

Resistance of Eight Cultivars of Citrus Rootstock to a Larval Root Weevil, *Diaprepes abbreviatus* L. (Coleoptera: Curculionidae)

JEFFREY P. SHAPIRO AND TIM R. GOTTWALD

United States Horticultural Research Laboratory, USDA-ARS, 2120 Camden Road, Orlando, FL 32803

J. Econ. Entomol. 88(1): 148-154 (1995)

ABSTRACT To determine the potential for resistance (tolerance or antibiosis) to root weevil attack on citrus rootstocks, seedlings of eight citrus rootstock cultivars were challenged with young larvae of a root weevil, *Diaprepes abbreviatus* L. After 6 wk of feeding and development on root systems of potted plants in a greenhouse, soil and larvae were separated from roots. Larval survival and weight, and the weight and volume of root systems, were measured and compared between infested and uninfested plants and among cultivars. For morphometric comparison of root damage, photographs of root systems were digitally imaged and integrated, and relative imaged areas were compared. Imaging by subsoil stratum indicated that most damage occurred 4-12 cm below the surface. Larvae survived equally well on all cultivars, but larval weight showed significant variation among cultivars. Nonetheless, mean weight gain ranged from 7-fold (on Cleopatra rootstock) to 13-fold (on sour orange). Damage indexes and statistical comparison of damage indicated a wide range of tolerance among rootstock cultivars to larval feeding. Cleopatra, Carrizo, *Citrus macrophylla*, and a Flying Dragon × Nakon hybrid showed significant damage by larvae. Sour orange, Pummelo × *Poncirus trifoliata* (2N and 4N), and Swingle had insignificant damage. At least in reference to young ungrafted rootstocks, results indicated little antibiosis in seven of the eight rootstock cultivars studied. The exception was Swingle rootstock, on which larvae gained significantly less weight than on sour orange or Pummelo × *P. trifoliata* (4N). Because the damage index of Swingle was very low and differences between infested and uninfested seedlings were insignificant regarding root mass, volume, and area, a degree of both antibiosis and tolerance was indicated. Tolerance alone was indicated in rootstocks showing insignificant differences between infested and uninfested seedlings, although lack of significance was also the result of variability within cultivars.

KEY WORDS host-plant resistance, citrus, weevil

LARVAE OF A root weevil *Diaprepes abbreviatus* L. infest and destroy the root systems of both seedling and mature citrus trees in Florida and the Caribbean (Beavers et al. 1979). *Diaprepes* is by far the most destructive of five root weevil species that attack citrus in Florida and the Caribbean (Schroeder & Beavers 1977). Although many plants will not support larval development of *D. abbreviatus*, a surprisingly large range of host plants will support development; these host plants include citrus, cedar, juniper, and sugarcane (Schroeder et al. 1979). No fully effective methods are available for control of larval or adult weevil populations, and more effective integrated methods will be needed to manage the spread of populations. Novel biological controls are now being developed and used (Schroeder 1988), new insecticides are being tested, and changes in cultural practices are being considered. Rootstock breeding and engineering for

resistance are not immediate options but may play critical roles in the future. Studies on rootstock resistance and tolerance are limited. Despite the number of available rootstock cultivars (Castle et al. 1989), no rootstock to date has been shown to be resistant to *D. abbreviatus* or other root weevil (Norman et al. 1974, Beavers & Hutchison 1985).

Discovery of active natural products in roots complements the testing of germplasm for resistance against root weevil larvae. The roots of citrus and other Rutaceae contain many natural products that may serve as allelochemicals (Shapiro 1991). Coumarins are especially prevalent in citrus roots (Nordby & Nagy 1981) and are excellent examples for study of phytochemical interactions with the root weevils. A synthetic coumarin has been shown to be absorbed and bound to proteins in hemolymph of *D. abbreviatus*, and may serve as a model for absorption, transport, and disposition of natural coumarins (Shapiro et al. 1988; Shapiro 1989 & 1991).

Effective plant defense strategies may involve several types of resistance of plants to insect pests.

Table 1. Rootstocks used caused by *D. abbreviatus*

Common name	
Pummelo × <i>Poncirus trifoliata</i> (2N)	Citr...
Pummelo × <i>Poncirus trifoliata</i> (4N)	C. g...
Flying Dragon × Nakon Swingle citrumelo	P. tr...
<i>Citrus macrophylla</i>	C. m...
Cleopatra mandarin	C. r...
Carrizo citrange	C. s...
Sour orange	C. o...

Antibiosis concerns qualities that adversely affect an insect, formerly termed *nonproletarian* (Ber 1980) concerns qualities of alternate hosts by the inability of the host plant to resist insect damage (Smith 1980). Such resistance factors as phytochemicals contrast with the dramatic effects of insecticides against insects, which must apply defensive mechanisms over long periods. Allelochemicals may act through dynamic mechanisms to affect insect population pressures, and are graded and complex responses that can be studied by parameters other than simple mortality or survival.

Concurrent study of both host plants and insect responses may increase chances of detecting antibiosis or antixenosis and tolerance in eight rootstock cultivars.

Materials and Methods

Insects. Larvae were reared on a colony maintained for >4 years. Occasional infusion of adult weevils from Florida citrus groves. Newly hatched larvae were added to 30 ml of a standard diet (Bio-Serv) for rearing as described by Shapiro (1989). Larvae per cup were used at room temperature for 29 days.

Rootstocks. All citrus rootstocks were grown in a greenhouse from trees at the L.A.H. Horticultural Research Laboratory, Leesburg, FL. Hybrid rootstocks (Flying Dragon × Nakon; Pummelo × *P. trifoliata* (4N)) were obtained from

This article reports the results of research only. Mention of a proprietary product does not constitute an endorsement or a recommendation for its use by USDA.

Table 1. Rootstocks used in the study of damage caused by *D. abbreviatus*

Common name	Taxonomic name and reference
Pummelo × <i>Poncirus trifoliata</i> (2N)	<i>Citrus grandis</i> (L.) Osb. × <i>Poncirus trifoliata</i> (2N) (L.) Raf
Pummelo × <i>Poncirus trifoliata</i> (4N)	<i>C. grandis</i> (L.) Osb. × <i>P. trifoliata</i> (4N) (L.) Raf
Flying Dragon × Nakon	<i>P. trifoliata</i> × <i>C. grandis</i>
Swingle citrumelo	<i>C. paradisi</i> Macf. × <i>P. trifoliata</i> (L.) Raf.
<i>Citrus macrophylla</i>	<i>C. macrophylla</i> (Christm.)
Cleopatra mandarin	<i>C. reticulata</i> Blanco
Carrizo citrange	<i>C. sinensis</i> (L.) Osb. × <i>P. trifoliata</i>
Sour orange	<i>C. aurantium</i> L.

Antibiosis concerns qualities of the host plant that adversely affect an insect pest, antixenosis (formerly termed nonpreference; Painter 1951, Horber 1980) concerns qualities that lead to selection of alternate hosts by the insect, and tolerance is the ability of the host plant to withstand or recover from insect damage (Smith 1989). Activities of such resistance factors as allelochemicals often contrast with the dramatic toxicities exhibited by pesticides against insects. Especially in trees, which must apply defensive pressures against insect populations over long individual life spans, allelochemicals may act through relatively subtle and dynamic mechanisms to attenuate or surmount insect population pressures (Schultz 1983). Such graded and complex responses can only be detected by studying parameters that are more sensitive than simple mortality or survival.

Concurrent study of both plant responses to insects and insect responses to plants will also increase chances of detecting and differentiating antibiosis or antixenosis and tolerance. In our study, eight rootstock cultivars were challenged with young *D. abbreviatus* larvae to determine the growth and survival of both root systems and larvae when measured after 6 wk.

Materials and Methods

Insects. Larvae were obtained from a weevil colony maintained for >4 yr in isolation with occasional infusion of adult weevils from central Florida citrus groves. Neonate larvae (within 2 d of hatch) were added to 30-ml cups containing 20 ml of a standard diet (Bio-Serve, Frenchtown, NJ; rearing was as described by Beavers [1982]). ≈10 larvae per cup were used; they were reared at room temperature for 29–32 d.

Rootstocks. All citrus rootstocks used (Table 1) were grown in a greenhouse from seed derived from trees at the L.A.H. Whitmore U.S. Horticultural Research Laboratory Foundation farm in Leesburg, FL. Hybrid rootstocks (Flying Dragon × Nakon; Pummelo × *Poncirus trifoliata* [2N and 4N]) were obtained from the USDA-ARS Breed-

Table 2. Initial and final mean larval weights (mean ± SD of larvae from 7 replicate seedlings, *n* = 7) and multiple of increase in weights during the 44-d infestation period

Cultivar	Mean larval weights, mg		
	Initial	Final	Increase
Carrizo	17.7 ± 1.7	153.4 ± 21.1	8.7
<i>C. macrophylla</i>	19.6 ± 2.3	165.0 ± 27.9	8.4
Cleopatra	18.4 ± 1.1	126.4 ± 21.1	6.9
Flying Dragon × Nakon	15.9 ± 1.2	159.5 ± 18.6	10.0
Pummelo × <i>P. trifoliata</i> (2N)	16.9 ± 2.0	156.6 ± 28.5	9.3
Pummelo × <i>P. trifoliata</i> (4N)	16.9 ± 1.9	176.7 ± 17.7	10.5
Sour Orange	16.3 ± 1.9	207.2 ± 34.3	12.7
Swingle	15.5 ± 1.0	127.3 ± 26.6	8.2
Means	17.2	159.0	9.3

Each replicate represents the mean weight of surviving larvae from that replicate.

ing Program, U.S. Horticultural Research Laboratory, Orlando, FL. Rootstock seeds were planted in cone trays in January 1991, and seedlings were transferred to 3.8-liter pots (20 by 21 cm in diameter; soil to ≈16 cm from bottom) ≈4 mo later. Potting soil consisted of 10% Florida peat: 50% peat moss: 10% sand: 30% Sunshine Mix (Fisons Horticulture, Vancouver, BC) by volume.

Infestation of Seedlings. Seedlings were infested 13.5 mo after planting in March 1992 with 10 larval *D. abbreviatus* per seedling during a 3-d period. Larvae were weighed individually and those weighing 10–30 mg were selected and combined in 64 groups of 10 each, with similar mean and range of weights among groups (range of group mean weights was 15–20 mg; Table 2). One larva was placed at the bottom of each of 10 holes (9 cm deep) in the 3.8-liter pots at a radius of 5 cm from the seedling trunk. Seven seedlings of each of eight cultivars were infested during 3 d, and seven control seedlings of each cultivar were left uninfested. An eighth seedling of each cultivar was infested and periodically sacrificed to estimate damage. At 44 d after infestation (a period from early March through mid-April 1992), each plant was harvested and larvae were collected. Timing of the harvest was determined from results of a preliminary study and by the periodic sacrifice of seedlings. We purposely selected the date to result in moderate damage to most cultivars for optimal comparison with uninfested controls.

Sample Collection and Analysis. At collection, seedling heights were measured and plants were removed from pots. Soil and larvae were carefully separated from the roots, and roots were thoroughly rinsed. The root mass was photographed under bidirectional strobes on Tmax-100 black and white negative film (Kodak, Rochester, NY) against a black velvet background. Soil was carefully sieved and larvae were separated and weighed. Root volume displacements in milliliters were determined

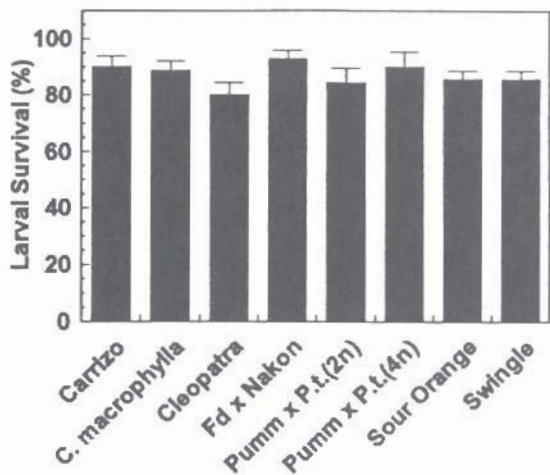


Fig. 1. Survival of larval *D. abbreviatus*. Ten larvae were originally introduced into each of 7 potted plants per cultivar (means \pm SEM; $n = 7$). No significant differences among means were found.

by submerging root masses to the soil line in water in a graduated cylinder. Roots were separated from trunks at the soil line and roots were weighed (seven replicates, one tree per replicate).

For analysis, the mean weight of larvae recovered from each pot was computed; the mean \pm SEM were calculated for each group of seven pots per cultivar (7 replicates, 10 larvae per replicate). Seedling damage indexes were calculated as $1 - I/U$, where I is the root weight, volume, or imaged area from infested plants and U is the same from uninfested plants. Thus, 1 indicates total destruction of roots and 0 indicates no damage.

Differences between means of infested and uninfested rootstocks were examined by paired t -tests. For comparison of means among rootstocks, a single factor analysis of variance (ANOVA) was used (SAS version 6.04, SAS Institute [1987]). Tukey's honestly significant difference (HSD) test (CoStat version 4.2, CoHort Software [1994]) was used to compare larval weights among cultivars. Except where noted, all tests were performed at $P = 0.05$.

Digital Image Analysis. Negative images of root masses were captured and digitized directly from black and white photographic negatives with a model 1 HR video camera (MicroImage) of 1.0×1.0 resolution. All images to be analyzed were photographed at a constant distance (61.5 cm) and focal length. Acquired images were captured using an M8 (Targa) video card and stored as TIFF-image files with image acquisition and analysis software (OPTIMAS version 1.0, BioScan, Washington, DC). Stored images were analyzed by integrating root areas delimited by overlays with a standardized grayscale density thresholding procedure. Total integrated root area or root area delimited by subsoil stratum was recorded for each;

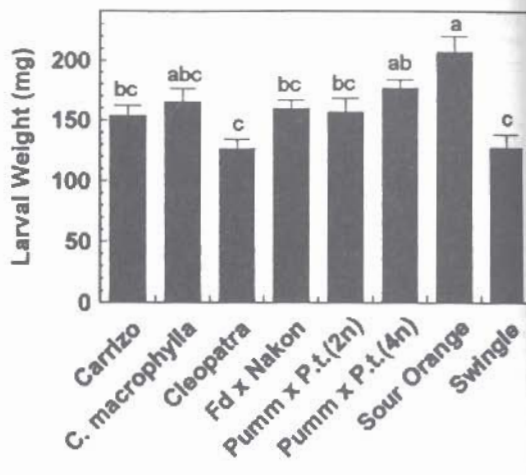


Fig. 2. Weights of recovered larvae. Bars denoted by the same letter are not significantly different ($P > 0.05$; Tukey's HSD test [CoHort Software 1994]). Mean \pm SEM, $n = 7$, as in Fig. 1.

mean and SD for each cultivar (one seedling per replicate, seven replicates per infested or uninfested treatment) were calculated.

Results

Larval Survival and Growth on Rootstocks. The small (≈ 17 mg) larvae initially added to potted rootstocks survived (80–94%) and grew very well on the rootstock seedlings, with no significant differences in mean survival of larvae among rootstock cultivars (Fig. 1; $F = 1.03$; $df = 7, 48$; $P > 0.4$). We found no evidence of larval cannibalism as the cause of the minor mortality. Larvae had developed from the initial overall mean (\pm SEM) weight of 17 ± 0.5 to 159 ± 11 mg, an average increase of 9.3-fold (Table 2) (Fully mature larvae weigh ≈ 400 – 800 mg.). By one-way ANOVA, larval weights showed significant differences among cultivars (Fig. 2; $F = 6.91$; $df = 7, 48$; $P < 0.0001$). Final weights of larvae on sour orange were significantly greater than those on all other rootstocks, and weights on Swingle and Cleopatra were significantly lower than on sour orange or Pummelo \times *P. trifoliata* (4N). Larvae gained the most weight on sour orange rootstock (12.7-fold increase), and the least on Cleopatra (a 6.9-fold increase; Table 2).

Root Weights and Volumes. Root masses of five rootstocks were reduced by feeding of larvae. Comparison of root weights between infested and uninfested plants showed damage indexes ranging from 0.11 (Swingle) to 0.75 (Cleopatra) on a scale of 0 to 1 (Fig. 3). Differences between infested and uninfested seedlings of Carrizo ($t = 3.80$, $df = 7.6$), *C. macrophylla* ($t = 3.44$, $df = 7.0$), Cleopatra ($t = 7.98$, $df = 6.4$), and the Flying Dragon \times Nakon hybrid ($t = 3.18$, $df = 9.7$) were highly significant ($P < 0.01$), and significant ($P < 0.05$)

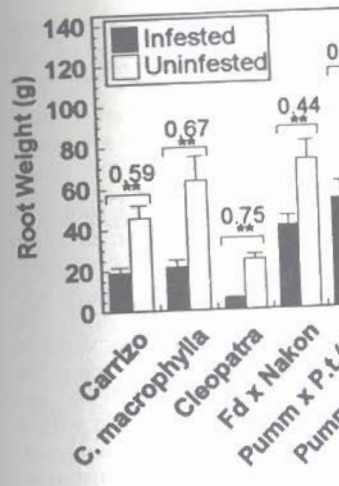


Fig. 3. Weights of root masses of infested and uninfested plants (mean \pm SEM). Damage indexes calculated from comparison of infested and uninfested roots (0, no damage; 1, total destruction). Numbers above bars are damage indexes. **, Highly significant difference ($P < 0.01$; t -test [SAS Institute 1987]). *, Significant difference ($P < 0.05$).

for sour orange ($t = 2.41$, $df = 6.4$) and Swingle ($t = 2.41$, $df = 6.4$) root volumes (Fig. 4) correlated with root weights, although slightly higher in insignificant differences ($P > 0.05$) between infested and uninfested sour orange (0.60) and *C. macrophylla* (0.45) root volumes (damage indexes were determined from root weights and volumes). The lowest damage (0.02) and highest root volume (0.82) were found on Swingle (0.82).

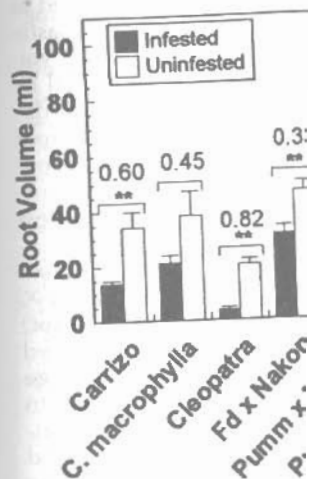


Fig. 4. Volumes of root masses of infested and uninfested plants (mean \pm SEM). Damage indexes calculated from comparison of infested and uninfested roots (0, no damage; 1, total destruction). Numbers above bars are damage indexes. **, Highly significant difference ($P < 0.01$; t -test [SAS Institute 1987]). *, Significant difference ($P < 0.05$).

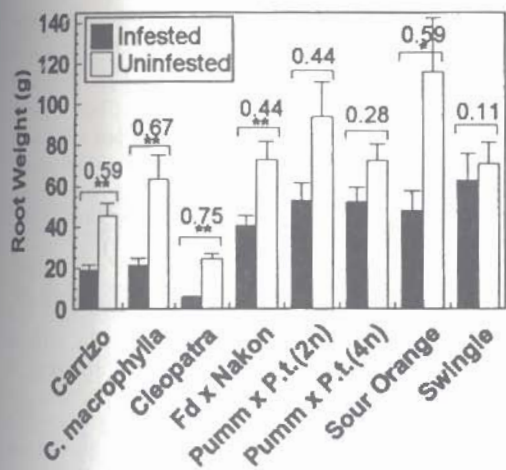


Fig. 3. Weights of root masses from larval-infested and uninfested plants (mean \pm SEM; $n = 7$), and damage indexes calculated from comparison between infested and uninfested roots (0, no damage; 1, total root loss; numbers above bars). **, Highly significant difference ($P < 0.01$; t -test [SAS Institute 1987]) between means of infested and uninfested roots; *, significant difference ($P < 0.05$).

for sour orange ($t = 2.41$, $df = 7.6$). As expected, root volumes (Fig. 4) correlated well with root weights, although slightly higher variation resulted in insignificant differences ($P > 0.05$) between infested and uninfested sour orange ($t = 2.14$, $df = 7.4$) and *C. macrophylla* ($t = 1.92$, $df = 7.3$). Root-volume damage indexes were similar to those determined from root weights, with Swingle showing the lowest damage (0.02) and Cleopatra the highest (0.82).

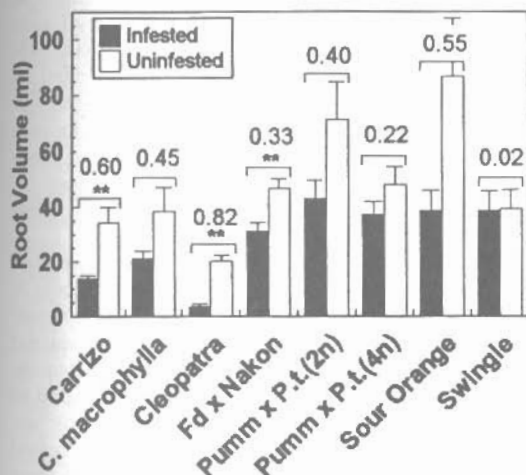


Fig. 4. Volumes of root masses determined by water displacement and calculated damage index. **, Highly significant difference ($P < 0.01$; t -test [SAS Institute 1987]) between means of infested and uninfested roots; *, significant difference ($P < 0.05$).

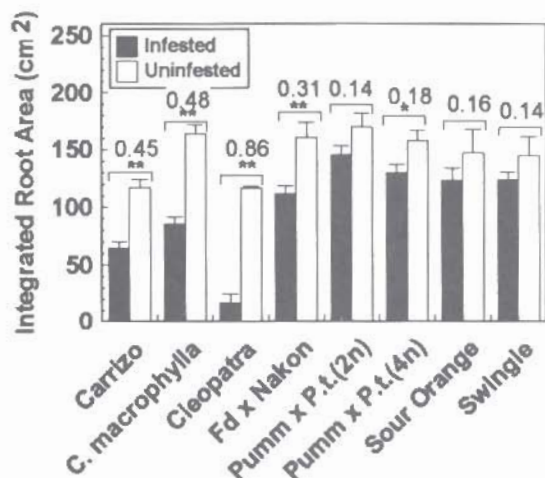


Fig. 5. Total integrated root areas. Areas were digitally acquired and integrated from negative photographic images of root masses and damage index calculated from integrated areas. **, Highly significant difference ($P < 0.01$; t -test [SAS Institute 1987]) between means of infested and uninfested roots; *, significant difference ($P < 0.05$).

Imaged Areas: Comparison with Root Mass and Volume. Comparisons of total integrated root areas among cultivars yielded results similar to comparisons of root weights and volumes (Fig. 5). Differences in total integrated root areas between infested and uninfested roots were seen in Carrizo, *C. macrophylla*, Cleopatra, and Flying Dragon \times Nakon rootstocks, as with root weight (although root volume was not significantly affected by infestation in *C. macrophylla*; see above). Comparisons of damage indexes among cultivars from integrated root areas were also similar to indexes of root weight and volume, except on Pummelo \times *P. trifoliata* (2N) and sour orange rootstocks (Table 3). Those cultivars had much lower damage index-

Table 3. Summary of damage indexes from Figs. 3-5 tabulated by method of measurement, including significance of weights, volumes, or areas of infested versus uninfested roots

Cultivar	Damage index			
	Wt	Vol	Area	Total Rank
Carrizo	0.59**	0.60**	0.45**	1.64 2
<i>C. macrophylla</i>	0.67**	0.45	0.48**	1.60 3
Cleopatra	0.75**	0.82**	0.86**	2.43 1
Flying Dragon \times Nakon	0.44**	0.33**	0.31**	1.08 5
Pummelo \times <i>P. trifoliata</i> (2N)	0.44	0.40	0.14	0.98 6
Pummelo \times <i>P. trifoliata</i> (4N)	0.28	0.22	0.18*	0.68 7
Sour Orange	0.59*	0.55	0.16	1.30 4
Swingle	0.11	0.02	0.14	0.27 8
Mean Index	0.48	0.42	0.34	1.25

Cultivars were ranked by sum total of the indexes ("Total") calculated by each method. Levels of significance as determined by t -test were marked at $P < 0.05$ (*) or $P < 0.01$ (**).

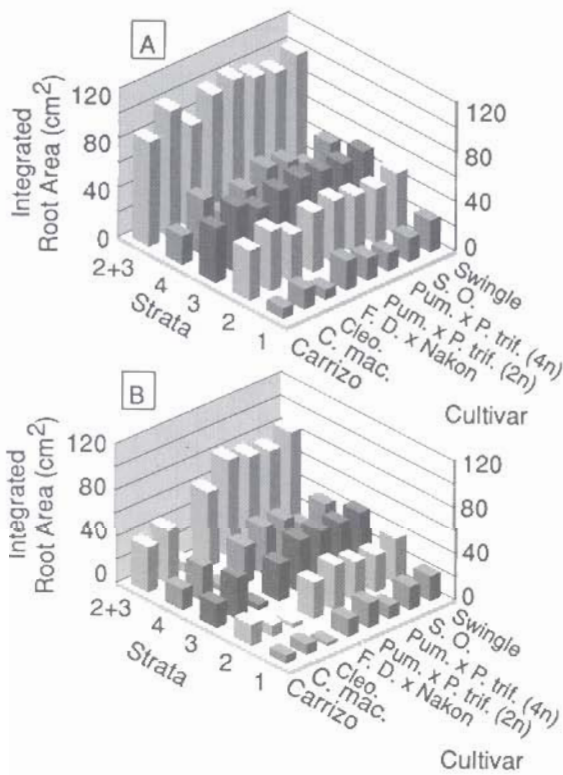


Fig. 6. Integrated root areas of strata 1 through 4 and combined strata 2 + 3 for uninfested (A) and infested root systems (B). Strata are each ≈ 4 cm thick; stratum 1 represents the top 4 cm below the soil surface and stratum 4 represents the bottom 4 cm.

es for integrated area than for weight or volume. Despite the comparability between integrated root area and weight or volume of infested versus uninfested roots, the range of integrated areas among rootstock cultivars was relatively small. For example, the ratio of largest to smallest mean integrated root areas of uninfested plants was ≈ 1.5 -fold, versus a 4-fold ratio between largest and smallest rootstocks in mean weight or volume. This difference results from the fact that image analysis uses a two-dimensional representation of a three-dimensional root system, and differences increase as a square rather than a cubic function.

We assessed root profiles below the soil surface by area imaging. To do so, the digitized photographic images were integrated as four strata. The digitized root area of each stratum and combined strata 2 + 3 are shown in Fig. 6. Because larvae were initially placed 9 cm below the soil surface, areas of strata 2 + 3 (≈ 4 –12 cm below the surface) are shown combined and separately.

The root area of Cleopatra rootstock was most reduced by larval feeding, in comparison with area of uninfested roots (Fig. 6B versus 6A). Reductions of Carrizo and *C. macrophylla* roots were also evident, especially in a comparison of combined strata

2 + 3 between infested and uninfested root systems. Flying Dragon \times Nakon roots were also clearly reduced, whereas the remaining rootstocks show less obvious damage.

Discussion

Earlier studies on larval survival and rootstock damage have shown no clear indication of resistance to *D. abbreviatus* larvae in any citrus rootstock tested (Norman et al. 1974, Beavers & Selhime 1975, Beavers & Hutchison 1985). Such lack of clear resistance was probably caused by variable and high mortality rates (3–22%; Beavers & Hutchison 1985) when neonate larvae were applied to trees, to variability among seedlings within a cultivar, and to the subjective nature of analyses. To increase the likelihood of detecting resistance, we used older larvae, seven replicates, and three independent and quantitative methods of analysis.

We detected apparent resistance against weevil larvae in several of the eight tested rootstocks, and differentiated between antibiosis/antixenosis and tolerance. Larval mortality (the clearest indication of antibiosis) did not differ significantly among cultivars (Fig. 1). However, antibiosis (or antixenosis) may also be implicated by reduced larval growth rates. In contrast with survival, larval growth during the 44 d of infestation significantly differed among rootstocks (Fig. 2; Table 2). Larvae on sour orange rootstock seedlings grew significantly more ($F = 6.91$, $df = 48$, $P < 0.05$; Tukey's HSD test [CoHort Software 1994]) than those on any other rootstock, whereas larvae on Swingle and Cleopatra grew significantly less than those on sour orange.

Root mass of uninfested rootstocks generally coincided with the descriptions of Castle & Youtsey (1977) and Castle et al. (1989), with the exception of Cleopatra. Sour orange rootstocks, described as having a vigorous spreading root system by Castle & Youtsey (1977), had the largest root mass, volume, and integrated area. Of all cultivars cited by Castle & Youtsey (1977), Carrizo had the least-developed root system and was also low in root weight and volume in our study. However, Cleopatra, which was described as similar to sour orange by Castle & Youtsey (1977), had the smallest root system by all measures in our study.

Measurements of root damage were more variable than larval growth and mortality. Pummelo \times *P. trifoliata* (2N) sustained slight (insignificant) damage, Pummelo \times *P. trifoliata* (4N) sustained significant damage based on digitized area; damage to sour orange was significant only with regard to root weight. Swingle rootstocks were nearly undamaged by infestation; root masses of infested trees differed little from those of uninfested trees regarding volume, weight, or digitized area.

Because larvae on Swingle survived well, the best explanation for the lack of damage to Swingle is either rootstock tolerance or compensatory growth. However, slower larval growth on Swingle

than on all other rootstocks also suggest a degree of tolerance. Swingle may be a nutritional source for larvae. Cleopatra Swingle: root masses of uninfested seedlings were the smallest obtained heavy damage during infestation with Swingle seedlings. Swingle seedlings were entirely stripped of small roots. Although substantial damage to larvae on Swingle seedlings was observed, masses of Cleopatra seedlings were quantitatively undernourished. Rootstocks may prove to be more tolerant to damage than Swingle. Lemon, a widely used rootstock, showed strong tolerance in a preliminary study. Published data. Roughly available for the current study.

The three independent methods of analysis were fairly comparable. Comparison of methods of analysis is difficult, this is evident from Figs. 3–5 in Table 1. Four cultivars showed significant differences between infested and uninfested plants. Integrated root areas and root volumes of four cultivars showed no significant differences whether analyzed by area or by weight. Root volume is a measure of damage (as a percentage of displacement) and error in displacement result that only three cultivars were significantly damaged. The third in damage index is total, but a distant second. This ambiguity is reflected in the significance of damage measures except weight (Table 1).

Integrated areas and root volumes gave similar results. Although root volume was not directly proportional to root volumes, it has the advantage of being a morphometric method. Data below the soil surface are a limitation about larval feeding. Larvae were introduced to the soil surface. The greater damage in uninfested trees may indicate a preference for an inter-cultivar.

By all measures, there is a strong potential for resistance in Pummelo \times *P. trifoliata* (raploid). In our study, the first generation (Ortman & Petraitis) had a higher than average damage index because *P. trifoliata* is a polyploid. In other cultivars, results may indicate a preference to insect damage. Further testing, the

than on all other rootstocks except Cleopatra may also suggest a degree of resistance. Alternatively, Swingle may be nutritionally deficient as a food source for larvae. Cleopatra seedlings contrast with Swingle: root masses of uninfested Cleopatra seedlings were the smallest of all rootstocks and sustained heavy damage during infestation compared with Swingle seedlings. Some Cleopatra were entirely stripped of small roots and of bark on tap roots. Although substantial root mass was available to larvae on Swingle seedlings, the declining root masses of Cleopatra seedlings may have left larvae quantitatively undernourished. Other untested rootstocks may prove to have even higher tolerance to damage than Swingle. For example, rough lemon, a widely used rootstock in Florida, showed strong tolerance in a preliminary study (J.P.S., unpublished data). Rough lemon germplasm was not available for the current study).

The three independent measures of root destruction were fairly consistent. Because direct comparison of methods for determining damage indexes is difficult, this information is compiled from Figs. 3–5 in Table 3. An equal number of cultivars showed significant difference between infested and uninfested plants when analyzed by integrated root areas and by root weights. The same four cultivars showed highly significant ($P < 0.01$) differences whether analyzed by digital integration or by weight. Root volume was the least sensitive measure of damage (as a result of higher variability and error in displacement measurements), with the result that only three cultivars were identified as significantly damaged. By rank, sour orange was third in damage index by weight, volume, and index total, but a distant sixth by integrated area. This ambiguity is reflected in the lack of statistical significance of damage to sour orange by all measures except weight (Table 3).

Integrated areas and weights are apparently the most sensitive measures that we used, and they gave similar results. Although integrated root area was not directly proportional to root weights or volumes, it has the additional advantage of being a morphometric method. Root area profiles in strata below the soil surface gave more detailed information about larval feeding patterns. In this study, larvae were introduced at an intermediate depth. The greater damage in the middle strata show that larvae remained near the depth of introduction. This may indicate lack of vertical movement, or preference for an intermediate soil depth in pots.

By all measures, three rootstocks show outstanding potential for resistance: Swingle and the two Pummelo \times *P. trifoliata* crosses (diploid and tetraploid). In our study, all three cultivars fit the definition (Ortman & Peters 1980) of showing lower than average damage by the insect (Table 3). Because *P. trifoliata* is a common parent to all three cultivars, results may indicate a propensity for tolerance to insect damage by that germplasm. With further testing, the outstanding rootstock among

the three—Swingle—may serve as a standard in further screening for resistance against *D. abbreviatus*.

Our study represents the most detailed analysis of root weevil effect on citrus root systems to date, and of various rootstocks on weevil larvae. Very little is known about effects of root-feeding insects on trees. For example, we know "virtually nothing about the effect of root- and mycorrhiza-feeding insects" on population dynamics of plants (Crawley 1989). The effects of roots on insects are also unexplored, because detailed measurements of such parameters as growth, feeding rates, assimilation, and excretion are difficult (Slansky & Scriber 1985).

Studies such as ours may have several uses. First, they can serve as the basis for screening of rootstock germplasm (e.g., germplasm readily available through the USDA-ARS breeding program or other programs) for cultivars that may be resistant or tolerant to root weevil attack. Second, they begin to describe the feeding patterns of root weevil larvae, to date a little-illuminated phenomenon. Third, the quantitative study of both plant and insect growth parameters could yield insight that cannot be derived from qualitative or semi-quantitative study of one organism or the other. Finally, they could serve as the basis for discovering subtle chemical responses in the plant that may eventually be used in defense against root-damaging insects and pathogens.

Acknowledgments

We thank Angela R. Williams (USDA-ARS, USHRI) for excellent technical assistance in all stages of experimental design and data collection; Dean Price (USDA-ARS, USHRL) for technical assistance in preliminary trials; Patricia Bell (USDA-ARS, USHRL) for digital image acquisition and analyses and statistical analyses of results; D. J. Hutchison, C. J. Hearn, and H. Wutscher (USDA-ARS, USHRL) for input concerning rootstock cultivars; and H. C. Barrett (USDA-ARS, USHRL) for furnishing hybrid rootstocks.

References Cited

- Beavers, J. B. 1982. Biology of *Diaprepes abbreviatus* (Coleoptera: Curculionidae) reared on an artificial diet. Fla. Entomol. 65: 263–269.
- Beavers, J. B. & D. J. Hutchison. 1985. Evaluation of selected *Citrus* spp. and relatives for susceptibility to root injury by *Diaprepes abbreviatus* larvae (Coleoptera: Curculionidae). Fla. Entomol. 68: 222–223.
- Beavers, J. B. & A. G. Selhime. 1975. Development of *Diaprepes abbreviatus* on potted citrus seedlings. Fla. Entomol. 58: 271–273.
- Beavers, J. B., R. E. Woodruff, S. A. Lovestrand & W. J. Schroeder. 1979. Bibliography of the sugarcane rootstalk borer weevil, *Diaprepes abbreviatus* Bull. Entomol. Soc. Am. 25: 25–29.
- Castle, W. S. & C. O. Youtsey. 1977. Root system characteristics of citrus nursery trees. Proc. Fla. State Hort. Soc. 90: 39–44.

- Castle, W. S., D.P.H. Tucker, A. H. Krezdorn & C. O. Youtsey. 1989. Rootstocks for Florida citrus. Publication no. SP42, University of Florida IFAS, Gainesville, FL.
- CoHort Software. 1994. CoStat, version 4.21. CoHort Software, Berkeley, CA.
- Crawley, M. J. 1989. Insect herbivores and plant population dynamics. *Annu. Rev. Entomol.* 34: 531-564.
- Horber, E. 1980. Types and classification of resistance, pp. 15-21. In F. G. Maxwell & P. R. Jennings [eds.], *Breeding plants resistant to insects*. Wiley, New York.
- Nordby, H. E. & S. Nagy. 1981. Chemotaxonomic study of neutral coumarins in roots of *Citrus* and *Poncirus* by thin-layer, gas-liquid and high-performance liquid chromatographic analyses. *J. Chromatogr.* 207: 21-28.
- Norman, P. A., A. G. Selhime & R. A. Sutton. 1974. Feeding damage to five citrus rootstocks by larvae of *Diaprepes abbreviatus* (Coleoptera: Curculionidae). *Fla. Entomol.* 57: 296.
- Ostwan, E. E. & D. C. Peters. 1980. Introduction, pp. 3-21. In F. G. Maxwell & P. R. Jennings [eds.], *Breeding plants resistant to insects*. Wiley, New York.
- Painter, R. H. 1951. *Insect resistance in crop plants*. University Press of Kansas, Lawrence.
- SAS Institute. 1987. SAS/STAT guide for personal computers, version 6 ed. SAS Institute, Cary, NC.
- Schroeder, W. J. 1988. Entomogenous nematodes for root weevil control in citrus, pp. 1223-1226. In R. Goren & K. Mendel [eds.], *Proceedings, Sixth International Citrus Congress*, Tel Aviv, Israel, March 6-11, 1988. Balaban, Philadelphia.
- Schroeder, W. J. & J. B. Beavers. 1977. Citrus root weevils in Florida: identification, biology, and control. *Proc. Int. Soc. Citric.* 2: 498-500.
- Schroeder, W. J., R. A. Hamlen & J. B. Beavers. 1979. Survival of *Diaprepes abbreviatus* larvae on selected native and ornamental Florida plants. *Fla. Entomol.* 62: 309-312.
- Schultz, J. C. 1983. Impact of variable plant defensive chemistry on susceptibility of insects to natural enemies, pp. 37-54. In P. A. Hedin [ed.], *Plant Resistance to Insects*. American Chemical Society Symposium Series, Washington, DC.
- Shapiro, J. P. 1989. Xenobiotic absorption and binding by proteins in hemolymph of the weevil *Diaprepes abbreviatus*. *Arch. Insect Biochem. Physiol.* 11: 65-78.
1991. Phytochemicals at the plant-insect interface. *Arch. Insect Biochem. Physiol.* 17: 191-200.
- Shapiro, J. P., R. T. Mayer & W. J. Schroeder. 1988. Absorption and transport of natural and synthetic toxins mediated by hemolymph proteins, pp. 997-1005. In F. Sehnal, A. Zabza & D. L. Denlinger [eds.], *Endocrinol. frontiers in physiol. insect ecology*. Wroclaw Technical University, Wroclaw, Poland.
- Slansky, F., Jr. & J. M. Scriber. 1985. Food consumption and utilization, pp. 87-163. In G. A. Kerkut & L. I. Gilbert [eds.], *Comprehensive insect physiology biochemistry and pharmacology*. Pergamon, New York.
- Smith, C. M. 1989. *Plant resistance to insects: a fundamental approach*. Wiley, New York.

Received for publication 17 January 1994; accepted 14 September 1994.

Action Th Mana (Col

GEOFFREY

Department of En

ABSTRACT Sn
'Superior' potato
(Say), defoliation
defoliation rating
feeding. Prelimin
in large plot expe
program in which
the defoliation th
sprays with no si
Action threshold
and 60% (late bl
the maximum le
given the usual,
use of the defoli
presented

KEY WORDS

ON THE EASTERN shore of the eastern U.S. potato-growing areas, insecticides used in potato production on the Colorado potato beetle (*Leptoglossus neata* (Say)). Survey data indicate that insecticides for potato beetle control average \$140 per hectare (Evanylo 1989) and may be as high as \$200 per season on some farms (unpublished data). Because of the high cost, growers do not rotate Colorado potato beetle in rotated fields adjacent to Colorado potato beetle. Ginning at plant emergence, before, as many as 12 or more applications are applied throughout the season. This is based on the presence of Colorado potato beetle damage without regard to the availability of potato plants to be treated. Incorporating knowledge of the susceptibility of potato plants to beetle feeding at various stages of plant growth may allow unnecessary insecticide applications to be eliminated, resulting in increased profit.

¹ Division of Biological Sciences, University of Georgia, GA 30602-2601.

² Virginia Polytechnic and State University, Agricultural Experiment Station, Blacksburg, VA 24061.