Effects of Nutrient Supply on Citrus Resistance to Root Herbivory by *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae)

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ABSTRACT We treated two citrus cultivars with a complete fertilizer diluted to 25, 100, 200, or 400 ppm N to test whether increasing fertilizer concentration alters root and leaf chemistry and decreases resistance of citrus to root-feeding larvae of *Diaprepes abbreviatus* L. Roots and leaves of better-nourished 'sour orange' (*Citrus aurantium* L.) had larger amounts of total proteins and increased activities of enzymes associated with resistance than did plants given 25 ppm N. The fertilizer effect was less consistent for 'Swingle citrumelo' (*C. paradise* Macf. × *Poncirus trifoliate* L.), which has greater resistance to *D. abbreviatus*. Herbivory increased root protein content and peroxidase but decreased activities of chitinase and β-1,3-glucanase, which are enzymes associated with resistance to microbial pathogens. When significant, the effect of root herbivory on enzyme activities in leaves was opposite the effects on roots. Fertilizer and herbivory rarely interacted, indicating enzyme induction was not a function of nutrient supply. Fertilizer did not affect total phenolics in roots of either citrus, but root herbivory increased levels in 'sour orange'. Despite elevated levels of putative defense proteins, 'sour orange' given ≥100 ppm N produced 50% greater total larval mass per pot than did plants given 25 ppm N. Fertilizer concentration did not affect mass of larvae on 'Swingle citrumelo' roots and did not affect larval mortality for either citrus cultivar. Our results concerning a root herbivore are consistent with the body of studies of folivores that have demonstrated that increased fertilizer has no effect or increases herbivore performance.

KEY WORDS *Diaprepes abbreviatus*, nutrition, pathogenesis-related proteins, root weevil

A BURGEONING BODY OF literature indicates that increased soil nutrients generally have no effect on, or improve, performance of a wide variety of herbivores (reviewed by Kytö et al. 1996, Herms 2002). Despite the significance of roots for water and nutrient uptake and storage, few studies have examined interactions between woody plants and root herbivores (Kytö et al. 1996, Hunter 2001, Blossey and Hunt-Joshi 2003). It is not clear whether increased soil nutrient supply increases performance of tree root herbivores and how root herbivory and nutrient supply interact to affect plant chemistry.

Insect herbivores are nourished by some plant proteins and deterred by others, and variation in nutrient supply to plants may affect the balance between nutritional versus resistance properties of plant tissue (Mattson 1980). Proteins associated with resistance include "pathogenesis-related" proteins, which are induced by pathological conditions including pathogen infection and insect herbivory (Joosten and DeWit 1989, Mayer et al. 1995, McCollum et al. 1995, Sticher et al. 1997, Inbar et al. 1998, Datta and Muthukrishnan 1999, Van Loon and Van Strien 1999). Elevated activities of these proteins have been associated with reduced disease progression and insect performance (Thaler et al. 1996, Inbar et al. 1998, Cipollini and Redman 1999). The focus of this study is on how production of several resistance-related compounds varies with nutrient supply and whether variation in such chemicals affects larval growth of the root weevil, *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae).

Diaprepes abbreviatus larvae feed on roots of >40 plant species and constitute a major threat to agronomic crops (Simpson et al. 1996). After hatching from eggs deposited on leaves, larvae drop to the ground and feed on roots. Young trees or heavily infested mature trees can die after girdling of the root crown by larvae. Lower levels of infestation by root weevils reduce citrus growth and predispose roots to invasion by opportunistic pathogens, especially *Phytophthora* spp. (Rogers et al. 1996). Currently *D. abbreviatus* larvae are considered the primary pest of citrus in Florida, causing production losses estimated

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to be $75 million annually (Diaprepes Task Force 1997).

Soil-inhabiting insects such as *D. abbreviatus* are particularly difficult to control with insecticides. Innovative pest and phytopathogen control includes manipulation of host plant chemical defenses by means of genetic engineering and application of chemical elicitors that induce plant defenses including peroxidases and other pathogenesis-related proteins such as chitinases and β-1,3-glucanase (McCollum et al. 1995). Peroxidases cause lignification and act on phenolics to cause denaturation and precipitation of dietary protein, thus reducing the nutritive value of plant tissues (Duffey and Felton 1989). Citrus chitinases affect fungal pathogens and have been shown to degrade peritrophic membranes of *D. abbreviatus* (Mayer et al. 1995). Increased chitinase activity from increased fertilizer or induction by larval feeding could reduce digestive efficiency and increase vulnerability of larvae to pathogens. β-1,3-Glucanase has no known effects on insects but may act synergistically with chitinase to inhibit fungal growth (Leah et al. 1991, Melchers et al. 1993). If *D. abbreviatus* larvae affect production of β-1,3-glucanase, they may influence vulnerability of citrus to pathogens.

In an experiment in which citrus seedlings were each infested with10 *D. abbreviatus* larvae, Mayer et al. (1995) showed that larval feeding increased chitinase activities in roots of three cultivars, including 'Swingle citrumelo' (*C. paradisi* Macf. × *Poncirus trifoliata* L.) and 'sour orange' (*Citrus aurantium* L.) but reduced β-1,3-glucanase activities regardless of cultivar. Larvae gained significantly less mass after 6 wk of feeding on 'Swingle citrumelo' than on 'sour orange', but mortality of larvae did not differ among the eight cultivars tested (Shapiro and Gottwald 1995). These experiments demonstrate that some pathogenesis-related proteins can be induced by root herbivory, but cultivars vary in response to and resistance to *D. abbreviatus* larvae. Thus, manipulation of pathogenesis-related proteins could aid in pest control in citrus. However, before manipulation of pathogenesis-related proteins can be an effective pest control, it is important to understand how plant-pest interactions vary over a range of environmental conditions (Bostock 1999). Here we extend our understanding of the interaction between *D. abbreviatus* and citrus by examining how nutrient supply to plants and root herbivory by larvae interact to affect herbivore performance and root and leaf chemistry in 'sour orange' and 'Swingle citrumelo'.

Materials and Methods

'Sour orange' and 'Swingle citrumelo' were examined in separate experiments. Because of the logistics of our research program, the 'Swingle citrumelo' experiment commenced 5 wk after the start of the 'sour orange' experiment and followed the same procedures with the exceptions noted below. For both experiments, 3-yr-old trees grown from seed in U.S. Horticultural Research Laboratory greenhouses were transplanted to 3.75-liter pots lined with nylon screen and containing steamed sand. In each experiment, plants were randomly assigned to fertilizer treatment (four levels) and to root weevil treatment (0 versus 10 larvae).

Fertilizer Treatment. Plants were fully wetted to field capacity five or six times per week with Plantex (20–10-20 N-P-K), a complete citrus fertilizer that was diluted to yield 25, 100, 200, or 400 ppm N. Based on the manufacturer's recommended concentration of 200 ppm N for average conditions, fertilizer levels were intended to span a range of nutritional conditions from significant stress to excess fertilization. Because of differences in growth, the volume of fertilizer required to achieve field capacity varied across time and among fertilizer treatments. Pots were flushed with tap water once a week to prevent fertilizer build up and for the final 2 d before harvest to minimize contamination of root tissue with fertilizer. By the time insect treatment commenced, 8 wk after initiation of fertilizer treatments, plants receiving the lowest concentration had little new shoot growth and were visibly pale compared with better-nourished plants.

Insect Treatment. *Diaprepes abbreviatus* larvae were obtained from a colony reared on artificial media at the U.S. Horticultural Research Laboratory (Fort Pierce, FL). In each experiment, 10 larvae were placed singly into 10-cm-deep holes located 5 cm from the trunk of randomly assigned plants. Control plants were handled identically but without addition of larvae. Each hole was brushed closed, and every pot was gently watered. For the 'sour orange' experiment, larvae were sorted into five mass categories that ranged from 10 to 30 mg. Two larvae from each of the five mass categories were added to each randomly assigned pot. We anticipated greater variability among plants treated with root weevils and therefore assigned more plants to this treatment. Seven replicate 'sour orange' plants from each fertilizer treatment received larvae. Six plants receiving 25 ppm N, six plants receiving 100 ppm N, five plants receiving 200 ppm N, and five plants receiving 400 ppm N served as controls for root weevil grazing, giving a total of 50 plants in this experiment. Because we had more available, a total of 63 'Swingle citrumelo' plants were used in the second experiment. Six plants from each fertilizer level received no larvae. Root weevil larvae were added to 11 pots receiving 25 ppm N, 10 pots receiving 100 ppm N, 10 pots receiving 200 ppm N, and 8 pots receiving 400 ppm N. This distribution of replicates was caused by mortality associated with transplanting and happened well before addition of root weevils. To have sufficient numbers of larvae for the larger number of root weevil-treated pots in the 'Swingle citrumelo' experiment, we extended the size range. Two larvae from each of four size categories between 10 and 29.9 mg and one each from 30 to 34.9 mg and 35 to 40 mg were added to each pot assigned the root weevil treatment. All treatments were randomly assigned to plants.

Because growth conditions vary throughout greenhouses, a blocking variable was used to account for

temporal and spatial heterogeneity and to increase statistical power (Potvin 2001). One or more plants from each treatment was assigned to each of three blocks for the 'sour orange' experiment. Four blocks were used for the Swingle experiment. Plants assigned to the same block were grouped together within the greenhouse, received larvae on the same day, and were harvested on the same day.

Harvest. Based on results of Shapiro and Gottwald (1995), we harvested plants 7 wk after addition of larvae to ensure damage to infested plants and to allow for significant growth of larvae. The number of larvae found in each pot was recorded, and each larva was weighed. Samples of young (not fully expanded) and mature (fully expanded) leaves were stored at −80°C for later analysis. We analyzed mature and immature leaves separately because root proteins change with leaf age (McCollum et al. 1995), and *D. abbreviatus* adults avoid mature citrus leaves (Fennah 1942). Because plants had been pot-bound before transplanting, there was a clear demarcation between older roots and fine roots produced during this experiment. These attached fine roots were severed from the older roots, washed, patted dry, and weighed. Samples were stored at −80°C for biochemical analyses.

Sample Preparation. Frozen leaves (≈1 g fresh weight) were extracted with an electric roller press (Ravenel Specialties Co., Seneca, SC). Extracts were washed from the rollers with ice-cold extraction buffer (0.1 M sodium phosphate buffer, pH 7.4) into tubes containing 0.6 g hydrated polyvinylpolypyrrolidone (PVPP; Sigma, St. Louis, MO) to give a final volume of 20 ml. PVPP binds with alkaloids and phenolics to prevent them from modifying proteins (Gegenheimer 1990). Samples were mixed for at least 30 min at 4°C. Roots were ground in liquid N_2 using an Omni-mixer (OCI Instruments, Waterbury, CT). One gram of the resulting powder was suspended in 20 ml ice-cold extraction buffer containing 0.6 g hydrated PVPP and mixed overnight at 4°C. Leaf and root samples were centrifuged at 20,000 × *g* for 15 min, and the supernatants were filtered through one layer of Miracloth (Calbiochem, La Jolla, CA) into dialysis tubing (Spectrum, Laguna Hills, CA) with a molecular cutoff of 6000–8000 da. Samples were dialyzed overnight against deionized water at 4°C and lyophilized. Lyophilized samples were resuspended in water (1 ml/g fresh tissue) and centrifuged (10,000 × *g*) for 10 min. Supernatants were used for analyses.

Biochemical Analyses. Total protein was measured according to Bradford (1976) using bovine serum albumin (fraction V) as the standard. Chitinase activities were measured colorimetrically at 550 nm using soluble dye-labeled chitin (CK-Chitin-RBV; Loewe Biochemica, Munich, Germany) as the substrate (Wirth and Wolf 1990) and expressed as ΔA_{550}/min/g tissue. Reactions were conducted in sodium acetate buffer (pH 5.0) for 10 min at 37°C. β-1,3-Glucanase activities were determined by measuring the rate of reducing sugar production using laminarin (*Laminaria digitata;* Sigma) as the substrate and glucose (Glc) as the standard (Abeles and Forrence 1970, Mayer et al. 1995). Reactions were conducted in sodium acetate buffer (pH 5.0) at 50°C, and activities were expressed as micromoles Glc per minute per gram of tissue. Peroxidase activities were measured as described in the Worthington Enzyme Manual (Worthington Biochemical 1978). Reactions were conducted in potassium phosphate buffer (pH 7.0) for 4 min at room temperature using 4-aminoantipyrine as the hydrogen donor and were expressed as ΔA_{510}/min/mg tissue. Total phenolics were determined using a modified version of the Folin-Ciocalteu method (Waterman and Mole 1994), using tannic acid as the standard, and expressed as milligrams tannic acid equivalents per gram root.

Statistical Analyses. Statistical analysis followed recommendations from Scheiner and Gurevitch (2001). Sets of response variables were analyzed by multivariate analysis of variance (MANOVA) with block, which accounted for potential spatial and temporal effects as a random factor and root weevil treatment, fertilizer concentration, and their interaction as fixed experimental factors. MANOVA allowed us to examine the magnitude and direction of response to experimental factors for protein determinations and for larval survival and total mass. Significant MANOVA tests were followed by multiple comparisons of means (Tukey procedure; Neeter and Wasserman 1974).

Unidentified mites infested the 'Swingle citrumelo' plants toward the end of the experiment, and all plants were treated with insecticidal soap 5–8 d before harvest. Before treating plants with insecticide, mite abundance was estimated by ranking each plant from 0 (no mites) to 4 (mites and webbing over >50%), and the rank was used as a covariate in analyses of 'Swingle citrumelo' data. Initial analyses showed that the covariate did not interact with any main effect. Subsequently, all interactions involving mite abundance were dropped, and MANOVA and follow-up analyses were performed.

Results

Root Proteins. Fertilizer increased the amounts of total proteins and the activities of all three enzymes in 'sour orange' and 'Swingle citrumelo' roots, although chitinase in 'Swingle citrumelo' roots increased only at intermediate concentrations of fertilizer (Figs. 1 and 2). The increase in enzyme activities relative to total protein varied among enzymes (compare fertilizer standardized canonical coefficients in Table 1). The overall effect of fertilizer in 'sour orange' was mostly caused by the response of peroxidase (Table 1), which increased across all levels of fertilizer (Fig. 1). For 'Swingle citrumelo', β-1,3-glucanase contributed most to the fertilizer effect (Table 1).

Root weevil feeding increased total proteins, but the direction and magnitude of feeding effects varied among the defensive enzymes (Table 1; Figs. 1 and 2). Feeding depressed expression of β-1,3-glucanase and chitinase but increased peroxidase in 'sour orange' and 'Swingle citrumelo' roots (Figs. 1 and 2). For 'Swingle citrumelo', a significant interaction between *D. ab-*

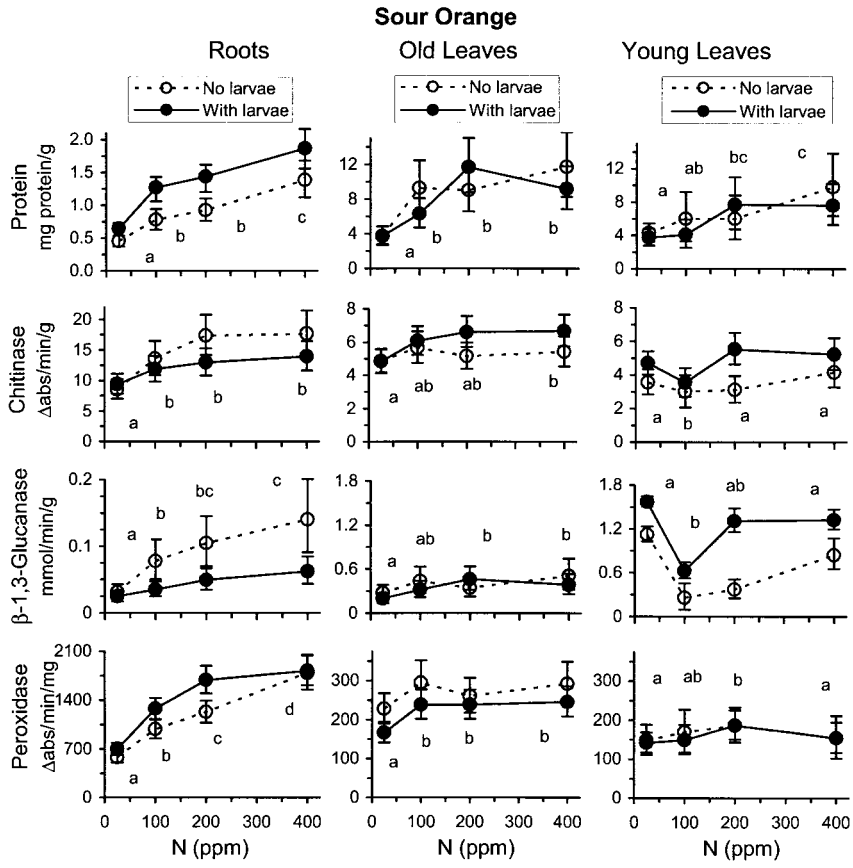

Fig. 1. Effects of fertilizer concentration and herbivory by *D. abbreviatus* larvae on total protein and on chitinase, β-1,3-glucanase, and peroxidase activities in roots, old leaves, and young leaves of 'sour orange' plants. Back-transformed least squares means (±2 SEM) are shown. Letters refer to means for fertilizer averaged across herbivory treatment; means with the same letter do not differ significantly.

breviatus and fertilizer in the MANOVA stems from opposite effects of herbivory at the midrange of fertilizer for total proteins versus β-1,3-glucanase (Table 1; Fig. 2). Mite abundance did not influence the protein assays of roots (Table 1).

Foliar Proteins. Similar to roots, fertilizer significantly affected the pattern of proteins in old (mature, fully expanded) leaves (Table 2; Figs. 1 and 2). For both 'sour orange' and 'Swingle citrumelo', significant differences were primarily caused by change in total proteins (Table 2), suggesting that increased activity of enzymes was mainly a product of increased total proteins. The fertilizer effect was also significant in MANOVA for young (immature, not fully expanded) leaves (Table 3), but the pattern was less consistent, especially for individual enzyme activities (Figs. 1 and 2). The significant effect in 'Swingle citrumelo' was mainly caused by an increase in total proteins, whereas β-1,3-glucanase contributed most strongly to the pattern of proteins in 'sour orange' leaves (Table 3). Although total proteins increased with increasing fertilizer concentration in both rootstocks, β-1,3-glucanase activity was significantly lower at 100 than at 25 or 400 ppm N in young 'sour orange' leaves (Fig. 1).

Feeding by root weevil larvae strongly influenced the overall pattern of investment in proteins in old and young 'sour orange' leaves and in old 'Swingle citrumelo' leaves (Tables 2 and 3). In 'sour orange', the significant herbivore effect was primarily caused by effects on total proteins and chitinase in old leaves (Table 2) and chitinase in young leaves (Table 3). In 'Swingle citrumelo', chitinase and β-1,3-glucanase contributed strongly to the pattern of proteins in old leaves (Table 2), whereas total protein was the dominant factor for young leaves (Table 3). When herbivory affected resistance-related proteins, the effect was opposite that observed for roots (Figs. 1 and 2). In 'sour orange', feeding increased chitinase activities in old and young leaves, increased β-1,3-glucanase in young leaves, and decreased peroxidase in mature leaves (Fig. 1). For 'Swingle citrumelo', the effect of root weevil treatment was limited to enhanced activity of β-1,3-glucanase in old and young leaves (Tables 2 and 3).

Mite abundance did not influence any of the enzyme activities of 'Swingle citrumelo' older leaves (Table 2). Mite abundance had a marginal effect on the overall responses of proteins in young 'Swingle

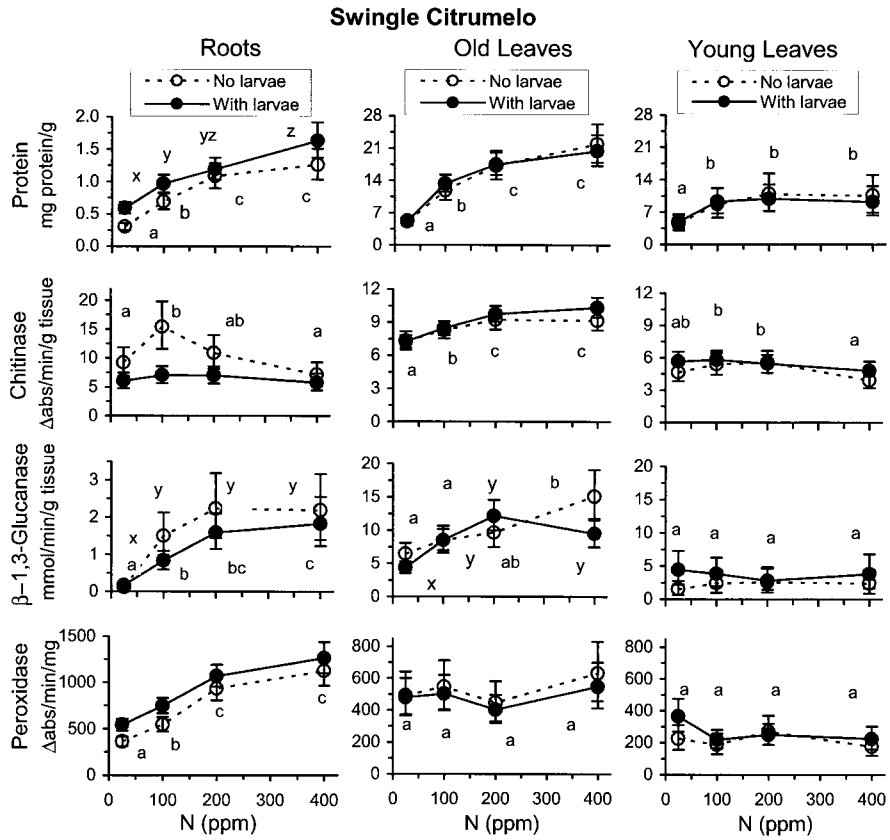

Fig. 2. Effects of fertilizer and root herbivory by *D. abbreviatus* on total protein and on chitinase, β-1,3-glucanase, and peroxidase activities in roots, old leaves, and young leaves of 'Swingle citrumelo' plants. Back-transformed least squares means (±2 SEM) are shown. Letters refer to means for fertilizer averaged across herbivory treatment unless a significant interaction required tests to be performed within a level of herbivory treatment. Fertilizer levels with the same letter do not differ significantly.

citrumelo' leaves, with chitinase and β-1,3-glucanase contributing most to the effect (Table 3).

Root Phenolics. Fertilizer significantly affected total phenolics in 'sour orange' roots ($F_{3,40} = 4.30$, $P = 0.0102$), but the effect was not consistent across levels. Only plants given 100 versus 400 ppm N fertilizer differed significantly (backtransformed mean [upper, lower SEM]: 25 ppm N = 674.5 [36.7, 34.8], 100 ppm N = 608.1 [34.5, 32.7], 200 ppm N = 732.8 [39.9, 37.8], 400 ppm N = 796.2 [45.2, 42.8]). Root weevil feeding elevated total phenolics across all levels of fertilizer ($F_{1,40} = 8.98$, $P = 0.0047$; backtransformed mean [upper, lower SEM]: no larvae = 644.2 [27.3, 26.2], with larvae = 758.6 [28.5, 27.4]).

Table 1. MANOVA results of sour orange and Swingle citrumelo root proteins from plants fertilized with one of four concentrations of fertilizer and grown with or without *D. abbreviatus* larvae

Source	Pillai's trace			Standardized canonical coefficients			
	df	*F*	*P*	Protein	Chitinase	β-1,3-Glucanase	Peroxidase
				Sour orange			
Block	8, 76	1.11	0.3685	−1.4810	−0.7921	−0.6473	3.7909
Herbivory (H)	4, 37	27.16	0.0001	−2.3425	0.3942	1.2289	0.1498
Fertilizer (F)	12, 117	5.15	0.0001	−0.3753	−0.1295	0.3838	3.0393
H × F	12, 117	1.52	0.1260	−0.4723	1.0692	0.5473	−2.0843
				Swingle citrumelo			
Block	12, 150	1.43	0.1561	−1.3875	−1.1388	1.3674	2.4267
Herbivory (H)	4, 48	22.40	0.0001	0.9916	−0.3490	−1.9769	2.3376
Fertilizer (F)	12, 150	13.17	0.0001	0.4284	−0.6191	2.3166	0.3742
Mite rank	4, 48	1.39	0.2511	0.9836	−0.4040	−1.8781	2.2642
H × F	12, 150	2.35	0.0086	2.1875	0.6529	1.6036	−2.4351

Table 2. MANOVA results of proteins in old (mature fully expanded) leaves from sour orange and Swingle citrumelo plants fertilized with one of four concentrations of fertilizer and grown with or without *D. abbreviatus* root weevil larvae

Source	Pillai's trace			Standardized canonical coefficients			
	df	*F*	*P*	Protein	Chitinase	β-1,3-Glucanase	Peroxidase
				Sour orange			
Block	8, 76	1.52	0.1655	−2.3188	−1.2057	1.2693	0.7038
Herbivory (H)	4, 37	7.31	0.0002	−1.3254	−1.2491	0.7055	1.0908
Fertilizer (F)	12, 117	3.98	0.0001	2.2847	1.0689	−0.9244	−0.0865
H × F	12, 117	1.12	0.3515	2.0650	1.0371	−0.4351	−0.4351
				Swingle citrumelo			
Block	12, 150	1.73	0.0663	−1.0801	−1.0195	1.9151	−0.2863
Herbivory (H)	4, 48	3.79	0.0093	0.0795	−1.6162	1.7614	0.3730
Fertilizer (F)	12, 150	6.64	0.0001	2.8792	−0.2808	0.2512	0.0521
Mite rank	4, 48	0.73	0.5732	0.5879	0.7532	0.8639	−0.5276
H × F	12, 150	1.95	0.0330	0.5225	−1.3635	1.9879	0.0910

Total phenolics in 'Swingle citrumelo' roots were not significantly affected by fertilizer ($F_{3,48} = 1.73, P = 0.1742$). Total phenolics were also not affected by root weevil treatment ($F_{1,48} = 2.90$, $P = 0.0951$; Fig. 3) in contrast to the results with 'sour orange'.

Larval Performance. Mortality of root weevil larvae, determined by counting the number of larvae remaining from the initial 10 added to each pot, was ≈50% across all fertilizer levels and was unaffected by soil fertility (Table 4; Fig. 3). Total mass of larvae per pot increased significantly with fertilizer for 'sour orange', but the top three levels did not differ among themselves (Fig. 3). Thus the most nutrient-stressed plants supported the smallest mass of larvae. We did not examine mean larval mass because it is not independent of total mass and percentage of survival. However, a significant Pillai's trace (Table 4) and large, positive canonical coefficient for total mass paired with a smaller, negative canonical coefficient for survival indicates that increased fertilizer is associated with increased average mass of larvae.

Fertilizer did not influence insect performance on 'Swingle citrumelo' rootstocks (Table 4), although inspection of Fig. 3 suggests a tendency toward reduced mass of larvae reared on the most nutrient-stressed plants. Mite abundance was not significant in the MANOVA of insect performance (Table 4) but was marginally significant in univariate ANOVA of total weight of larvae ($F_{1,31} = 4.28$, $P = 0.047$). The significant mite effect on larval mass indicates that mites contributed to the variance in the plant-insect interactions.

Mite Abundance. Using our ranking of mite abundance as a response variable, we examined the effects of root weevil and fertilizer treatments on mite abundance. Root weevils did not significantly affect mite abundance ($F_{1,52} = 0.30$, $P = 0.5873$). However, mite abundance increased significantly with fertilizer ($F_{3,52} = 7.13$, $P = 0.0004$), with abundance at 400 ppm N significantly greater than abundance at either 25 or 100 ppm N ($P < 0.05$).

Discussion

Insect performance on 'sour orange', measured as total mass of larvae per pot, exhibited a similar trend to many studies of primarily folivorous insects (Kytö et al. 1996, Herms 2002): performance increased with improved host-plant nutrition. Here, insects gained the most mass when raised on plants that had high concentrations of total proteins *and* high activities of resistance-related enzyme activities in roots. Thus, protein concentration seems to have been an important determinant of larval growth, and a decline in insect performance with increased defense chemistry was not observed. Total protein of grazed roots ex-

Table 3. MANOVA results of proteins in young (immature not fully expanded) leaves from sour orange and Swingle citrumelo plants fertilized with one of four concentrations of fertilizer and grown with or without *D. abbreviatus* root weevil larvae

Source	Pillai's trace			Standardized canonical coefficients			
	df	*F*	*P*	Protein	Chitinase	β-1,3-Glucanase	Peroxidase
				Sour orange			
Block	8, 66	0.60	0.7716	0.0099	−1.1960	1.6190	−0.0807
Herbivory (H)	4, 32	14.32	0.0001	−0.2795	−1.7443	0.0935	−0.5274
Fertilizer (F)	12, 102	8.02	0.0001	1.1812	0.9465	−1.4841	0.0269
H × F	12, 102	1.27	0.2496	0.7043	1.7963	−0.5548	−0.3077
				Swingle citrumelo			
Block	12, 150	0.72	0.7321	1.0781	0.7929	0.1230	−0.8527
Herbivory (H)	4, 48	2.56	0.0507	−1.3156	0.2318	1.1293	0.2706
Fertilizer (F)	12, 150	7.42	0.0001	−1.5981	0.4309	−0.4022	1.0377
Mite rank	4, 48	2.07	0.0999	−0.0947	0.6118	0.7981	−0.1781
H × F	12, 150	0.69	0.7575	−1.1258	1.3705	−0.6868	0.7094

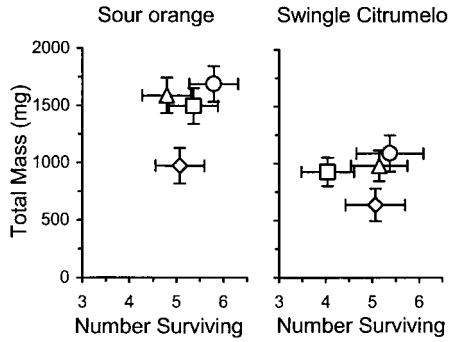

Fig. 3. Number of surviving *D. abbreviatus* larvae per pot and total mass of surviving larvae per pot raised on 'sour orange' and 'Swingle citrumelo' plants given different fertilizer treatments. Each potted plant was initially challenged with 10 larvae. Least squares means ± 1 SEM are presented. Diamond, 25 ppm N; square, 100 ppm N; triangle, 200 ppm N; circle, 400 ppm N.

hibited somewhat greater increase than did chitinase and β-1,3-glucanase activities, suggesting that nutrient quality of roots increased faster than did defense. However, peroxidase activities increased strongly across fertilizer levels and were especially high in roots grazed by weevils. Peroxidases oxidize phenolics in a chain of events that result in polymerization of proteins and loss of food value (Duffey and Felton 1989). Because phenolics did not increase consistently across fertilizer levels, peroxidase may have been substrate-limited at greater amounts of N, limiting its effects on proteins and growth of root weevil larvae.

Although larvae reared on better-nourished 'Swingle citrumelo' tended to grow larger, differences among fertilizer levels were not significant. There may be no significant pattern of nutrient supply and insect growth for this cultivar. However, significant effects of mite abundance on larval mass, and marginally, on plant chemistry suggest that variance caused by mite infestation may have obscured a significant pattern of insect performance.

Interestingly, mite abundance increased with fertilizer concentration. Thus, in our study, two different types of arthropods feeding on different plant parts with different modes of feeding exhibited the same general response to changes in plant quality. This positive response of mites is consistent with other studies that have demonstrated increased performance or damage by phytophagus mites with increased nitrogen application (Wilson 1994, Wood and Reilly 2000).

Insect herbivory may have varying effects on host plant defenses (Felton and Eichenseer 1999, Walling 2000). Induced resistance, suppression of jasmonate-mediated responses to insect herbivory, and induction of resistance to phytopathogens may result depending on the herbivore. Plant chitinases and β-1,3-glucanases inhibit microbial growth (Leah et al. 1991, Melchers et al. 1993). Thus, *D. abbreviatus* larvae constitute a triple threat to citrus: they reduce plant growth, open roots to invasion by opportunistic root pathogens, and reduce resistance to soil-borne pathogens through reduction of chitinase and glucanase activities. This may explain why citrus trees infested with both *Phytophthora* spp. and *D. abbreviatus* experience rapid decline (Rogers et al. 1996). Furthermore, because β-1,3-glucanase plays a role in plant growth and reproduction (Hinton and Pressey 1980), feeding by root weevil larvae may have as yet unknown effects on citrus physiology.

The effects of *D. abbreviatus* root herbivory on leaf chemistry may not be relevant to a commercial setting because most commercial citrus plantings use rootstocks with grafted scions. Molecular signals and/or messengers produced by *D. abbreviatus* larvae feeding on roots may or may not cross the graft to aerial portions of the tree. Even if signals do translocate, it is possible that a scion may not respond to a particular signal because of varietal differences, i.e., the receptor for the signal is not present. Nevertheless, when herbivory of roots significantly affected resistance-related chemistry of leaves the effect was opposite of the effect on roots: feeding elevated chitinase and β-1,3-glucanase and depressed peroxidase in leaves. Thus, feeding influences not only growth of shoots but may also alter resistance of leaves to pathogens and herbivores, including adult *D. abbreviatus.* Furthermore, if the reverse is true, i.e., induction of proteins in leaves reduces activities in roots, application of elicitors to elevate levels of proteins in leaves could have unintended consequences for biotic interactions involving roots. Evaluation of elicitor efficacy should include examination of nontarget tissues.

Brown and Gange (1990) observed that root-feeding insects have important effects on productivity of

Table 4. Results of MANOVA of *D. abbreviatus* larval survival and mass for sour orange and Swingle citrumelo plants fertilized with one of four concentrations of fertilizer

Source	Pillai's trace			Standardized canonical coefficients	
	df	*F*	*P*	Number	Mass
			Sour orange		
Block	4, 44	1.61	0.1890	−1.3008	1.3208
Fertilizer	6, 44	2.90	0.0181	−0.8815	1.5744
			Swingle citrumelo		
Block	6, 62	0.94	0.4715	0.3097	0.8091
Fertilizer	6, 62	1.65	0.1494	0.9364	−1.0632
Mite rank	2, 30	2.16	0.1330	−0.2332	1.1060

agricultural systems and the dynamics of natural systems, but also noted that studies of root herbivory are rare. Although the range of research on root herbivory has expanded (e.g., Ganade and Brown 1997, Coffin et al. 1998, Maron 1998, Strong et al. 1999, Gange 2001), studies on root herbivory are far fewer than studies of folivory (Kytö et al. 1996, Hunter 2001). Our research demonstrates that root herbivory and soil nutrition influence the defensive chemistry of citrus and helps explain why root weevil infestation makes citrus more vulnerable to root pathogens. Furthermore, our research shows that effects of root herbivory on root defensive chemistry may not parallel its effects on leaf chemistry, underscoring the need for research that integrates above- and belowground interactions (Van der Putten et al. 2001, Blossey and Hunt-Joshi 2003).

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