PLANT RESISTANCE

Is There Meaningful Plant Resistance to *Diaprepes abbreviatus* (Coleoptera: Curculionidae) in Citrus Rootstock Germplasm?

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ABSTRACT Host plant resistance to the Diaprepes root weevil, *Diaprepes abbreviatus* (L.) was assessed for seedlings of 54 *Poncirus trifoliata* (L.) Raf. selections and two families of progeny from crosses between *Citrus* and *P. trifoliata.* Weight gain was consistently lower when larvae were reared in pots containing the progeny of *Citrus reticulata* Blanco 'Sunki' x *P. trifoliata* 'Flying Dragon' compared with larvae reared on progeny of 'Pearl' (*C. reticulata x C. paradisi* Macf.) x 'Flying Dragon'. This is the first evidence of genetic control of resistance to the Diaprepes root weevil within sexually compatible citrus rootstock germplasm. There was a significant positive correlation between percentage root loss and larval weight gain within the resistant progeny, indicating a possible antixenotic effect. Two varieties of *P. trifoliata* were identified as more resistant than 'Flying Dragon' based on larval weight gain.

KEY WORDS citrus, host plant resistance, Diaprepes root weevil, *Diaprepes abbreviatus, Poncirus trifoliata*

THE DIAPREPES ROOT WEEVIL, *Diaprepes abbreviatus* (L.), apparently evolved in the Caribbean where it has been a major pest of principal crops such as sugarcane and citrus (O'Brien and Wibmer 1982). On Puerto Rico, *D. abbreviatus* is considered the single most damaging pest of agricultural commodities (R. Franqui, personal communication). This weevil, typical of the broad-nosed weevils of the curculionid subfamilies Brachyderinae and Otiorhynchinae, has a wide host range (Simpson et al. 1996). Hutson (1917) described *D. abbreviatus* as an important pest in the Caribbean of sugar-cane, corn, limes, cotton, sweet potatoes, onions, and ground nuts. Today, *D. abbreviatus* is found on Puerto Rico and Hispaniola and in the Lesser Antilles from Grenada and Barbados in the south to the Virgin Islands in the north. By 1933, it was recognized that the various forms of *Diaprepes* found throughout Hispaniola, Puerto Rico, and the Lesser Antilles were most likely a single species, fundamentally similar both structurally and behaviorally (Wolcott 1933). Puerto Rico is the apparent center of origin of *D. abbreviatus* because of the high degree of stable phenotypic diversity on the island. The weevil was first reported from the United States in 1964 when it was discovered in Florida (Woodruff 1964). Since that time, it has slowly colonized a major portion of the Florida peninsula and has become a major limitation to citrus production throughout the state. It is now reported from Texas (Texas Department of Agriculture 2001) and must be considered a threat to invade California.

In addition to the damage caused by root feeding, larval *D. abbreviatus* contribute to tree decline by providing infection courts for root rot pathogens such as *Phytophthora* spp., particularly in heavier, poorly drained soils. In Florida, such soil types have been highly valued for production of fresh grapefruit. The combination of the Diaprepes root weevil and *Phytophthora* now threatens that industry.

The orange subfamily, Aurantioideae, of the plant family Rutaceae, is large and taxonomically complex. The subfamily contains *Citrus* and 32 other genera with varying degrees of relatedness to *Citrus,* totaling >200 species (Swingle and Reece 1967). Within *Citrus,* taxonomic classifications have varied widely in the number of species proposed. Swingle (1946) recognized only 16 species while Tanaka (1977) listed 162 species of *Citrus.* Recent phylogenetic studies based on molecular analyses (Nicolosi et al. 2000) support most of Tanaka's groups, but it seems likely that many of these groups do not merit the status of botanical species. There are few genetic barriers to interspecific hybridization within *Citrus,* making the concept of species difficult to apply. There are also mechanisms, associated with a long history of cultivation and selection, which act to reduce intraspecific variability (Federici et al. 1998). Almost universal propagation of cultivated citrus by apomictic seed and grafting has resulted in a very narrow range of variability among

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the principal cultivated forms (e.g., sweet orange, grapefruit, and lemon) (Kijas et al. 1995, Fang and Roose 1997).

Attempts to identify sources of plant resistance in citrus rootstocks to *D. abbreviatus* have focused on sexually compatible species within the subtribe Citrinae, with little success (Norman et al. 1974, Beavers and Hutchison 1985, Shapiro and Gottwald 1995, Grosser and McCoy 1996). Recently, representatives of the remote citroid fruit trees (Clauseninae, sensu Swingle) have been identified as resistant to larval *D. abbreviatus* (Shapiro et al. 1997, 2000; Lapointe et al. 1999; Bowman et al. 2001). *Glycosmis pentaphylla* (Retzius) Correa was shown to inhibit larval growth and survival of *D. abbreviatus* due to the presence in its roots of the amide dehydrothalebanin B (Shapiro et al. 1997, 2000). *Murraya koenigii* (L.) Sprengel, an ornamental citroid fruit tree, also depressed larval growth and survival compared with true citrus rootstocks (Lapointe et al. 1999). Unfortunately, these species are sexually incompatible with true citrus and do not make good rootstocks themselves, although near-citrus relatives may be sources of resistance characters amenable to manipulation by molecular methods.

Despite the relative lack of success to date in finding resistance within true citrus rootstocks, the citrus subtribe (Citrinae) has not been adequately surveyed for resistance to *D. abbreviatus,* due in part to difficulties involved in conducting bioassays with this long-lived, subterranean insect feeding on roots of slow-growing trees. Of the six sexually compatible genera included in the group designated by Swingle as "true citrus fruit trees," he considers the monotypic genus *Poncirus* Raf. to be the most genetically distinct genus based on unique characteristics such as trifoliate deciduous leaves and cold-hardiness (Swingle and Reece 1967). Here we report results of a screen of *Poncirus trifoliata* (L.) Raf. germplasm for resistance to larval feeding by *D. abbreviatus,* and the segregation of resistance to larval feeding in progeny of *P. trifoliata* crosses. These results represent the first evidence of genetic control of resistance in citrus rootstocks to root-feeding weevils.

Materials and Methods

Seeds of selected citrus rootstocks, hybrids, and citrus relatives were harvested from fruit, treated with 8-quinolinol sulfate (Eastman Kodak, Rochester, NY) as a preservative, dried, and stored at 4°C until use. Seeds were planted directly into individual plastic cells (4 by 21 cm with a rooting depth of ≈15 cm) (SC-10 super cell Cone-tainers, Stuewe and Sons, Corvalis, OR) containing sterile sand. A square of plastic screen was placed over the drain holes and each cell was nested into another to hold the screen in place and thereby prevent larvae from escaping. Seedlings were maintained throughout the experiment on elevated benches in a greenhouse with an average diurnal temperature cycle of 35°C maximum and 23°C minimum in the summer, and a diurnal cycle of 32 and 20°C in the winter. No supplemental light was supplied. Maximum photosynthetic photon flux in the greenhouse was 800 mol $\cdot$ s^{-1} $\cdot$ m^{-2}. Plants were watered with a dilute fertilizer mix weekly using water soluble (N:P:K, 20:10:20) at a rate of 150 mg $\cdot$ $liter^{-1}$ N. We selected 21 uniform seedlings of each genotype at 3.5–5 mo after germination. Fourteen plants were infested and seven plants served as uninfested controls.

Larvae of *D. abbreviatus* were obtained from an artificial colony maintained at the U.S. Horticultural Research Laboratory (USHRL), Orlando, FL, and reared as described by Lapointe and Shapiro (1999). For the infested treatment, two 3-wk-old larvae weighing between 10 and 40 mg were placed in each plastic cell with one healthy plant. This level of infestation had previously been determined to be optimal for this test because it minimizes escapes and provides sufficient root mass for continuous feeding throughout the infestation period (Lapointe et al. 1999). Larvae and roots were recovered after 28 d and weighed. To calculate the percentage of weight increase, the weights of larvae recovered from each cone were compared with the mean initial weight of the two larvae infesting the respective cone. The means for fresh weight of larvae were analyzed by Fisher protected least significant difference (LSD) after a significant analysis of variance (ANOVA) (Abacus Concepts 1996). Larval weight gain was compared by using the mean square term for plastic cell. Percentage of root loss was calculated by comparing the weight of infested roots with the mean weight of uninfested controls for each genotype. The angular transformation (arcsine) was applied to the data to stabilize variance. Transformed means were compared by Fisher Protected LSD after a significant ANOVA (Abacus Concepts 1996).

Trial I: First Bioassay of Hybrid Progeny from Two Crosses. Selected rootstock progeny of two crosses between varieties of *Citrus* and *P. trifoliata* were tested for resistance to *D. abbreviatus.* These included progeny from a cross between Pearl tangelo (*C. paradisi* x *C. reticulata* 'Pearl') and *P. trifoliata* 'Flying Dragon', and progeny from the cross *C. reticulata* L. Blanco 'Sunki' x 'Flying Dragon'. Common commercial rootstock cultivars, 'Swingle' (*C. paradisi* Macf. x *P. trifoliata*), 'Carrizo' citrange [*C. sinensis* (L.) Osbeck x *P. trifoliata*], and the resistant species *G. pentaphylla* were included as controls (Lapointe et al. 1999) (Table 1). Seedlings in this trial were infested 5 mo after germination. Seedlings used in trial I were considered older than the optimal age for this bioassay because the seedlings were nearly root-bound in the plastic cells at the time of infestation with larvae. Younger seedlings were selected for trial II.

Trial II: Second Bioassay of Hybrid Progeny. The first trial was repeated with a similar set of progeny (Table 1) to confirm the results of trial I. Seedlings in trial II were infested at 3.5 mo after germination. In addition, to the progeny, the parents of the two crosses ('Flying Dragon', 'Pearl', and 'Sunki') were included. Five progeny genotypes from trial I were unavailable and not tested in trial II.

Table 1. Genotypes tested for resistance to the Diaprepes root weevil in three trials (numbers refer to number of entries [genotypes])

Genotype	Cultivar or accession	Trial 1	Trial 2	Trial 3
C. paradisi x *P. trifoliata*	'Swingle'	1	1	1
C. reticulata	'Sunki'		1	
C. reticulata x *C. paradisi*	'Pearl'		1	
C. sinensis x *P. trifoliata*	'Carrizo'	1	1	
G. pentaphylla		1	1	1
M. koenigii		1		
P. trifoliata	'Flying Dragon'		1	1
'Sunki' x 'Flying Dragon' progeny		14	13	
'Pearl' x 'Flying Dragon' progeny		12	9	
P. trifoliata				53

Trial III: Survey of varieties of *P. trifoliata*. Fifty-four varieties of *P. trifoliata* were tested including 'Flying Dragon' (Table 1). Controls consisted of *G. pentaphylla* and 'Swingle' Citrumelo. Seedlings were infested 4.5 mo after germination. Final root volume was estimated instead of root weight because the seedlings at the end of the feeding trial were used in another bioassay, to be reported elsewhere. Root volume was measured by inserting the root mass into a graduated cylinder to measure water displacement. Root loss was then calculated as described above.

Results

Trial I: First Bioassay of Hybrid Progeny from Two Crosses. Weight gain of larval *D. abbreviatus* was least on *M. koenigii* and *G. pentaphylla* compared with the remaining genotypes (Table 2). These species have been reported as resistant (Lapointe et al. 1999, Shapiro et al. 1997) and were included here as controls. Of the other controls, larvae reared on 'Carrizo' gained the most weight and larvae reared on 'Swingle' were intermediate in weight between the resistant controls and 'Carrizo'. The distribution of weight gain data for the progeny of 'Sunki' x 'Flying Dragon' and 'Pearl' x 'Flying Dragon' tended to a bimodal distribution and therefore a posthoc analysis by ANOVA was done using "cross" ('Pearl' x 'Flying Dragon' and 'Sunki' x 'Flying Dragon') as a descriptive variable for the progenies. Both final larval weight and larval weight gain varied significantly by cross. The mean final weight (±SEM) of larvae reared on progeny of 'Sunki' x 'Flying Dragon' (63.9 ± 1.5 mg) was reduced by 25% compared with that of larvae reared on progeny of 'Pearl' x 'Flying Dragon' (85.2 ± 2.2 mg) ($F = 72.2$; df = 1, 539; $P < 0.01$). Similarly, mean weight gain of larvae reared on progeny of 'Sunki' x 'Flying Dragon' (45.3 ± 1.5 mg) was reduced by 35% compared with that of larvae reared on progeny of 'Pearl' x 'Flying

Table 2. Mean survival and weight gain (±SEM) of Diaprepes root weevil larvae reared for 28 d on roots of citrus seedlings in 21-cm plastic cells in a greenhouse (trials I and II)

Trial I			Trial II		
Genotype	Survival (%)	Weight gain (mg)	Genotype	Survival (%)	Weight gain (mg)
M. koenigii	70.8	4.7 ± 2.0a	*G. pentaphylla*	67.9	31.3 ± 3.6a
G. pentaphylla	70.8	5.0 ± 1.9a	5-48-13	64.3	70.1 ± 6.9b
5-48-19	67.9	33.3 ± 5.8b	'Flying Dragon'	46.4	70.7 ± 12.5bc
5-48-22	71.4	36.7 ± 4.9b	5-48-19	46.4	78.9 ± 9.6bcd
5-49-7	78.6	36.8 ± 4.1bc	5-49-7	57.1	81.0 ± 11.4bcd
5-48-2	82.1	40.6 ± 5.1bcd	5-48-23	46.4	83.9 ± 11.2bcde
5-48-26	85.7	41.0 ± 4.9bcde	5-48-10	57.1	84.3 ± 9.5bcde
5-49-16	82.1	41.4 ± 5.9bcde	5-48-11	46.4	92.4 ± 10.6bcdef
5-48-32	85.7	43.0 ± 6.0bcde	5-48-31	42.9	94.6 ± 9.7bcdefg
'Swingle'	85.7	43.2 ± 4.6bcde	5-73-32	53.6	94.8 ± 9.3bcdefg
5-48-10	88.5	43.6 ± 5.7bcde	5-48-24	50.0	99.5 ± 11.4bcdefg
5-48-11	78.6	48.8 ± 5.0bcdef	5-48-22	42.9	99.8 ± 11.5bcdefgh
5-49-15	96.4	49.5 ± 3.7bcdefg	'Sunki'	50.0	101.7 ± 9.7cdefgh
5-48-31	78.6	49.8 ± 7.3bcdefg	5-48-26	42.9	101.9 ± 8.4cdefgh
5-48-13	67.9	52.5 ± 4.7cdefg	'Pearl'	64.3	102.4 ± 15.6defgh
5-73-32	89.3	53.2 ± 5.1cdefg	5-74-16	46.4	102.8 ± 16.5defgh
5-74-40	67.9	55.1 ± 5.6defgh	5-48-20	57.1	105.2 ± 12.4defgh
5-74-9	71.4	55.9 ± 8.5defgh	'Swingle'	46.4	106.3 ± 14.6defgh
5-48-23	82.1	56.7 ± 5.9efgh	5-75-6	71.4	106.7 ± 8.3defgh
5-75-3	46.4	57.0 ± 4.5efgh	5-75-24	67.9	107.4 ± 8.7defgh
5-48-24	92.3	57.6 ± 6.0efghi	5-48-32	50.0	110.2 ± 12.9defgh
5-74-2	89.3	62.6 ± 8.1fghij	5-49-16	53.6	112.8 ± 14.0efgh
5-74-5	75.0	65.6 ± 7.2ghijk	5-74-5	39.3	120.0 ± 19.5fgh
5-74-16	82.1	71.1 ± 7.7hijkl	5-75-3	50.0	125.9 ± 16.5gh
5-75-6	85.7	76.4 ± 5.5ijkl	5-74-40	42.9	127.9 ± 13.4gh
5-75-1	57.1	78.2 ± 5.2jkl	'Carrizo'	60.7	132.3 ± 8.6h
5-74-37	75.0	80.6 ± 8.7kl	5-74-9	46.4	134.0 ± 9.7h
'Carrizo'	92.9	84.3 ± 7.9l	5-74-2	39.3	174.2 ± 14.6i
5-75-24	82.1	86.3 ± 6.9l			
5-75-22	71.4	88.3 ± 9.3l			

Means followed by the same letter are not significantly different at $\alpha = 0.05$ by Fisher's protected LSD after a significant ANOVA (trial I: $F = 10.4$; df = 29, 627; $P < 0.01$; trial II: $F = 5.0$; df = 27, 378; $P < 0.01$).

Table 3. Mean ± SEM reduction of root mass of citrus seedlings infested with Diaprepes root weevil larvae for 28 d in 21-cm plastic cells in a greenhouse (trials I and II)

Trial I			Trial II		
Genotype	*n*	Root loss (%)	Genotype	*n*	Root loss (%)
5-75-1	14	−34.0 ± 12.5a	5-73-32	14	55.5 ± 8.5a
5-75-22	14	−31.9 ± 22.7a	5-48-20	14	60.4 ± 4.9ab
5-48-22	14	−29.4 ± 15.7a	5-74-5	14	60.9 ± 9.3abc
5-49-16	14	−18.6 ± 13.2ab	5-48-32	14	62.5 ± 6.5abc
5-48-19	14	−16.1 ± 12.5abc	5-49-7	14	64.3 ± 6.7abcd
5-74-40	14	−9.8 ± 8.7abcd	5-74-9	14	66.6 ± 4.7abcd
5-74-37	14	3.7 ± 8.2bcde	5-48-10	14	68.6 ± 5.7abcde
5-49-7	14	4.0 ± 7.5bcde	5-75-3	14	68.6 ± 6.0abcde
'Swingle'	14	4.1 ± 7.9bcde	'Swingle'	14	69.8 ± 4.0abcdef
5-48-20	14	6.3 ± 7.8bcde	5-48-23	14	70.6 ± 5.6bcdefgh
5-74-9	14	7.1 ± 7.6bcde	5-75-6	14	71.0 ± 4.1abcdefg
5-75-3	14	11.3 ± 6.2cdef	5-48-19	14	71.3 ± 5.3bcdefgh
5-48-32	14	11.4 ± 8.0cdef	'Sunki'	14	72.3 ± 7.3bcdefghi
5-74-5	14	13.4 ± 6.2def	'Flying Dragon'	14	73.0 ± 4.1bcdefghi
5-73-32	14	15.8 ± 5.6defg	5-48-24	14	73.6 ± 5.5cdefghi
5-48-10	13	16.6 ± 7.6defg	5-74-16	14	73.7 ± 4.6cdefghi
5-48-31	14	17.1 ± 7.6defg	5-48-13	14	75.1 ± 4.0cdefghij
5-75-24	14	17.7 ± 9.1defg	5-48-22	14	76.4 ± 2.4cdefghij
G. pentaphylla	12	18.0 ± 17.6defg	5-75-24	14	78.0 ± 2.6defghij
5-48-11	14	21.0 ± 7.6efg	'Carrizo'	14	78.1 ± 2.9defghij
5-48-26	14	21.2 ± 8.6efg	5-48-31	14	80.4 ± 4.1fghij
5-49-15	14	22.2 ± 7.1efg	5-48-11	14	80.5 ± 2.4efghij
5-74-16	14	24.6 ± 7.0efg	'Pearl'	14	81.1 ± 4.5ghij
5-48-13	14	25.2 ± 8.3efg	5-49-16	14	81.2 ± 5.0hij
5-75-6	14	27.9 ± 10.3efg	5-48-26	14	82.4 ± 2.3fghij
5-48-23	14	29.0 ± 7.7efg	5-74-40	14	84.1 ± 2.4ij
M. koenigii	12	30.3 ± 10.0efg	5-74-2	14	86.3 ± 2.5j
5-48-24	13	36.7 ± 17.0fgh			
5-74-2	14	42.1 ± 5.9gh			
'Carrizo'	14	62.1 ± 5.4h			

Means followed by the same letter are not significantly different at $\alpha = 0.05$ by Fisher's protected LSD after a significant ANOVA (trial I: $F = 4.6$; df = 29, 384; $P < 0.01$; trial II: $F = 4.6$; df = 26, 351; $P < 0.01$). Data are untransposed means.

Dragon' (69.4 ± 2.2 mg) ($F = 93.0$; df = 1, 539; $P < 0.01$).

Survival of the larvae ranged between 13 (46%) and 27 (96%) larvae recovered from an initial infestation of 28 although the design of the trial (2 larvae per pot) did not allow for statistical comparison of survival data (Table 2). The mean percentage root loss in trial I ranged from −34 to 62% (Table 3).

There was no significant correlation between percentage root loss and larval weight gain (linear regression, $\alpha = 0.05$) when all data points were included. However, there was a significant positive correlation ($y = 2.1x-84.2$, $r^2 = 0.66$, $t = 4.9$, $P < 0.01$) between these two variables for the progeny of the 'Sunki' x 'Flying Dragon' cross (Fig. 1).

Trial II: Second Bioassay of Hybrid Progeny. Weight gain of larval *D. abbreviatus* was least on *G. pentaphylla* compared with the remaining genotypes (Table 2). As in trial I, there was a statistically significant separation of the final weight and weight gain of larvae reared on the progeny of 'Sunki' x 'Flying Dragon' and 'Pearl' x 'Flying Dragon' when grouped by cross. Both final larval weight and larval weight gain varied significantly by cross. The mean final weight (±SEM) of larvae reared on progeny of 'Sunki' x 'Flying Dragon' (122.9 ± 3.1 mg) was reduced by 17% compared with that of larvae reared on progeny of 'Pearl' x 'Flying Dragon' (148.4 ± 4.5 mg) ($F = 28.2$; df = 1, 290; $P < 0.01$). Similarly, mean weight gain of larvae reared on progeny of 'Sunki' x 'Flying Dragon' (92.9 ± 3.1 mg) was reduced by 22% compared with that of larvae reared on progeny of 'Pearl' x 'Flying Dragon' (118.8 ± 4.4 mg) ($F = 30.2$; df = 1, 290; $P < 0.01$). Survival of the larvae ranged between 11 (39%)

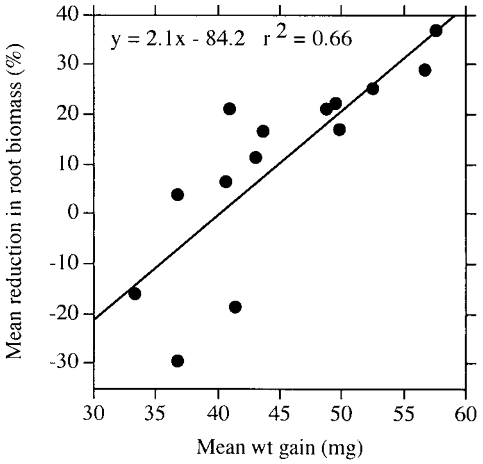

Fig. 1. Correlation of mean weight gain of larvae feeding on roots, and mean percentage reduction in root biomass for 14 selections from the progeny of the cross 'Sunki' x 'Flying Dragon'.

Table 4. Mean survival and weight gain ± SEM of Diaprepes root weevil larvae reared for 28 d on seedlings, and mean ± SEM reduction of root mass of seedlings of 56 varieties of *P. trifoliata* in 21-cm plastic cells in a greenhouse (trial III)

Genotype	Survival (%)	Weight gain (mg)	Root loss (%)
G. pentaphylla	0.0		26.3 ± 14.5abcd
Marks-1 4n	57.1	40.1 ± 10.3a	56.8 ± 10.8ijklm
Marks-11	50.0	47.9 ± 11.2ab	53.6 ± 8.7fghijklm
English Large	42.9	55.1 ± 16.4abc	37.4 ± 7.4bcdefgh
Gainesville (71)	60.7	58.0 ± 11.7abc	66.2 ± 6.8klm
Christiansen 4n	67.9	58.8 ± 12.8abc	55.2 ± 6.5hijklm
Jacobson	50.0	58.9 ± 12.4abcd	46.8 ± 6.6cdefghij
Ronnse	53.6	59.4 ± 16.8abcd	52.4 ± 6.5efghijk
ARB 9-6	71.4	60.1 ± 9.6abcd	52.4 ± 7.1efghijk
Rubidoux	60.7	60.7 ± 9.3abcd	49.7 ± 6.3cdefghij
Marks-1 2n	39.3	62.5 ± 12.5abcde	51.1 ± 9.4fghijkl
Large Flower (China) 4n	57.1	62.8 ± 17.6abcde	32.1 ± 10.5abcdefg
English Dwarf	50.0	63.3 ± 15.3abcde	30.1 ± 6.2abcde
Rich (12-2)	71.4	64.0 ± 11.1abcde	44.4 ± 8.3bcdefghij
Davis (a)	75.0	64.2 ± 11.8abcde	33.6 ± 6.7abcdef
Kryder (43-3)	60.7	65.5 ± 11.1abcde	26.8 ± 7.6abc
Towne G	46.4	66.8 ± 14.9abcdef	50.3 ± 6.0cdefghij
Chambers	57.1	66.9 ± 13.8abcdef	46.8 ± 8.3cdefghij
Pomeroy	64.3	67.0 ± 12.8abcdef	72.2 ± 3.8m
Rich (21-3)	64.3	67.3 ± 10.0abcdef	62.2 ± 6.3ijklm
Marks-13	64.3	68.2 ± 8.4abcdef	52.7 ± 8.7fghijklm
English Small	57.1	69.8 ± 12.1abcdef	44.3 ± 5.3bcdefghi
Kryder (8-5)	57.1	73.5 ± 13.6abcdefg	23.8 ± 8.9ab
Gotha Road #2	28.6	74.1 ± 15.2abcdefgh	69.6 ± 6.6klm
Marks Small	64.3	74.5 ± 16.1abcdefgh	12.7 ± 10.4a
Gotha Road #1	57.1	74.9 ± 12.9abcdefgh	51.2 ± 6.4defghij
Rich (16-6)	71.4	75.0 ± 10.9bcdefgh	53.6 ± 6.3fghijklm
Kryder (25-4)	71.4	75.5 ± 9.9bcdefgh	28.6 ± 8.2abcd
Swingle TO	50.0	75.9 ± 17.1bcdefgh	37.7 ± 8.2bcdefgh
Large Flower (Aust.)	53.6	79.2 ± 13.0bcdefgh	33.3 ± 6.8abcdef
Kryder (15-3)	71.4	81.7 ± 14.8bcdefgh	44.6 ± 4.5bcdefghi
Gainesville (70)	57.1	81.8 ± 13.0bcdefgh	56.5 ± 8.7ijklm
Medium	53.6	83.2 ± 13.1bcdefgh	49.8 ± 7.9cdefghij
Large Flower (China) 2n	67.9	86.7 ± 10.9cdefgh	50.7 ± 7.6defghij
Yamaguchi	85.7	87.4 ± 10.5cdefgh	66.0 ± 5.1jklm
Rich (22-2)	53.6	88.3 ± 10.2cdefgh	71.9 ± 5.5klm
Large Flower	67.9	88.3 ± 11.2cdefgh	46.1 ± 6.2cdefghij
Kryder Medium	53.6	88.5 ± 12.1cdefgh	25.7 ± 13.5abc
Rubidoux-123 2n	75.0	89.3 ± 11.9cdefgh	58.3 ± 5.2hijklm
Argentina (Rusk)	64.3	90.5 ± 12.5cdefgh	36.5 ± 8.7bcdefgh
Rich (6-6)	75.0	90.5 ± 10.9cdefgh	62.5 ± 3.6ijklm
Towne F 2n	35.7	92.5 ± 12.5cdefgh	56.2 ± 6.9ghijklm
Rich (7-5)	71.4	93.9 ± 11.6defgh	53.0 ± 5.6fghijkl
'Flying Dragon'	57.1	95.0 ± 17.3defgh	49.0 ± 7.9cdefghij
Small Flower	60.7	97.6 ± 12.3efgh	53.6 ± 6.5fghijklm
Small Flower #23	78.6	99.6 ± 9.6efgh	48.4 ± 4.7cdefghij
Benecke	67.9	101.6 ± 12.9efgh	53.4 ± 4.7efghijkl
'Swingle' Citrumelo	78.6	101.6 ± 9.1fgh	64.3 ± 8.4lm
Christiansen 2n	42.9	104.5 ± 16.2fgh	40.1 ± 8.5bcdefghi
Towne F 4n	67.9	106.0 ± 11.8gh	63.9 ± 5.2jklm
Kryder (55-1)	71.4	113.3 ± 13.4h	55.1 ± 7.8hijklm
Kryder (28-3)	67.9	113.3 ± 12.8h	48.3 ± 5.7cdefghij
Small Flower (China)	71.4	114.2 ± 16.6h	51.7 ± 6.6fghijklm
Rich (5-2)	35.7	114.2 ± 19.5h	55.1 ± 5.3fghijklm
Kryder (5-5)	57.1	114.9 ± 11.9h	46.8 ± 7.1cdefghij
Argentina	60.7	117.4 ± 15.1h	44.3 ± 10.5cdefghij

Means followed by the same letter are not significantly different at $\alpha = 0.05$ by Fisher's protected LSD after a significant ANOVA (weight gain: $F = 2.2$; df = 54, 876; $P < 0.01$; root loss: $F = 2.8$; df = 55, 728; $P < 0.01$).

and 20 (71%) larvae recovered from an initial infestation of 28 (Table 2). The mean percentage root loss in trial II ranged from 56–86% (Table 3).

As in trial I, there was no significant correlation between the percentage root loss and larval weight gain (linear regression, $\alpha = 0.05$) when all data points were included. There was a significant positive correlation ($y = 0.004x + 0.4$, $r^2 = 0.33$, $t = 2.3$, $P = 0.04$) between these two variables for the progeny of the 'Sunki' x 'Flying Dragon' cross. Data for the genotypes common to trials I and II were pooled to examine genotype x trial and cross x trial interactions for larval weight gain. There was a significant interaction between genotype and trial ($F = 3.4$; df = 21, 836; $P < 0.01$). There was no significant interaction between cross and trial ($F = 0.08$; df = 1, 880; $P = 0.78$). The main effects of cross ($F = 94.6$; df = 1, 880; $P < 0.01$) and trial ($F = 356.0$; df = 1, 880; $P < 0.01$) were significant. The mean weight gain of larvae reared on progeny of the cross 'Sunki' x 'Flying Dragon' was 62.2 ± 1.8 mg compared with 85.9 ± 2.4 mg for larvae reared on progeny of the cross 'Pearl' x 'Flying Dragon'. The mean difference between the two groups of progenies was 28%.

Trial III: Survey of varieties of *P. trifoliata*. The mean weight gain of larval *D. abbreviatus* reared on 54 varieties of *P. trifoliata* (and the control, 'Swingle' Citrumelo) ranged from 40 to 117 mg and mean percentage root loss ranged from 13 to 72% (Table 4). Larvae reared on five varieties of *P. trifoliata* [Marks-1, Marks-11, English Large, and Gainesville (71)] gained significantly less weight than 'Flying Dragon' (Table 4). Two varieties [Marks Small and Kryder (8–5)] suffered significantly less root loss than 'Flying Dragon' (Table 4). There was no significant correlation between mean larval weight gain or final larval weight and percentage root loss (linear regression, $\alpha = 0.05$).

Discussion

It is particularly difficult to assess plant resistance in citrus trees to a subterranean, slow-growing univoltine insect such as the Diaprepes root weevil. A 28-d infestation period was selected to screen for plant resistance in citrus and citrus relatives (Lapointe et al. 1999) for convenience and because the period of infestation corresponds to a phase of continuous feeding and weight gain by the larvae (Lapointe 2000). Variables used to assess resistance include percentage larval survival, larval weight gain, and percentage root loss relative to uninfested controls (Lapointe et al. 1999, Bowman et al. 2001). The use of small plastic cells enables us to screen larger populations with reduced labor and materials. However, the design (infestation of two larvae per cell) does not allow for a statistical assessment of larval survival. This is not considered a problem because larvae of the Diaprepes root weevil are capable of surviving prolonged periods in soil without feeding (Lapointe and Shapiro 1999) and therefore larval survival over the period of infestation may not be a reliable indicator of plant resistance. Larval weight gain remains the most appropriate indicator of antibiotic or antixenotic resistance.

Trials I and II differed in the age of the plants at infestation. Trial I used older, slightly root-bound seedlings compared with those used in trial II. Perhaps as a result, the mean weight gain of larvae in trial I was significantly less than that in trial II. This suggests that larvae develop more quickly on roots of younger, actively growing seedlings. In trial I, the percentage root loss was less compared with trial II and, in some cases, infested plants actually had greater root mass at the end of infestation period compared with uninfested plants. This could be due to a stimulatory effect on root compensatory growth due to root pruning by larvae. This did not occur in trial II where the root mass was less at the beginning of the period of infestation. Despite these differences the results of the two trials were equivalent in terms of the differentiation of the two families of progeny for plant resistance.

In this study, there was a significant difference in the weight gain of larvae reared on the two families tested in both trial I and trial II. This is the first direct evidence of genetic control of resistance to the Diaprepes root weevil in true citrus. There was also a correlation between percentage root loss and weight gain within the resistant family ('Sunki' x 'Flying Dragon') suggesting that larvae were deterred from feeding on the more resistant genotypes. There was a high degree of variability in weight gain of larvae within families and weight gain data for genotypes within families were not consistent between trials (i.e., there was a significant genotype x trial interaction). This is likely due to a high degree of variability (noise) in the bioassay despite our efforts to control environmental and plant conditions and initial conditions of larval infestation. However, the consistent separation of the two progeny groups we tested demonstrates that they differed in one or more resistance factors, and indicates that breeding for root resistance to the Diaprepes root weevil within Citrinae is possible. The lack of any measurable difference in resistance between the parents that differed for the two progeny groups ('Sunki' and 'Pearl') suggests that recessive traits and/or combining ability may play important roles in resistance.

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