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Effects of nutrient supply and below-ground herbivory by *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae) on citrus growth and mineral content[☆]

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Abstract

Three-year-old citrus trees were grown in the greenhouse to study the effects of fertilizer concentration and root herbivory on plant growth and mineral concentration. In separate experiments, sour orange (*Citrus aurantium* L.) and Swingle citrumelo (*C. paradisi* Macf. × *Poncirus trifoliata* L.) plants were treated with a complete fertilizer diluted to provide 25, 100, 200, or 400 ppm N and grown for 7 weeks with or without *Diaprepes abbreviatus* L. larvae. Increased fertilizer concentration increased the shoot mass and the shoot:root ratio of both sour orange and Swingle citrumelo. Root herbivory also increased the shoot:root ratio by depressing root growth more than shoot growth. Effects of root herbivory on growth were consistent across the four levels of fertilizer concentration, indicating that tolerance is not a function of nutrient status. For both rootstocks, concentrations of nitrogen in roots and leaves increased with fertilizer concentration, and C:N ratios decreased. In sour orange, root herbivory most strongly affected the concentration of carbon in roots, whereas in Swingle citrumelo, root herbivory most strongly affected leaf nitrogen. In general, herbivory reduced mineral concentrations of roots but the strength, and sometimes the direction, of herbivore effects varied significantly among fertilizer treatments. This research indicates that application of excess, balanced fertilizer is unlikely to offset growth reductions due to root herbivory by *D. abbreviatus*, and suggests that supplementation of specific nutrients may be of value.

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Keywords: Root herbivory; Plant nutrition; Plant–herbivore interactions; C:N ratio

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1. Introduction

Although less studied than folivory, root herbivory significantly affects plants in both natural and managed systems (Brown and Gange, 1990; Blossey and Hunt-Joshi, 2003; Hunter, 2001). In addition to consuming stored photosynthates, root herbivores reduce surface area for water and nutrient uptake and open wounds for pathogen invasion of the host plant (Andersen, 1987; Brown and Gange, 1990; Blossey and Hunt-Joshi, 2003). Such damage by root-feeding herbivores can prompt a variety of responses in individual plants, including changes in physiology (Steinger and Müller-Schärer, 1992; Murray et al., 1996; Urias-Lopez et al., 2000), growth and storage (Karban, 1980; Murray et al., 1996; Morón-Ríos et al., 1997; Dunn and Frommelt, 1998; Nötzold et al., 1998), reproduction (Ganade and Brown, 1997; Maron, 1998, 2001), susceptibility to other insects and pathogens (Masters, 1995; Rogers et al., 1996), and mortality (Strong et al., 1995; Maron, 1998, 2001). The strength of these effects is variable and may depend on biotic and abiotic environmental factors (Dunn and Frommelt, 1998; Gange, 2001) and the temporal pattern and form of herbivory (Andersen, 1987).

Because plant nutrition affects how plants allocate resources to various functions, soil fertility can affect the ability of plants to compensate for herbivory (i.e., affect tolerance; Maschinski and Whitham, 1989; Strauss and Agrawal, 1999). Compared to well-nourished plants, nutrient-stressed plants may exhibit disproportionate reductions in growth due to herbivores (Fay et al., 1996; Houle, 1999). Nutrient deficiency or imbalances may also alter primary and secondary metabolism, and thus foster growth of herbivores (Beanland et al., 2003). In mixture modeling studies that manipulated proportions of minerals available to plants, herbivores performed best on plants grown with proportions of minerals that caused suboptimal plant growth (Busch and Phelan, 1999; Beanland et al., 2003).

In this study we held the proportions of minerals constant and varied the concentration of fertilizer delivered to young citrus to determine how fertilizer concentration and root herbivory affect growth and mineral concentrations of two citrus rootstock varieties. The herbivore, *Diaprepes abbreviatus* L. (*Diaprepes* root weevil), is the most important insect

pest of citrus in Florida (Diaprepes Task Force, 1997). Introduced in 1964, *D. abbreviatus* now causes annual losses in Florida estimated at \$ 75 million. These larvae attack roots of more than 40 plant species in 20 families and are considered a major long-term threat for many tropical and sub-tropical crops (Simpson et al., 1996). Larvae feed on bark of thicker roots and snip off thinner, nutrient-gathering roots. This damage reduces root surface area for water and nutrient uptake, induces defense-related proteins (Mayer et al., 1995; Borowicz et al., 2003), and opens wounds that promote invasion by opportunistic root pathogens, especially *Phytophthora* spp. (Rogers et al., 1996; Graham et al., 2003), which magnify the impact of *D. abbreviatus*.

Of the two rootstock varieties used in this study, sour orange (*Citrus aurantium* L.) is relatively susceptible to *D. abbreviatus*, and Swingle citrumelo (*C. paradisi* Macf. × *Poncirus trifoliata* L.) is more resistant (Shapiro and Gottwald, 1995). The results reported here are from a larger study that examined effects of fertilizer concentration on resistance to *D. abbreviatus* larvae (Borowicz et al., 2003). In that study, 3-year-old, well-nourished sour orange trees produced a greater mass of *D. abbreviatus* larvae compared to the most nutrient-stressed plants, and well-nourished sour orange and Swingle citrumelo plants had greater concentrations of total proteins and pathogenesis-related proteins than did severely nutrient-stressed plants. Here, we examine the effects of nutrient supply on growth of the young plants to determine whether or not host plant tolerance to root herbivory is a function of nutrient supply. We also performed mineral assays to determine how nutrient supply and herbivory of nutrient-gathering roots affect the carbon:nitrogen ratio and the balance of other minerals in citrus.

2. Methods

Three-year-old sour orange and Swingle citrumelo plants (approx. 60–100 cm tall) were grown from seed in U.S. Horticultural Research Laboratory greenhouses and were transplanted to 3.75 L pots lined with nylon screen and containing steamed sand. Plants of each rootstock were randomly assigned to fertilizer treatment (4 levels) and to root weevil treatment (0

versus 10 larvae). To allow for sufficient time to harvest plants, sour orange and Swingle citrumelo were examined in nearly identical, but separate experiments started 5 weeks apart.

2.1. Fertilizer treatment

Five or six times a week each plant was watered to field capacity with PlantexTM (20–10–20 N–P–K), a complete fertilizer that was diluted to yield 25, 100, 200, or 400 ppm N. The manufacturer recommends 200 ppm N for average conditions. These dilutions produced plants that ranged from significantly nutrient-stressed and visibly pale, to excessively fertilized. The volume of fertilizer required to achieve field capacity varied over time and among fertilizer treatments due to differences in plant growth. Each pot was flushed with tap water once a week to control fertilizer levels and once a day for two days immediately before harvest to minimize fertilizer contamination of roots.

2.2. Insect treatment

In the first experiment, seven sour orange plants from each fertilizer received *D. abbreviatus* larvae and five or six plants from each fertilizer level served as controls, giving a total of 50 plants. Of the sixty-three Swingle citrumelo plants in the second experiment, six from each fertilizer treatment received no larvae, and 8–11 plants from each fertilizer level received larvae. In each experiment, we assigned a greater number of plants to the *D. abbreviatus* treatment than to control because we anticipated greater variability among plants exposed to herbivory. In the Swingle citrumelo experiment, plant mortality following transplanting, but well before addition of larvae, led to unequal numbers of plants in the *D. abbreviatus* treatment.

Procedures for the insect treatment were based on Shapiro and Gottwald (1995). Ten root weevil larvae were added to pots assigned the root weevil treatment 8 weeks after fertilizer treatments commenced. Larvae were from the Fort Pierce, Florida, U.S. Horticultural Research Laboratory colony and had been reared on artificial media. Two larvae from each of five evenly spaced mass categories ranging from 10 to 30 mg were added to sour orange pots. Because the

larger number of plants required a greater total number of larvae, beginning larval mass in the Swingle citrumelo experiment spanned a slightly larger range, with the largest larva in each *D. abbreviatus*-treated pot between 35 and 40 mg. Each larva was placed in a 10-cm deep hole, 5 cm from the trunk covered with sand, and the pot was gently watered. Similar holes were also made and filled, followed by gentle watering, in pots of control plants.

Because light, temperature, and humidity vary within greenhouses, and because it took us several days to prepare and to harvest the experiments, we assigned plants from each treatment combination to blocks to increase statistical power (Potvin, 2001). There were three blocks in the sour orange experiment and four blocks in the Swingle citrumelo experiment. Plants within a block were arranged randomly on the greenhouse bench, inoculated with larvae at the same time, and were harvested simultaneously.

2.3. Harvest

Each experiment was terminated 7 weeks after larvae were added to pots. Due to the logistics of harvesting and the requirements of biochemical assays reported elsewhere (Borowicz et al., 2003), only fresh mass was taken for analyzing effects of treatments on plant growth. Plants were well hydrated at harvest. Fresh shoot mass consisted of the stems produced after the experiment was initiated and all leaves. After samples of immature and mature leaves were removed for biochemical assays the remaining leaves were pooled, dried at 60 °C, and ground. Fresh root mass included only roots that grew after the experiment commenced. The plants had been pot-bound before being transplanted, yielding a clear delineation between older versus newer roots. These newer, attached, fine roots were severed from the older roots, thoroughly rinsed in de-ionized water, patted dry, and weighed. Root weevil-treated pots also had detached fine roots that were included in the measurement of total root mass but not included in mineral assays. After weighing, the roots were frozen and later ground in liquid nitrogen. After samples were removed for biochemical assays the remaining tissue was dried at 60 °C.

2.4. Mineral assays

Due to the limited amount of dried material from some plants, several replicates were omitted from some assays. For each sample, 500 mg of dried plant tissue were digested with 10 ml concentrated nitric acid for 10 min under pressure at a reaction temperature of 170 °C following procedures outlined in U.S. EPA Method 3051A Revision 1 (1998). The digestion vessel was washed repeatedly with deionized distilled water and the wash water and digestate were combined and adjusted to 100 ml then filtered through Whatman No. 541 filter paper.

Root mineral concentrations were analyzed with a Thermo Jarrell Ash Iris 1000™ (Thermo Jarrell Ash Corp., Franklin, MA, USA) inductively coupled argon plasma (ICAP) emission spectrophotometer. Prior to any analysis, the ICAP was optimized according to manufacturer's instructions for each potential analytical wavelength using 50 ppm standards (SCP Science, Quebec, Canada) prepared with 10% nitric acid. After optimization, the system was calibrated using known standards. Calibration curves had a coefficient of determination (r^2) > 0.999 at the analytical wavelength and were verified by analyzing a midrange standard of all elements of interest prepared from stock solutions (Sigma Aldrich, St. Louis, MO, USA). These and other quality control procedures used in our analyses are outlined in U.S. EPA method 6010A (1997) and elsewhere in the SW-846 compendium. Digestate analysis by ICAP for the elements was conducted according to U.S. EPA method 6010A (1997). The mean of three sample readings served as the estimate of the concentration of each element analyzed.

Percentage carbon and nitrogen in root and leaf tissue were determined with a Thermoquest NC2100™ elemental analyzer (CE Elantech, Inc., Lakewood, NJ, USA) that was calibrated daily using pure acetanilide standards. Coefficients of determination (r^2) for the curves were always ≥ 0.999 . Calibration stability was assured by running a mid-range standard from the calibration standards bracketed by blanks after each curve and after every 10 tissue samples. Sample analysis was deemed acceptable if it was bracketed by calibration standards that were within 10% of the known concentrations of carbon and nitrogen in acetanilide. We also analyzed a

sample in duplicate in every group of 10 or fewer samples.

2.5. Statistical analyses

Sets of variables were analyzed by multivariate analysis of variance (MANOVA) using block as a random effect, and root weevil, fertilizer, and their interaction as fixed experimental factors. By considering the magnitude and direction of standardized canonical coefficients for sets of variables to experimental factors, MANOVA can reveal not only which variables are most important as contributors to effects but also correlations between variables across treatments (Scheiner, 2001). Because of the large number of minerals (15) included in the elemental analysis, principal components analysis was used to reduce the number of variables and to obtain uncorrelated variables. Principal components with eigenvalues >1 were retained for analysis. We analyzed the following sets of variables for sour orange and for Swingle citrumelo: (1) root and shoot mass; (2) root and leaf C and N; and (3) principal component (PC) scores for root minerals. The proportion of root mass detached by feeding was analyzed by ANOVA to determine whether fertilizer affected the intensity of feeding.

Swingle citrumelo became infested by unidentified mites near the end of the experiment. Mite abundance was estimated by scoring each plant from 0 (no mites) to 4 (mites and webbing over >50%). The pots and soil were then covered and the shoots were treated with Safer® (Safer, Inc., Newton, MA, USA), a contact insecticidal soap. When initial statistical analysis indicated that it was a significant effect, mite score was included as a covariate in analyses of Swingle citrumelo data. No interactions with mite score were significant and thus were not retained in any analyses.

3. Results

3.1. Citrus growth

Larval herbivory significantly reduced root and shoot mass for both sour orange and Swingle citrumelo but the effect was much greater on root

Table 1

Results of MANOVA of shoot and root mass of sour orange and Swingle citrumelo rootstocks fertilized with one of four concentrations of fertilizer and grown with or without *Diaprepes* root weevil larvae

Source	Pillai's trace		Standardized canonical coefficients	
	d.f.	P	Root	Shoot
Sour orange				
Block	4,80	0.0202	-0.4722	2.3472
Diaprepes (D)	2,39	0.0001	1.3365	0.6929
Fertilizer (F)	6,80	0.0001	-0.1723	2.3329
D × F	6,80	0.8765	1.4840	-1.2277
Swingle citrumelo				
Block	6,102	0.2583	0.2100	1.3952
Diaprepes (D)	2,50	0.0001	1.3929	-0.0196
Fertilizer (F)	6,102	0.0001	-0.8057	1.9413
Mite score	2,50	0.0551	-0.1944	1.6783
D × F	6,102	0.2226	1.7507	-1.0517

Data were log-transformed. Mite score describes mite abundance (0–4) on Swingle citrumelo plants and served as a covariate in these analyses.

mass (larger canonical coefficient, Table 1; Fig. 1). The proportion of root mass detached during larval feeding did not differ among fertilizer levels (sour orange: $F_{3,22} = 1.66$, $P = 0.2040$; Swingle citrumelo: $F_{3,32} = 1.38$, $P = 0.2657$), suggesting that feeding pressure was similar across levels of fertilizer.

Fertilizer significantly affected growth (significant MANOVA, Table 1) but this effect was almost entirely due to increased growth of shoots with increased fertilizer (larger canonical coefficients for shoot mass, Table 1; Fig. 2). Thus the shoot:root ratio increased with increased fertilizer concentration. Fertilizer level and root weevil treatment did not interact in either experiment (Table 1), indicating that nutrient supply

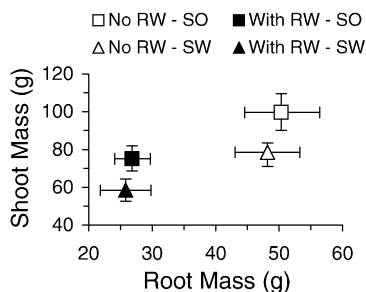


Fig. 1. Fresh mass of roots vs. shoots of sour orange (SO) and Swingle citrumelo (SW) undamaged or treated with root weevil larvae. Back-transformed least squares means (± 2 S.E.) are shown.

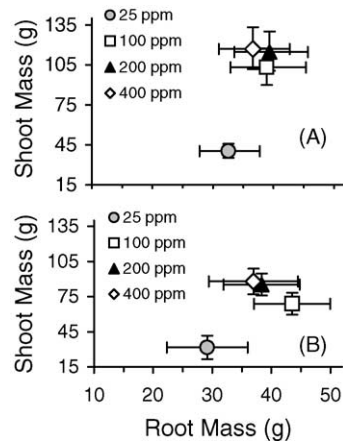


Fig. 2. Fresh mass of roots vs. shoots of (A) sour orange and (B) Swingle citrumelo treated with different concentrations of fertilizer. Back-transformed least squares means (± 2 S.E.) are shown.

for plants did not alter the effects of feeding on plant growth: well-nourished plants are no less affected by feeding than are nutrient-stressed plants. Mite infestation of Swingle citrumelo had a marginal effect on growth (Pillai's trace, Table 1) and this effect was primarily due to a reduced shoot mass with greater mite score (large canonical coefficient, Table 1).

3.2. Carbon and nitrogen

MANOVA indicated that herbivory marginally affected the carbon and nitrogen content in sour orange, primarily by increasing carbon in roots and secondarily by decreasing nitrogen in leaves (note opposite signs for canonical coefficients for nitrogen versus carbon, Table 2; Fig. 3). In Swingle citrumelo, herbivory of roots significantly reduced leaf nitrogen and increased carbon in leaves, thus increasing the leaf C:N ratio (Table 2; Fig. 3).

Increased fertilizer concentration resulted in decreased C:N ratio of sour orange and Swingle citrumelo roots and leaves (Table 2; Fig. 4). The C:N ratio of sour orange roots ranged from (least squares mean \pm S.E.) 10.6 (± 0.7) at 400 ppm to 24.2 (± 0.7) at 25 ppm N and sour orange leaves ranged from 10.4 (± 0.8) at 400 ppm N to 17.7 (± 0.8) at 25 ppm N. Similarly, Swingle citrumelo roots ranged from 9.2 (± 0.8) to 26.3 (± 0.7) and leaves ranged from 9.5 (± 0.3) to 14.5 (± 0.3) at 400 and 25 ppm N, respectively. The fertilizer effect was primarily due

Table 2

Results of MANOVA of log-transformed root and leaf carbon and nitrogen for sour orange and Swingle citrumelo rootstocks fertilized with one of four concentrations of fertilizer and grown with or without *Diaprepes* root weevil larvae

Source	Pillai's trace		Standardized canonical coefficients			
	d.f.	P	Root N	Root C	Leaf N	Leaf C
Sour orange						
Block	8,76	0.0963	-1.4153	0.0727	0.7206	0.7938
Diaprepes (D)	4,37	0.0652	-0.6969	1.2217	-0.8179	-0.1299
Fertilizer (F)	12,117	0.0001	2.7060 (1st)	-1.2472	0.3655	-0.4287
			0.2866 (2nd)	0.8467	-0.4145	-0.6627
			-0.1717 (3rd)	0.7725	0.5364	0.6188
D × F	12,117	0.2310	-2.7537	1.4226	0.8233	0.3397
Swingle citrumelo						
Block	12,150	0.5299	1.7949	0.2225	0.2421	-0.6064
Diaprepes (D)	4,48	0.0142	0.0609	0.5255	1.9353	-0.8578
Fertilizer (F)	12,150	0.0001	2.2960	-0.8609	1.2960	-0.3283
D × F	12,150	0.0361	2.8486	-0.8695	0.1411	-0.4640

Significant ($P < 0.05$) second and third standardized canonical coefficients for the fertilizer effect are listed on lines below the first canonical coefficient.

to increased nitrogen content of roots with higher concentrations of fertilizer (large canonical coefficients, Table 2), although N concentration of leaves also increased consistently (Fig. 4). The pattern of carbon content as a function of fertilizer was not consistent. Roots of plants given intermediate levels of fertilizer tended to have lower % carbon than did plants given the extremes (Fig. 4).

3.3. Other minerals

The first three principal components (PC) from analysis of sour orange roots explained 76% of the variation in mineral concentration. PC1 alone was responsible for 41% of the variation. Examination of factor loadings indicates high correlation among many minerals (Table 3). The first principal component was

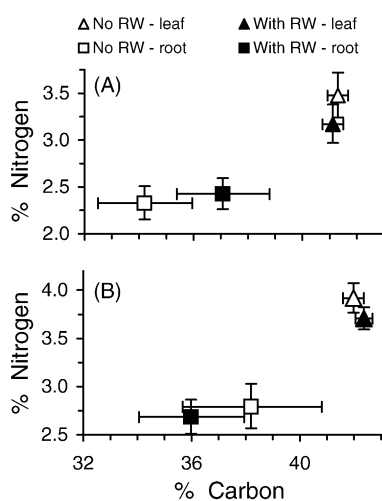


Fig. 3. Carbon vs. nitrogen concentration of leaves and roots of (A) sour orange and (B) Swingle citrumelo undamaged or treated with root weevil larvae. Back-transformed least squares means (± 2 S.E.) are shown.

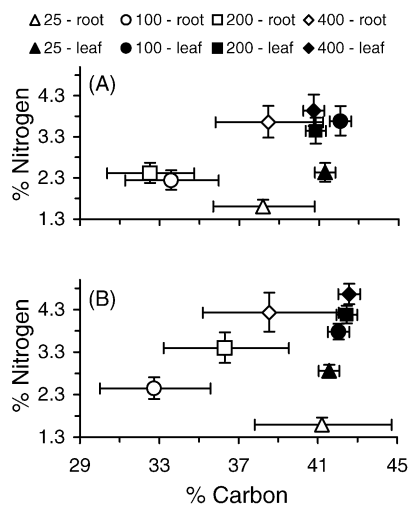


Fig. 4. Carbon vs. nitrogen concentration of leaves and roots of (A) sour orange and (B) Swingle citrumelo treated with different concentrations of fertilizer. Back-transformed least squares means (± 2 S.E.) are shown.

Table 3
Factor loadings from principal components analysis of sour orange roots

	Varimax rotated factor pattern		
	PC1 (% of variation = 41.3)	PC2 (% of variation = 18.0)	PC3 (% of variation = 16.2)
Aluminum	+0.17	+0.75	0.00
Boron	+0.83	-0.11	+0.15
Calcium	-0.01	-0.20	+0.79
Chromium	-0.07	+0.87	-0.18
Cobalt	-0.05	+0.90	+0.17
Copper	-0.40	-0.07	+0.81
Iron	+0.17	+0.87	-0.30
Magnesium	-0.16	-0.09	+0.75
Manganese	+0.76	+0.37	-0.41
Molybdenum	+0.55	+0.58	-0.54
Phosphorus	+0.75	+0.25	-0.40
Potassium	+0.75	-0.03	-0.07
Sodium	+0.29	+0.29	+0.78
Sulfur	+0.90	+0.17	-0.05
Zinc	+0.52	+0.60	+0.01
Minerals that co-vary strongly	B, Mn, Mo, P, K, S, Zn vs. other minerals	Al, Cr, Co, Fe, Mo, Zn vs. other minerals	Ca, Cu, Mg, Na vs. Mn, Mo, P

Boldface listings indicate original variables that load strongly on each principal component (loadings > 0.4).

strongly and positively correlated with several major elements (P, K, and S) and micronutrients (B, Mn, Mo, and Zn) (Table 3), indicating that treatments that increased concentrations of one of these minerals also tended to increase concentrations of the others. PC2 was positively correlated with several micronutrients (Fe, Mo, and Zn), trace elements (Cr and Co) and Al. PC3 was positively correlated with Ca, Cu, Mg, and Na but negatively correlated with Mn, Mo, and P (Table 3), indicating that treatments that produced higher concentrations of the first group of nutrients tended to decrease concentrations of the latter group.

MANOVA of these PCs yielded significant main effects and a significant interaction of *D. abbreviatus* and fertilizer (Table 4), indicating that effects of

Table 4
MANOVA for minerals in roots of sour orange

Source	d.f.	d.f. error	Pillai's trace	P	Standardized canonical coefficients		
					PC1	PC2	PC3
DRW (D)	3	37	0.713	0.0001	+0.388	+0.907	+2.538
Fertilizer (F)	9	117	1.765	0.0001	+3.134	+0.840	-3.312
D × F	9	117	0.553	0.0036	+2.947	+0.496	-1.132
Block	6	76	0.176	0.3033			

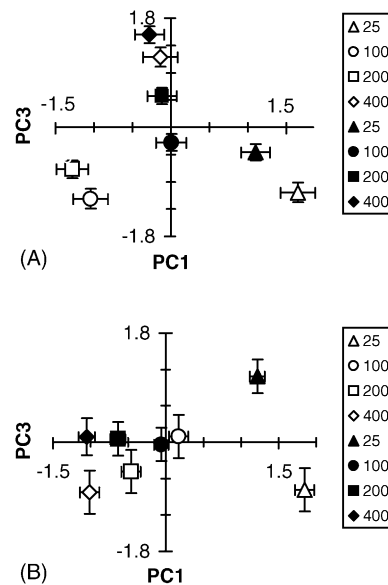


Fig. 5. PC1 vs. PC3 factors summarizing mineral concentrations in roots of (A) sour orange and (B) Swingle citrumelo plants grown without (open symbols) or with (filled symbols) root weevil larvae and treated with different concentrations of fertilizer. Least squares means (± 1 S.E.) are presented.

D. abbreviatus on mineral content of sour orange roots depended on how well nourished the plants were. Minerals summarized by PC1 (Table 3) contributed most to the significant interaction (Table 4). The minerals that loaded strongly on PC1 were most affected by *D. abbreviatus* when the plant received intermediate concentrations of fertilizer (Fig. 5A). Root weevil feeding decreased concentrations of these minerals when the plants were mildly undernourished or optimally nourished, had little effect when plants were excessively fertilized and, for some minerals, increased concentrations when plants were most nutrient-stressed (Fig. 6A–G). Plants damaged by *D. abbreviatus* separated out along PC3 axis in relation to fertilizer treatment better than did undamaged plants (Fig. 5A). Minerals with large negative scores for PC3 increased in concentration as

plants became better nourished (Fig. 6D and G) and minerals with large positive scores decreased in concentration (Fig. 6H–K).

The first four principal components from analysis of Swingle citrumelo roots summarized 80% of the variance associated with root mineral content. High scores for PC1 were due to highly correlated responses of Fe, Mn, Mo, and P and the opposite responses of Cu, Mg and Na (Table 5) indicating that treatments that increased concentrations of the first group of minerals tended to decrease Cu, Mg, and Na. High scores for PC2 resulted from positive correlation of Ca, Cr, Co, and Fe with each other and negative correlation of these minerals with B (Table 5). Al and the micronutrients Fe, Mn, and Zn loaded strongly and similarly on PC3 whereas several major elements (Ca, K, and S) and Na loaded strongly on PC4 (Table 5).

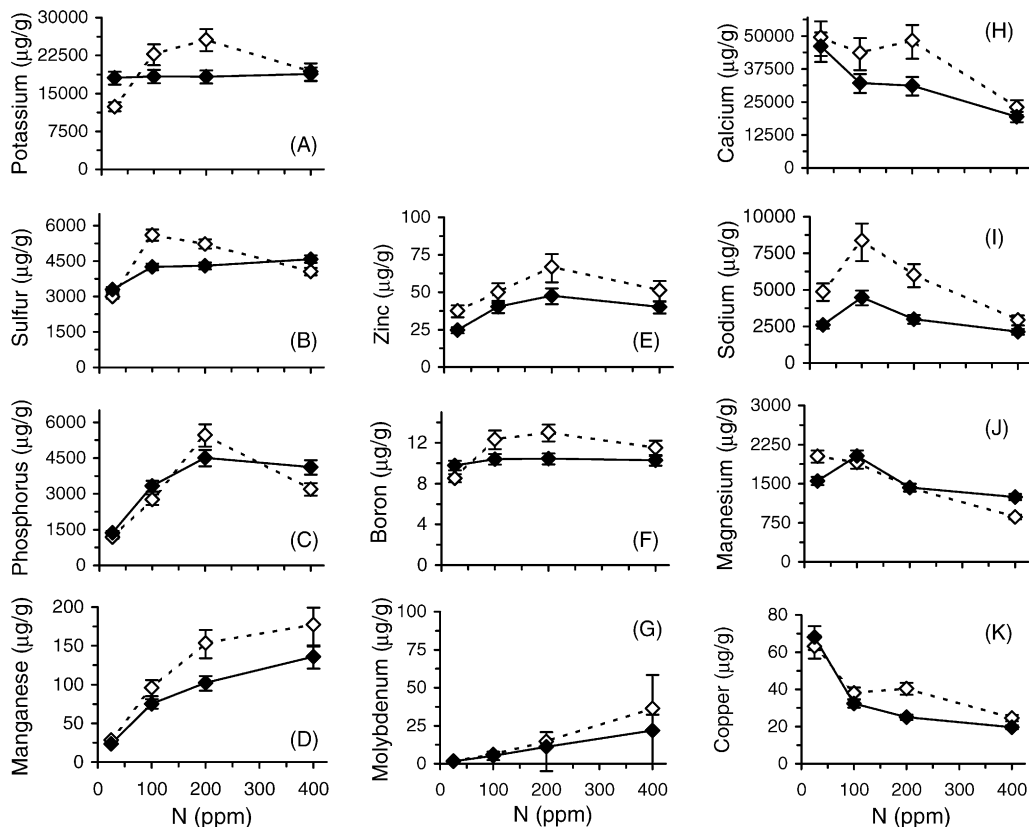


Fig. 6. Mineral concentration in roots of 3-year-old sour orange grown without (open symbols) or with (filled symbols) root weevil larvae and treated with complete fertilizer containing 25, 100, 200 or 400 ppm N. Back-transformed least squares means ($\pm 2S.E.$) are presented.

Table 5
Factor loadings for principal components analysis of minerals in Swingle citrumelo roots

	Varimax rotated factor loadings			
	PC1 (% of variation = 35.8)	PC2 (% of variation = 19.4)	PC3 (% of variation = 15.8)	PC4 (% of variation = 9.0)
Aluminum	−0.01	0.00	+0.93	+0.01
Boron	+0.11	−0.75	+0.26	+0.05
Calcium	−0.10	+0.50	−0.12	+0.43
Chromium	+0.34	+0.84	+0.29	−0.01
Cobalt	−0.01	+0.95	+0.13	0.00
Copper	−0.83	−0.05	0.00	+0.23
Iron	+0.45	+0.57	+0.50	−0.13
Magnesium	−0.89	+0.05	+0.11	+0.31
Manganese	+0.84	−0.04	+0.44	+0.06
Molybdenum	+0.90	−0.07	+0.32	−0.05
Phosphorus	+0.84	+0.23	+0.19	+0.25
Potassium	−0.10	−0.17	+0.08	+0.85
Sodium	−0.60	−0.14	+0.12	+0.56
Sulfur	−0.02	+0.17	+0.05	+0.88
Zinc	+0.31	+0.04	+0.82	+0.17
Minerals that co-vary strongly	Fe, Mn, Mo, P vs. Cu, Mg, Na	Ca, Cr, Co, Fe vs. B	Al, Fe, Mn, Zn vs. others	Ca, K, Na, S vs. others

Boldface listings indicate original variables that load strongly on each principal component (loadings > 0.4).

MANOVA of these PCs indicated that *D. abbreviatus*, fertilizer, and their interaction significantly affected concentrations of minerals in Swingle citrumelo roots and PC1 contributed most to the significant interaction (Table 6). Fertilizer treatments were ordered along PC1 and root weevil treatments were consistent within fertilizer (Fig. 5B). Minerals with large negative scores on PC1 declined in well-nourished plants (Fig. 7A–C) and minerals with large positive scores increased with fertilizer concentration (Fig. 7D–G). *D. abbreviatus* tended to decrease mineral concentrations but this trend was reversed for minerals summarized by PC3 (Table 5) when plants were fertilized with 100 ppm N (Fig. 7F–I).

4. Discussion

Herbivory reduced average growth of shoots and, especially, roots of each citrus cultivar, indicating that neither cultivar fully compensated for damage by root weevils over the course of the experiments. Although consistent with a study of beetles feeding on a grass (Morón-Ríos et al., 1997), this increase in the shoot:root ratio contrasts with results of other studies in which manipulation of root mass through pruning or herbivory resulted in relatively greater reduction of shoot growth (Schmid et al., 1990; Müller-Schärer, 1991; Steinger and Müller-Schärer, 1992; Murray et al., 1996; Nötzold et al., 1998) or had no effect on either root or shoot growth (Steinger and Müller-

Table 6
MANOVA for minerals in roots of Swingle citrumelo

Source	d.f.	d.f. error	Pillai's trace	P	Standardized canonical coefficients			
					PC1	PC2	PC3	PC4
DRW (D)	4	48	0.450	0.0001	−1.988	+0.625	+0.284	+1.263
Fertilizer (F)	12	150	1.780	0.0001	+4.825	−0.859	+1.163	−0.600
D × F	12	150	0.482	0.0024	−3.286	+1.155	−1.245	+1.154
Block	12	150	0.202	0.5449				

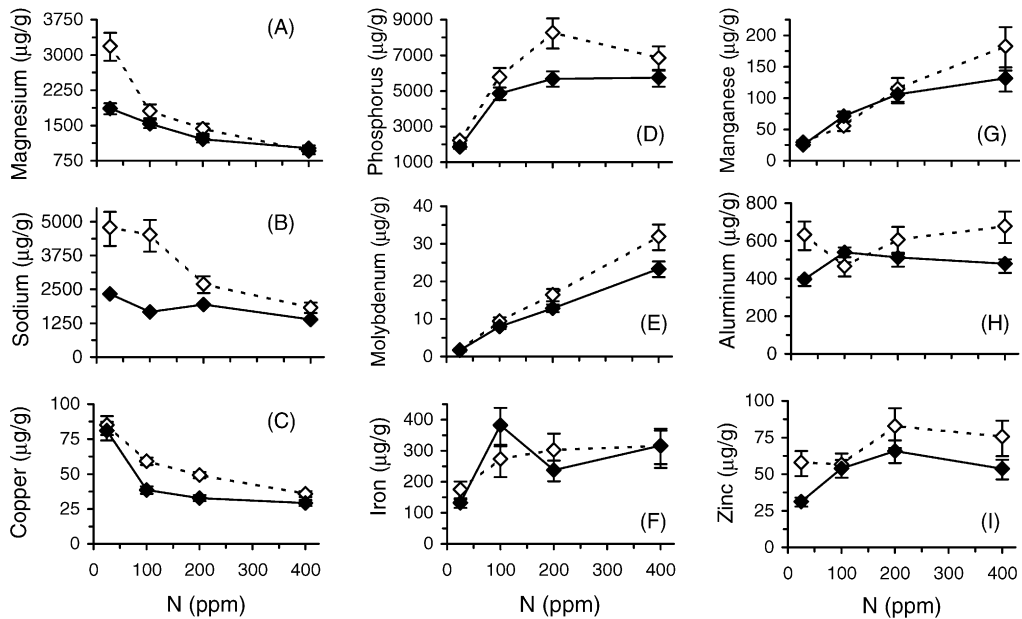


Fig. 7. Mineral concentration in roots of 3-year-old Swingle citrumelo grown without (open symbols) or with (filled symbols) root weevil larvae and treated with complete fertilizer containing 25, 100, 200 or 400 ppm N. Back-transformed least squares means (± 2 S.E.) are presented.

Schärer, 1992). Because studies of below-ground herbivory cannot account for root mass consumed by herbivores, it is difficult to assess compensatory growth, which includes mass lost to the herbivore (Belsky, 1986), accurately. Had we been able to account for the mass of roots consumed by larvae, the shoot:root ratio would have been less biased towards reduced root growth. Our study differs from others in that we assessed effects of herbivory on woody species. The pattern of growth in citrus trees, which exhibit alternating growth of shoot versus roots, may cause plant responses to herbivory to be seasonally dynamic, such that assessing growth at a single point in the growing season cannot capture the full effects of root herbivory.

Increased fertilizer produced a larger increase in shoot mass than root mass, resulting in greater shoot:root ratio. This result is consistent with observations that plants invest most heavily in the organs that capture the resource in shortest supply (Bloom et al., 1985). As mineral supply increased, there was greater investment in light capture (i.e., greater shoot mass). Although herbivory by weevils reduced root mass more strongly, shoot mass was also reduced, as expected if plants became nutrient limited.

Generally, lower levels of mineral concentrations in roots and lower concentrations of nitrogen in leaves of plants treated with root weevils also suggest that herbivory limited nutrient uptake. Root herbivory also increased the C:N ratio in Swingle citrumelo leaves, which agrees with observations that plants accumulate storage carbohydrates in leaves when attacked by root herbivores (Steinger and Müller-Schärer, 1992, but see Dunn and Frommelt, 1998) or when otherwise nutrient limited (Bloom et al., 1985).

Effects of herbivory on mass were consistent across fertilizer concentrations in both experiments, indicating that increased nutrient supply increased growth of nutrient-stressed plants but did not compensate for damage by root weevil larvae. This result contrasts with other studies in which increased nutrient supply reduced effects of herbivory (Steinger and Müller-Schärer, 1992; Meyer, 2000) or exacerbated effects of herbivory (Schmid et al., 1990). The lack of interaction between herbivory and fertilizer that we observed has practical consequences. It does not appear that growers can offset root weevil damage to nutrient-gathering roots by adding more fertilizer. Although increased fertilizer concentration produced roots with significantly higher concentrations of

nitrogen, well-nourished sour orange plants also produced larger *D. abbreviatus* larvae (Borowicz et al., 2003). Thus, application of excess complete fertilizer provides no benefit in terms of either vegetative growth of citrus (tolerance) or resistance to *D. abbreviatus*.

We held the proportional make-up of the fertilizer constant and manipulated fertilizer concentrations through dilution. Despite constant proportions in the fertilizer, proportional representation of minerals in roots and nitrogen in leaves varied across levels of fertilizer and root weevil treatment. Although much of the variation in nitrogen concentration of tissue was probably due to increased nitrogen uptake at higher fertilizer concentration, some of this variation in leaf nitrogen was probably due to developmental differences among treatments. The timing and extent of leaf production differed among treatments (pers. obs.), resulting in varying proportions of mature versus immature leaves included in pooled samples within plants. Greater growth by well-nourished plants would result in a greater proportion of younger leaves, which typically have higher nitrogen concentration than more mature leaves (Hanlon et al., 1995).

D. abbreviatus significantly altered the concentrations of minerals in roots but that effect differed among minerals and varied as a function of fertilizer levels. There was no indication that herbivory increased concentrations of elements that are toxic at elevated levels, e.g., Al, Cr, or Co (Jones, 1998). Rather, herbivory generally decreased mineral concentrations, suggesting that even well-fertilized plants may become deficient in particular minerals when damaged by root herbivores. Although we concluded that adding excess complete fertilizer may not improve plant growth, the interacting effects of fertilizer and herbivory on the balance of nutrients suggests that supplementing infested plants with specific minerals depressed by herbivory may be of value.

Alonso and Herrera (2003) evaluated leaf mineral content in natural populations of the evergreen shrub *Daphne laureola* L. and found a significant relationship between nutrient covariation patterns and defoliation. They suggested that herbivores may discriminate in favor of well-nourished plants, e.g., in their study, leaves with balanced, high concentrations of N, P, K, and Ca. However, in other systems insect damage is often greater on nutrient-stressed

plants (reviewed by Dale (1988)) and proportions of minerals that are optimal for herbivores may not coincide with proportions that maximize plant growth (Busch and Phelan, 1999; Beanland et al., 2003). *D. abbreviatus* adults feed on foliage of many species including citrus, and females may sample trees prior to ovipositing on leaves. We are not aware of any studies that have evaluated whether or not preference of oviposition sites in citrus groves by *D. abbreviatus* corresponds to the mineral profile of a well-nourished tree. If the preferred mineral profile of *D. abbreviatus* does not match the profile characteristic of an optimally nourished tree, nutrient manipulation of selected “trap” trees within groves may have management potential.

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References

- Alonso, C., Herrera, C.M., 2003. Developmental and spatial covariation of nutrients in growing leaves of *Daphne laureola* and their relationships with herbivory. *New Phytol.* 159, 645–656.
- Andersen, D.C., 1987. Below-ground herbivory in natural communities: a review emphasizing fossorial animals. *Q. Rev. Biol.* 62, 261–286.
- Beanland, L., Phelan, P.L., Salminen, S., 2003. Micronutrient interactions on soybean growth and the developmental performance of three insect herbivores. *Environ. Entomol.* 32, 641–651.
- Belsky, A.J., 1986. Does herbivory benefit plants? A review of the evidence? *Am. Nat.* 127, 870–892.
- Bloom, A.J., Chapin, F.S., Mooney, H.A., 1985. Resource limitation in plants—an economic analogy. *Ann. Rev. Ecol. Syst.* 16, 363–392.
- Blossey, B., Hunt-Joshi, T.R., 2003. Belowground herbivory by insects: influence on plants and aboveground herbivores. *Annu. Rev. Entomol.* 48, 521–547.
- Borowicz, V.A., Albrecht, U., Mayer, R.T., 2003. Effects of nutrient supply on citrus resistance to root herbivory by *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae). *Environ. Entomol.* 32, 1242–1250.
- Brown, V.K., Gange, A.C., 1990. Insect herbivory below ground. *Adv. Ecol. Res.* 20, 1–58.

- Busch, J.W., Phelan, P.L., 1999. Mixture models of soybean growth and herbivore performance in response to nitrogen–sulphur–phosphorous nutrient interactions. *Ecol. Entomol.* 24, 1–14.
- Dale, D., 1988. Plant-mediated effects of soil mineral stresses on insects. In: Heinrichs, E.A. (Ed.), *Plant Stress-Insect Interactions*, Wiley, New York, pp. 35–110.
- Diaprepes Task Force, 1997. Diaprepes Root Weevil Workshop Report and Strategic and Operational Plan. October 15, Winter Haven, FL.
- Dunn, J.P., Frommelt, K., 1998. Effects of below-ground herbivory by *Diabrotica virgifera virgifera* (Coleoptera) on biomass allocation and carbohydrate storage of maize. *Appl. Soil Ecol.* 7, 213–218.
- Fay, P.A., Hartnett, D.C., Knapp, A.K., 1996. Plant tolerance of gall-insect attack and gall-insect performance. *Ecology* 77, 521–534.
- Ganade, G., Brown, V.K., 1997. Effects of below-ground insects, mycorrhizal fungi and soil fertility on the establishment of *Vicia* in grassland communities. *Oecologia* 109, 374–381.
- Gange, A.C., 2001. Species-specific responses of a root- and shoot-feeding insect to arbuscular mycorrhizal colonization of its host plant. *New Phytol.* 150, 611–618.
- Graham, J.H., Bright, D.B., McCoy, C.W., 2003. Phytophthora-Diaprepes weevil complex: *Phytophthora* spp. Relationship with citrus rootstocks. *Plant Dis.* 87, 85–90.
- Hanlon, E.A., Obreza, T.A., Alva, A.K., 1995. Tissue and soil analysis. In: Tucker, D.P.H., Alva, A.K., Jackson, L.K., Wheaton, T.A. (Eds.), *Nutrition of Florida Citrus Trees*, University of Florida, IFAS, Gainesville, pp. 13–16.
- Houle, G., 1999. Nutrient availability and plant gender influences on the short-term compensatory response of *Salix planifolia* ssp. *planifolia* to simulated leaf herbivory. *Can. J. For. Res.* 29, 1841–1846.
- Hunter, M.D., 2001. Out of sight, out of mind: the impacts of root-feeding insects in natural and managed systems. *Agric. For. Entomol.* 3, 3–9.
- Jones Jr., J., 1998. *Plant Nutrition Manual*, CRC Press, New York.
- Karban, R., 1980. Periodical cicada nymphs impose periodical oak tree wood accumulation. *Nature* 287, 326–327.
- Maron, J.L., 1998. Insect herbivory above- and belowground: individual and joint effects on plant fitness. *Ecology* 79, 1281–1293.
- Maron, J.L., 2001. Intraspecific competition and subterranean herbivory: individual and interactive effects on bush lupine. *Oikos* 92, 178–186.
- Maschinski, J., Whitham, T.G., 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. *Am. Nat.* 134, 1–19.
- Masters, G.J., 1995. The impact of root herbivory on aphid performance: field and laboratory evidence. *Acta Ecol.* 16, 135–142.
- Mayer, R.T., Shapiro, J.P., Berdis, E., Hearn, C.J., McCollum, T.G., McDonald, R.E., Doostdar, H., 1995. Citrus rootstock responses to herbivory by larvae of the sugarcane rootstock borer weevil (*Diaprepes abbreviatus*). *Physiol. Plant.* 94, 164–173.
- Meyer, G.A., 2000. Interactive effects of soil fertility and herbivory on *Brassica nigra*. *Oikos* 88, 433–441.
- Morón-Ríos, A., Dirzo, R., Jaramillo, V.J., 1997. Defoliation and below-ground herbivory in the grass *Muhlenbergia quadridentata*: effects on plant performance and on the root-feeder *Phyllophaga* sp. (Coleoptera, Melolonthidae). *Oecologia* 110, 237–242.
- Müller-Schärer, H., 1991. The impact of root herbivory as a function of plant density and competition: survival, growth and fecundity of *Centaurea maculosa* in field plots. *J. Appl. Ecol.* 28, 759–776.
- Murray, P.J., Hatch, D.J., Cliquet, J.B., 1996. Impact of insect root herbivory on the growth and nitrogen and carbon contents of white clover (*Trifolium repens*). *Can. J. Bot.* 74, 1591–1595.
- Nötzold, R., Blossey, B., Newton, E., 1998. The influence of below ground herbivory and plant competition on growth and biomass allocation of purple loosestrife. *Oecologia* 113, 82–93.
- Potvin, C., 2001. ANOVA: experimental layout and analysis. In: Scheiner, S., Gurevitch, J. (Eds.), *Design and Analysis of Ecological Experiments*, 2nd ed. Oxford University Press, New York, pp. 63–76.
- Rogers, S., McCoy, C.W., Graham, J.H., 1996. Insect plant pathogen interactions: Preliminary studies of Diaprepes root weevil injuries and Phytophthora infections. *Proc. Flor. State Hort. Soc.* 109, 57–62.
- Scheiner, S., 2001. MANOVA: Multiple response variables and multispecies interactions. In: Scheiner, S., Gurevitch, J. (Eds.), *Design and Analysis of Ecological Experiments*, 2nd ed. Oxford University Press, New York, pp. 99–115.
- Schmid, B., Miao, S.L., Bazzaz, F.A., 1990. Effects of simulated root herbivory and fertilizer application on growth and biomass allocation in the clonal perennial *Solidago canadensis*. *Oecologia* 84, 9–15.
- Shapiro, J.P., Gottwald, T.R., 1995. Resistance of eight cultivars of citrus rootstock to a larval root weevil, *Diaprepes abbreviatus* L. (Coleoptera Curculionidae). *J. Econ. Entomol.* 88, 148–154.
- Simpson, S.E., Nigg, H.N., Coile, N.C., Adair, R.A., 1996. *Diaprepes abbreviatus* (Coleoptera Curculionidae): host plant associations. *Environ. Entomol.* 25, 333–349.
- Steinger, T., Müller-Schärer, H., 1992. Physiological and growth responses of *Centaurea maculosa* (Asteraceae) to root herbivory under varying levels of interspecific plant competition and soil nitrogen availability. *Oecologia* 91, 141–149.
- Strauss, S.Y., Agrawal, A.A., 1999. The ecology and evolution of plant tolerance to herbivory. *Trends Ecol. Evol.* 14, 179–185.
- Strong, D.R., Maron, J.L., Connors, P.G., Whipple, A., Harrison, S., Jeffries, R.L., 1995. High mortality, fluctuation in numbers, and heavy subterranean insect herbivory in bush lupine, *Lupinus arboreus*. *Oecologia* 104, 85–92.
- Urias-Lopez, M., Meinke, L.J., Higley, L.G., Haile, F.J., 2000. Influence of western corn rootworm (Coleoptera: Chrysomelidae) larval injury on photosynthetic rate and vegetative growth of different types of maize. *Environ. Entomol.* 9, 861–867.
- U.S. Environmental Protection Agency, 1997. Method 6010A: Inductively coupled plasma-atomic emission spectrometry. Test methods for evaluating solid waste, physical/chemical methods (SW-846). United States Environmental Protection Agency OSW/EMRAD, Washington, DC.
- U.S. Environmental Protection Agency, 1998. Method 3051A: Microwave assisted acid digestion of sediments, sludges, soils and oils, Revision 1. Test methods for evaluating solid waste, physical/chemical methods (SW-846). United States Environmental Protection Agency OSW/EMRAD, Washington, DC.