had no refuge leaves.

We believe that the effects reported in this study are more general, as a consequence of the keystone herbivore status (sensu Hunter, 1992) of WFs. Direct and plant-mediated mechanisms are involved in the negative effects of WF on cabbage loopers (*Trichoplusia ni*) when feeding on collards (Inbar et al., 1999). WFs are polyphagous pests that are found in many parts of the world, occasionally in high densities (Gerling and Mayer, 1996; Byrne and Bellows, 1991). There would be numerous occasions where they would share the same host plant and interact with other herbivores. The fate of the interactions between WFs and other herbivores will probably depend on the host plant, the mode of feeding of the potential competitor, WF density, and the timing of interactions.

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