Identification of Sources of Plant Resistance to *Diaprepes abbreviatus* (Coleoptera: Curculionidae) by Three Bioassays

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ABSTRACT Host plant resistance to the root weevil *Diaprepes abbreviatus* (L.) was assessed for 3 citrus rootstock cultivars, 5 promising hybrid rootstocks, and 3 citroid fruit trees using 3 bioassay methods: a pot bioassay with 1-yr seedlings; a new, 21-cm plastic cell bioassay with 5-mo seedlings; and a diet incorporation bioassay. The plastic cell bioassay is a more rapid screening method and is capable of evaluating a larger number of entries in a shorter period compared with current methods. The 3 bioassays yielded similar results. Larval growth was inhibited by 2 of the remote citroid fruit trees, *Murraya koenigii* (L.) Sprengel and *Glycosmis pentaphylla* (Retzius) Correa, compared with growth on commercial rootstock cultivars. Specifically, larvae allowed to feed on roots of *M. koenigii* or *G. pentaphylla* gained less weight compared with larvae fed on the commercial rootstock cultivar 'Swingle' [*Citrus paradisi* Macfayden × *Poncirus trifoliata* (L.) Rafinesque-Schmaltz]. The resistance of *G. pentaphylla* confirms previous reports. *M. koenigii* is a new source of resistance to *D. abbreviatus*.

KEY WORDS *Diaprepes abbreviatus*, citrus, host plant resistance, bioassay methods

The weevil *Diaprepes abbreviatus* (L.) causes losses in excess of $75 million yearly to citrus growers in 20 Florida counties (*Diaprepes* Task Force 1997). Of the 163,000 acres in the state considered infested as of July 1997, ≈20% represent citrus groves (Florida Department of Agriculture survey). In addition, to citrus, adults have been collected from ornamental nursery plants, native plants, and crops such as corn, peanut, sorghum, sugarcane, and sweet potato (Simpson et al. 1996). Because the larvae are subterranean, both the damage and area infested by this weevil are likely to be greatly underestimated.

Larval *D. abbreviatus* feed on roots of a wide range of host plants (Simpson et al. 1996). To date, no true citrus rootstock has been reported as resistant (Norman et al. 1974, Beavers and Hutchison 1985) with the possible exception of a low level of resistance in the rootstock 'Swingle' [*Citrus paradisi* Macfayden x *Poncirus trifoliata* (L.) Rafinesque-Schmaltz] (Shapiro and Gottwald 1995). Grosser and McCoy (1996) failed to find resistance in intergeneric somatic hybrids of citrus rootstocks with 4 genera of true citrus or near-citrus fruit trees within the subtribe Citrinae. However, at least 1 remote relative of citrus, *Glycosmis pentaphylla* (Retzius) Correa (subtribe Clauseninae of the orange subfamily Aurantioideae) is known to possess antibiotic resistance to *D. abbreviatus* as expressed by reduced larval weight gain (Shapiro et al. 1997). Although not sexually compatible with citrus, near-citrus relatives may be sources of resistance characters amenable to manipulation by molecular methods, or they may be sufficiently compatible to serve as rootstocks. Despite these efforts, the true citrus subtribe (Citrinae) has not been adequately surveyed for resistance to *D. abbreviatus*, caused in part by difficulties involved in conducting bioassays with this long-lived, subterranean insect feeding on roots of slow-growing trees. As sources of resistance are identified, rapid and reliable bioassays will be required to screen large numbers of progeny or to investigate in detail the effects of resistance on insect development.

We report here results from 3 bioassay methods and discuss their relative merits and applications. We also report the identification of antibiotic resistance in *Murraya koenigii* (L.) Sprengel (subtribe Clauseninae), an ornamental citroid fruit tree.

Materials and Methods

Three bioassays for assessing plant resistance to *D. abbreviatus* were compared in separate trials. Trial I compared larval growth and feeding damage to 1-yr-old seedlings of 8 citrus genotypes in 16-cm-diameter plastic pots (3.8 liters soil volume) following the protocol described by Shapiro and Gottwald (1995). Trial II compared the same parameters for 9 genotypes 5 mo after germination in 21-cm plastic cells used for rooting seedlings. For the trial in plastic cells, 3 levels of infestation were compared to determine optimal conditions for assessing insect survival and growth, and root damage. Trial III incorporated root powder from

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9 genotypes into artificial diet and compared larval weight gain on a defined diet.

Seeds of *M. koenigii*, *M. paniculata* (L.) Jack, and *C. pentaphylla* were provided by the USDA National Clonal Germplasm Repository for Citrus and Dates (Riverside, CA). Seeds of Swingle, 'Carrizo' citrange [*C. sinensis* (L.) Osbeck × *P. trifoliata*], 'Sun Chu Sha' mandarin (*C. reticulata* Blanco), 'Rough Lemon' (*C. jambhiri* Lushington), and the hybrid rootstocks HRS-801 (*C. reticulata* × *P. trifoliata*), HRS-802 [*C. grandis* (L.) Osbeck × *P. trifoliata*], HRS-812 (*C. reticulata* × *P. trifoliata*), HRS-896 (*C. reticulata* × *P. trifoliata*), and HRS-941 (*C. reticulata* × *P. trifoliata*) were harvested from trees at the USDA A.H. Whitmore U.S. Horticultural Research Laboratory Foundation Farm at Leesburg, FL. All seeds were extracted 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 in a peat/perlite/vermiculite potting mix (Pro-Mix BX, Premier Horticulture, Red Hill, PA) at a rate of 1 seed per cell in multicell trays (≈13 cm rooting depth) (Multi-Pots, Can-Am Containers, Springhill, Nova Scotia).

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).

Trial I: Pot Bioassay with 1-yr-old Seedlings. Seedlings of Carrizo, Swingle, *G. pentaphylla*, *M. koenigii*, *M. paniculata*, HRS-802, HRS 896, and HRS 941 were grown in a greenhouse from seeds planted in multipots and transferred to 3.8-liter pots (16 by 16 cm) 4 mo later as described by Shapiro and Gottwald (1995). Carrizo and Swingle are standard citrus rootstocks and served as susceptible controls. The HRS entries are promising USDA hybrids currently considered for release as rootstocks. Seedlings were infested 1 yr after planting when they were ≈1 m tall. 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 20-10-20 (N-P-K) at a rate of 150 mg $\cdot$ $liter^{-1}$ N. Each pot was infested with 10 larvae weighing between 15 and 35 mg each. Initial larval weights were recorded to obtain mean weight of larvae infesting each pot. Older larvae were used instead of neonates to assure a uniform number of larvae per pot and to avoid movement of active neonates between pots. There were 7 replications (pots) of each treatment with an equal number of uninfested controls for each treatment. Larvae were removed and weighed 5 wk after infestation by sieving the soil in the pots. The change in fresh weight of individual larvae over the course of the experiment was estimated by subtracting final weight from mean initial weight of larvae infesting each pot. Larval weight gain was analyzed for larvae nested within pots by analysis of variance (ANOVA) and means were compared by the Tukey honestly significant difference (HSD) test (Abacus Concepts 1996). Sources of variation were genotype and pot and the mean-square term for 'pot' was used for comparison of larval weight gain. Roots were washed, allowed to air-dry, and weighed. Percentage of root loss and percentage of recovery of larvae were calculated and the angular transformation (arcsine) was applied to the data to stabilize variance. Transformed means were compared by the Tukey HSD after a significant ANOVA (Abacus Concepts 1996). Because initial root weights were variable between plant genotypes, final root weights for infested and noninfested plants were compared by Student *t*-test for unpaired samples.

Trial II: Plastic Cell Bioassay. To determine an optimal level of infestation for the volume of soil and root mass in plastic cells, a trial was conducted with 4 levels of infestation: 0, 1, 2, or 4 larvae per plastic cell containing individual seedlings of 3 rootstock cultivars: Carrizo, Swingle, or Rough Lemon. Healthy seedlings were selected 5 mo after germination and transplanted 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). During transplanting, a square of plastic screen was placed over the drain holes and a small amount of a soilless potting mix containing peat/vermiculite/pine bark (Metro Mix 500, Scotts Sierra Horticultural Products, Marysville, OH) was added to each plastic cell to prevent larvae from escaping. Plants were maintained in a greenhouse as described for trial I. Weevil larvae weighing between 8 and 28 mg were placed in each plastic cell. A 3 × 4 (cultivar by level of infestation) factorial design was used with 14 replications (plant). Plants, roots, and larvae were removed from the plastic cells 30 d after infestation. Fresh weights of roots and larvae were recorded and analyzed by ANOVA. Means were compared by the Tukey HSD test after a significant ANOVA. Percentage of root loss and percentage of recovery of larvae were transformed (arcsine) and compared by the Tukey HSD after a significant ANOVA (Abacus Concepts 1996).

We determined that 2 larvae per plastic cell was an optimal level of infestation from the results of the trial comparing levels of infestation. Nine genotypes were then compared using the same protocol described above for plastic cells: *G. pentaphylla*, *M. koenigii*, Swingle, Sun Chu Sha, and 5 promising rootstock hybrids (HRS 801, 802, 812, 896, and 941). Seed of *M. paniculata* was unavailable for this test. Two larvae weighing between 8 and 28 mg were placed in each plastic cell. Larvae and roots were recovered after 31 d and weighed. To calculate percentage of weight increase, the weights of recovered larvae were compared with the mean initial weight of larvae infesting the respective repetition (plastic cell). Plants were selected to be as uniformly sized as possible at infestation. A minimum of 12 plants were infested with an equal number of uninfested controls. The means for fresh weight of larvae were analyzed by the Tukey HSD test after a significant ANOVA (Abacus Concepts 1996). Larval weight gain was compared by using

the mean-square term for plastic cell. Mean fresh weight of roots was analyzed by ANOVA.

Trial III: Diet Incorporation Bioassay. Roots of 9 genotypes were collected from uninfested seedlings grown in 16 by 16-cm pots: Carrizo, *G. pentaphylla*, *M koenigii*, *M. paniculata*, and 5 hybrid rootstocks (HRS 801, 802, 812, 896, and 941). After storage at −80°C, roots were milled in a centrifugal mill (Retsch ZM-1000, Brinkmann, Westbury, NY) at 10,000 × *g* to <0.5 mm particle size. Diet for incorporation was prepared by first adding 14 g of agar to ≈800 ml water, and heating to ≈100°C while mixing with a Braun (Lynnfield, MA) type 4169 hand-held homogenizer. As the agar cooled, 184 g of citrus root weevil diet premix #1675 F (Bio-Serve, Frenchtown, NJ) was added. The mixture was thoroughly mixed and diluted to 1 liter with water. Diet was distributed to 100-ml beakers, and 10 g of roots were blended with 100 ml of diet when diet had cooled to a temperature of ≈50°C, the melting point of the agar used in the diet. Approximately 15 ml of diet were rapidly poured into each plastic 30-ml shot cup (Jet Plastica, Hatfield, PA), allowed to cool, and dried for ≈6 h under a laminar flow hood. Controls consisted of diet only with no root material added, and diet containing 10 g of cellulose.

Diet cups infested with neonate larvae of *D. abbreviatus* were obtained from the USHRL colony and held at 26°C for 3 wk. Larvae were removed from the cups and weighed until we had obtained 330 larvae weighing between 25 and 30 mg each. These were assigned to treatments in a completely randomized design. One larva was added to each of 10 cups of diet per treatment. Cups were covered, and larvae were allowed to feed for 32 d at an approximate temperature of 29°C at a photoperiod of 10:14 (L:D) h. Larvae were then separated from the diet and individually weighed. One-way ANOVA and posthoc means comparison (Tukey HSD) were performed using the Statistica version 5.0 Basic Statistics module (StatSoft 1995).

Results

Trial I: Pot Bioassay with 1-yr-old Seedlings. When allowed to feed on 1-yr-old seedlings, larvae of *D. abbreviatus* gained the most weight on HRS-802, followed by the cultivars Carrizo and Swingle and the hybrids HRS 896 and HRS 941 (Table 1). Compared with these, larval growth was significantly reduced on *G. pentaphylla* and the 2 species of *Murraya*. Larval weight gain on *M. koenigii* was only 4% of that on HRS 802 and 6% of that on the commercial rootstock Swingle. Fewer larvae were recovered from the soil in pots containing seedlings of *M. koenigii* and *G. pentaphylla* compared with pots containing Swingle (Table 2). However, given the difficulty of finding small larvae in the soil and the reduced growth of larvae reared on *M. koenigii*, reduced recovery may not be an accurate measure of survival in this bioassay. Significant root loss occurred for all genotypes tested except *M. koenigii* (Table 3). *M. koenigii* root weight was reduced by only 12% compared with a 58% reduction of the root mass of Swingle.

Trial II: Plastic Cell Bioassay. In the trial to determine an appropriate level of infestation, final root weight was affected by cultivar ($F = 31.4$; df = 2, 156; $P < 0.01$) and level of infestation ($F = 31.2$; df = 3, 156; $P < 0.01$) and there was no interaction between these factors ($F = 0.9$; df = 6, 156; $P = 0.52$). Regardless of level of infestation, fresh weight of roots was greatest for Swingle followed by Rough Lemon and Carrizo (Table 4). Infestation with a single larva per plastic cell resulted in a 30% reduction of root mass compared with the uninfested control, whereas infestation with 2 or 4 larvae per plastic cell increased the loss to 53 and 63%, respectively. Root damage was no greater with 4 larvae than with 2 larvae per plastic cell (Table 4). The level of infestation significantly affected the final weight of individual larvae. Larval weight declined by 19% in the treatment receiving 4 larvae, compared with the treatment receiving a single larva per plastic cell (Table 5). Cultivar had no effect on larval weight ($F = 1.1$; df = 2, 159; $P = 0.34$) and there was no interaction between level of infestation and cultivar ($F = 1.1$; df = 4, 159; $P = 0.38$). Similarly, the percentage of larvae recovered from each plastic cell at the end of the trial was affected by the initial level of infestation (Table 5) but not by cultivar ($F = 0.1$; df = 2, 117; $P = 0.86$), and there was no interaction ($F = 0.5$;

Table 1. Mean ± SEM fresh weight gain of larval *D. abbreviatus* reared for 35 d on roots of citrus seedlings in 3.8-liter pots in a greenhouse

Genotype	Weight gain, mg	*n*	Increase, %
HRS-802	215.7 ± 8.9a	52	726
Carrizo	152.4 ± 6.4b	57	523
Swingle	146.1 ± 4.8b	63	584
HRS 941	143.9 ± 5.6b	56	471
HRS 896	116.2 ± 7.0b	55	399
Murraya paniculata	55.1 ± 3.8c	45	187
Glycosmis pentaphylla	34.6 ± 2.6c	43	114
Murraya koenigii	8.9 ± 2.1c	32	30

Means followed by the same letter are not significantly different at $P = 0.05$ by the Tukey HSD after a significant ANOVA ($F = 70.3$; df = 7, 6; $P < 0.01$).

Table 2. Mean ± SEM percentage recovery of larval *D. abbreviatus* from soil after 35 d in 3.8-liter pots (trial I) and 30 d on roots of citrus seedlings in 21-cm plastic cells (trial II) in a greenhouse

Genotype	% larval recovery	
	Trial I	Trial II
Swingle	90.0 ± 3.2a	86
Carrizo	81.4 ± 5.9ab	93
HRS-941	80.0 ± 3.7abc	92
HRS-896	78.6 ± 5.0abc	69
HRS-802	74.3 ± 5.4abc	75
M. paniculata	64.3 ± 6.5abc	No data
G. pentaphylla	61.4 ± 5.6bc	71
M. koenigii	45.7 ± 7.0c	71

Means followed by the same letter are not significantly different at $P = 0.05$ by Tukey HSD ($n = 7$) after a significant ANOVA ($F = 4.0$; df = 7, 48; $P < 0.01$). Data are untransformed means.

Table 3. Mean ± SEM (n = 7) fresh weight of infested (IF) and noninfested (NIF) roots of 8 genotypes after 35 d of feeding (IF) by *D. abbreviatus* in 3.8-liter pots in a greenhouse

Genotype	Root weight, g		% loss	$P > t$[a]
	IF	NIF		
Swingle	7.3 ± 1.0	18.3 ± 1.8	58.1 ± 6.6a	<0.01
HRS-802	12.4 ± 1.4	26.4 ± 1.7	50.5 ± 7.9ab	<0.01
Carrizo	6.3 ± 0.9	12.9 ± 0.7	48.7 ± 8.6ab	<0.01
HRS-896	9.5 ± 1.5	17.1 ± 1.0	45.7 ± 6.9ab	<0.01
G. pentaphylla	10.4 ± 1.2	21.3 ± 2.9	46.4 ± 8.3ab	<0.01
HRS-941	11.4 ± 2.1	20.8 ± 1.8	40.1 ± 13.9ab	<0.01
M. paniculata	8.2 ± 0.6	13.8 ± 1.8	33.1 ± 11.5ab	0.01
M. koenigii	30.4 ± 1.0	34.9 ± 1.9	12.0 ± 4.4b	0.05

Means followed by the same letter are not significantly different at P = 0.05 by Tukey HSD after a significant ANOVA (F = 2.3; df = 7, 48; P = 0.04). Data are untransformed means.
[a] Probability of a greater t statistic for IF vs NIF unpaired comparison. Student t-test.

df = 4, 117; P = 0.74). As expected, the initial levels of infestation resulted in significantly different numbers of larvae recovered from each plastic cell (F = 30.6, df = 2, 117; P < 0.01). However, survival of larvae declined to 49% when plastic cells were infested with 4 larvae each compared with 79 and 77% for 1 and 2 larvae, respectively. Moreover, the combined weight of larvae recovered from each plastic cell did not vary with level of infestation (Table 5).

When all genotypes were tested at an infestation rate of 2 larvae per cell, there was a significant effect of genotype on fresh weight gain of larval *D. abbreviatus*. Larvae reared in plastic cells with *M. koenigii* or *G. pentaphylla* gained less weight compared with larvae reared on the remaining genotypes (Table 6). Larvae reared on these 2 genotypes weighed ≈10% of those larvae reared on more susceptible genotypes. Mean recovery of larvae from plastic cells was 82% (range, 69–93%). Survival of larvae on the most resistant genotypes (*M. koenigii* and *G. pentaphylla*, as measured by weight gain) was 71% (Table 2). However, the design (2 larvae per pot) did not allow statistical comparison of larval survival per pot. There were no significant differences among genotypes in reduction of root weight (Table 7).

Trial III: Diet Incorporation Bioassay. There was a significant effect of genotype on larval weight gain when root powders were incorporated into artificial diet. Larvae fed diet containing root powder of *M. koenigii* and *G. pentaphylla* weighed 9 and 23%, respectively, compared with larvae reared on diet containing root powder of the rootstock Carrizo. No difference in larval weight gain was detected among the remaining treatments, including the controls (Table 8).

Table 4. Mean ± SEM fresh weight and percentage weight loss of 3 citrus rootstocks infested with larvae of *D. abbreviatus* in 21-cm plastic cells at 4 infestation levels

Cultivar	No. larvae/pot	Root weight, g[a]	% loss[b]
Carrizo		1.448 ± 0.116a	49.9 ± 4.9a
Rough Lemon		1.983 ± 0.165b	54.5 ± 4.4a
Swingle		3.005 ± 0.227c	41.2 ± 5.2a
	0	3.339 ± 0.220a	
	1	2.344 ± 0.195b	30.5 ± 4.8a
	2	1.602 ± 0.186c	52.6 ± 4.6b
	4	1.296 ± 0.157c	62.5 ± 3.9b

Means within columns for cultivar and infestation level followed by the same letter are not significantly different (α = 0.05, Tukey HSD)
[a] n = 56 for cultivars and 42 for infestation levels.
[b] n = 42. Data are untransformed means.

Discussion

Our results confirm the report by Shapiro et al. (1997) of a high level of resistance to *D. abbreviatus* in the remote citroid fruit tree *G. pentaphylla*. Larval weight gain of larvae fed on roots and on artificial diet containing root powder of *G. pentaphylla* was reduced compared with commercial rootstock Swingle or Carrizo. Curiously, this reduced weight gain did not result in reduced root loss to *G. pentaphylla* in our no-choice assay. When a secondary root is cut by larvae, all of the root tissue distal to that point is lost to the plant and results in reduced root mass regardless of how much of the root was actually consumed by the insect. For this reason, larval feeding assays on potted plants are not necessarily a good measure of larval consumption. How larvae of *D. abbreviatus* respond behaviorally to *G. pentaphylla* in terms of ingestion of plant tissue will require a more refined bioassay.

Our results constitute a new record of resistance to *D. abbreviatus* for *M. koenigii*. Larvae fed on *M. koenigii* consistently gained less weight and roots of *M. koenigii* were less damaged compared with the other genotypes tested. *M. paniculata* inhibited larval growth in trial I (1-yr-old seedlings in 3.8-liter pots) but failed to do so when root powder of this species was incorporated into artificial diet (trial III). Further research will be required to determine if this species is resistant, or, possibly, if it possesses a different resistance mechanism compared with its congener.

Common citrus rootstocks can be propagated by seedlings that are genetically uniform because of a type of apomixis called nucellar polyembryony (Swingle and Reece 1967). All of the common citrus rootstocks and new hybrid rootstocks described in this research reproduce by means of nucellar embryony. Although *G. pentaphylla*, *M. paniculata*, and *M. koenigii* probably do not produce apomictic seed, the seedlings

Table 5. Mean ± SEM final weight of larval *D. abbreviatus*, number of larvae surviving per 21-cm plastic cell, and total weight of all larvae per plastic cell at end of the trial (31 d) at 3 infestation levels

No. of larvae	Larval weight, mg[a]	n	% recovery[b]	n	Total larval weight/pot, mg[c]	n
1	167.6 ± 9.4a	33	79 ± 6a	42	167.6 ± 9.4a	33
2	145.2 ± 7.0ab	65	77 ± 4a	42	152.1 ± 7.0a	42
4	135.6 ± 6.3b	73	49 ± 4b	42	142.6 ± 7.4a	39

Means within columns followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey HSD)
[a] ANOVA ($F = 6.9$; df = 2, 13; $P < 0.01$).
[b] ANOVA ($F = 11.7$; df = 2, 117; $P < 0.01$). Data are untransformed means.
[c] ANOVA ($F = 2.5$; df = 2, 105; $P = 0.09$).

we tested appeared uniform. This suggests that our seed sources are self-pollinating and relatively homozygous, and that genetic variability among the plants tested did not contribute to variation in test results.

Disease and pest resistance traits of wild citrus relatives, although potentially valuable, are currently unavailable because of sexual incompatibility of related citroid genera with *Citrus*. Recent development of efficient protoplast-fusion methods allows for somatic hybridization of these genera (Louzada and Grosser 1994, Grosser et al. 1996). Emerging methods to overcome incompatibility place new emphasis on the need to systematically screen *Citrus* and related genera for sources of resistance to pests and diseases. Grosser and McCoy (1996) concluded that resistance to *D. abbreviatus* was unlikely even among wide hybrids because of the insect's wide host range. However, no somatic hybrid of *Citrus* with a genotype demonstrated to be resistant to *D. abbreviatus* was tested. Our results indicate greatly reduced larval weight gain of *D. abbreviatus* when reared on *G. pentaphylla* or *M. koenigii*. Additional work will be required to determine the effect of these genotypes on larval survival to adult and root consumption. Although root loss was significant (infested versus noninfested) for *G. pentaphylla*, there was no significant reduction of root mass of *M. koenigii* by larval *D. abbreviatus* (Table 3). *M. koenigii* is the most resistant citroid genotype we have tested to date.

Screening seedlings in plastic cells gave results comparable to those produced by the protocol of Shapiro and Gottwald (1995) using 1-yr-old seedlings in 3.8-liter pots. We found the plastic cell bioassay to be superior as a tool for large scale screening because it uses younger plants and allows testing of larger cohorts of seedlings over a shorter period. A larger number of entries can be processed because fewer larvae and smaller amounts of materials and bench space are required. Under our growing conditions, seedlings can be infested with larval *D. abbreviatus* at 3–4 mo after germination. By infesting with 2 larvae per 21-cm plastic cell, the chances of genotypes escaping infestation are reduced. Higher levels of infestation apparently resulted in competition between larvae, did not increase the feeding pressure as measured by the total weight of larvae per plastic cell at the end of the trial, and resulted in equivalent damage as measured by percentage of root loss. We intend to continue to use and refine the plastic cell technique to screen for resistance to *D. abbreviatus* both within genera of true citrus fruit trees and in related genera.

The diet incorporation assay can be used for testing root material collected from diverse field sites and is well suited for testing larval performance on potential host plant species when it would be difficult to control for plant growth characters such as age, root mass, and morphology in pot tests. Also, larvae can be observed closely when reared on diet allowing for more precise measurements of survival and weight gain. The high nutritional level of the artificial diet, however, may mask some resistance factors, particularly a nutritional deficiency in the host plant. In general, larvae gain more weight when reared on artificial diet compared with larvae reared on live plant roots. Despite this,

Table 6. Mean ± SEM fresh weight gain of larval *D. abbreviatus* reared for 30 d on roots of citrus seedlings in 21-cm plastic cells in a greenhouse

Genotype	Weight gain, mg	n	% increase
Murraya koenigii	4.7 ± 2.0a	17	37
Glycosmis pentaphylla	5.1 ± 1.9a	18	40
Swingle	43.2 ± 4.6b	24	290
HRS 812	45.5 ± 5.5b	25	308
HRS 896	47.6 ± 5.2b	19	328
HRS 941	56.1 ± 5.9b	25	336
HRS 801	56.9 ± 5.3b	26	358
HRS 802	57.9 ± 13.0b	10	477
Sun Chu Sha	64.9 ± 4.4b	24	362

Means followed by the same letter are not significantly different ($\alpha = 0.05$, Tukey HSD) after a significant ANOVA ($F = 11.3$; df = 8, 92; $P < 0.01$).

Table 7. Mean ± SEM reduction of root mass of citrus seedlings infested with *D. abbreviatus* larvae for 30 d in 21-cm plastic cells in a greenhouse

Genotype	Root weight, g		% loss[a]	n
	NIF	IF		
Murraya koenigii	7.28 ± 1.07	4.67 ± 0.67	36 ± 9	12
Glycosmis pentaphylla	1.72 ± 0.84	1.43 ± 0.31	17 ± 18	12
Swingle	5.65 ± 0.80	5.42 ± 0.45	4 ± 8	14
HRS 812	5.33 ± 0.68	4.60 ± 0.78	14 ± 15	12
HRS 896	2.89 ± 0.53	2.18 ± 0.42	25 ± 15	13
HRS 941	4.72 ± 0.78	2.98 ± 0.80	37 ± 17	13
HRS 801	3.92 ± 0.46	2.18 ± 0.32	44 ± 8	14
HRS 802	4.12 ± 0.28	3.32 ± 0.28	17 ± 7	20
Sun Chu Sha	4.09 ± 0.54	3.08 ± 0.46	25 ± 11	13

Means in column are not significantly different (ANOVA: $F = 115.1$; df = 8, 114; $P = 0.34$). Data are untransformed means.

Table 8. Mean ± SEM fresh weight gain of larval *D. abbreviatus* reared for 30 d on artificial diet containing ground roots of citrus plants

Root source	Weight gain, mg	% increase
Murraya koenigii	26.6 ± 4.1a	123
Glycosmis pentaphylla	68.2 ± 10.6a	265
HRS-802	211.8 ± 37.0b	960
Control (diet only)	230.3 ± 27.0b	970
Control + cellulose	233.2 ± 32.0b	1,061
HRS 896	242.4 ± 9.9b	1,000
Murraya paniculata	271.7 ± 24.2b	1,176
HRS-941	290.1 ± 17.7b	1,192
Carrizo	292.9 ± 30.0b	1,309
HRS-801	295.7 ± 19.9b	1,043
HRS-812	299.3 ± 19.3b	1,188

Means followed by the same letter are not significantly different at $P = 0.05$ by Tukey HSD ($n = 10$) after a significant ANOVA ($F = 16.0$; df = 10, 99; $P < 0.01$).

larvae reared on diet containing root powder of *G. pentaphylla* or *M. koenigii* were much smaller than controls (diet alone) or larvae fed diet containing root powder of susceptible genotypes, indicating the presence of a growth or feeding inhibitor in these species.

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