Phytophthora–Diaprepes Weevil Complex: Phytophthora spp. Relationship with Citrus Rootstocks

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ABSTRACT

Diaprepes abbreviatus (Coleoptera: Curculionidae) is a root weevil, introduced from the Caribbean Basin into Florida in 1964. The larval stage feeds on fibrous and structural roots of citrus, predisposing the injured root system to infection and girdling by Phytophthora spp. In citrus orchards, the rootstocks trifoliolate orange (Poncirus trifoliata) and hybrid ‘Swingle’ citrulmo (Citrus paradisi × P. trifoliata) are resistant to the complex of P. nicotianae with D. abbreviatus, while ‘Cleopatra’ mandarin (C. reticulata) is susceptible to this complex. When Phytophthora palmivora is coincident with P. nicotianae in fine-textured, poorly drained soils, Swingle citrulmo is more vulnerable to attack by the complex with P. palmivora than is Cleopatra mandarin. Infestation of 9-month-old seedlings with two, or five neonate larvae resulted in a wide range of fibrous root and taproot damage of trifoliolate orange and Cleopatra mandarin. Leakage of reducing sugars increased sharply as injury to the roots exceeded 75%. The relationship between feeding damage and root leakage was similar for the two rootstocks. Thus, reduced root damage was not based on host resistance to larval feeding. Root infection and rhizosphere populations of P. nicotianae were higher on Cleopatra mandarin than on trifoliolate orange. Root rot by P. nicotianae did not increase with severity of feeding injury on either rootstock. Root infection and root rot by P. palmivora was more severe on trifoliolate orange than on Cleopatra mandarin and increased with severity of larval damage. P. palmivora infected and rotted the taproot of both rootstocks if predisposed by larval feeding, but P. nicotianae did not.

Commercial rootstocks are severely damaged by larvae of D. abbreviatus; therefore, tolerance of the Phytophthora–Diaprepes weevil complex should be based on resistance of rootstocks to each Phytophthora sp.

Additional keywords: insect–fungus complex, Phytophthora nicotianae, P. palmivora, sour orange

In 1996 to 1997, a survey of weevil-affected orchards confirmed that Phytophthora spp. were associated with larval feeding injury on structural roots of all commercial rootstocks (5). Excavation of root systems revealed sloughing of root bark where Phytophthora spp. entered the injuries created by larval feeding, referred to as root etching or channeling of the bark. Infection of the wounds accelerated the girdling and collapse of roots from the crown of the tree outward. Greenhouse studies confirmed that larval feeding predisposed fibrous roots of seedlings to greater infection by P. nicotianae and promoted infection and higher rhizosphere populations of the pathogen (17). Subsequent investigations demonstrated that P. nicotianae was more severe on susceptible Cleopatra mandarin than on resistant trifoliolate orange (Poncirus trifoliata) selection DPI 50-7 (17,22). Severity of root rot caused by the Phytophthora–Diaprepes complex was not due to differences between the rootstocks in susceptibility to larval feeding because damage to Cleopatra mandarin and trifoliolate hybrid rootstocks Swingle citrulmo and ‘Carizzo’ citrange (C. sinensis (L.) Osbeck × Poncirus trifoliata) was similar (18,19).

The association of Phytophthora spp. with root damage by larvae was named the Phytophthora–Diaprepes complex (5). The potential importance of the complex in the decline of trees on different rootstocks prompted a survey of the east coast of Florida near Vero Beach (Indian River County) and Ft. Pierce (St. Lucie County), where trees rapidly declined despite aggressive management of the weevil. More severe damage was encountered where P. palmivora (Butler) Butler was the predominant pathogen in the complex with D. abbreviatus (5,7). The P. palmivora–Diaprepes complex was associated with fine-textured, poorly drained soils on rootstocks normally resistant to or tolerant of P. nicotianae. Swingle citrulmo and Carizzo citrange. In these situations, structural roots moderately damaged by larvae were extensively infected with P. palmivora. Bark infection caused rapid collapse of structural roots and expression of a gummy residue in the bark.

In the most recent surveys of Diaprepes-infested areas on the east coast (1,4), trees on Swingle citrulmo and Carizzo citrange rootstocks supported higher soil populations of P. palmivora and declined more...
rapidly than trees in adjacent blocks on Cleopatra mandarin and sour orange. This raised concern, because Swingle citrumelo is being used heavily as the rootstock for replanting of orchards on sour orange that are declining due to *Citrus tristeza virus* in this production area (4). These surveys were the first indication that rootstocks with trifoliate orange hybrid parentage might be more susceptible to the *P. palmivora*–*Diaprepes* sp. interaction. Susceptibility to *P. palmivora* was unexpected because the resistance of most commercial and experimental rootstocks to Phytophthora root rot is based on hybrids with trifoliate orange (6).

Hybrids appear to be more vulnerable to attack by *P. palmivora*; therefore, trifoliate orange, as well as Cleopatra mandarin, were compared for their relative resistance to the complex with each Phytophthora sp. We report the disease development of *P. nicotianae* and *P. palmivora* on these two rootstocks in the greenhouse after seedlings were infested with neonate larvae to establish a range of feeding damage.

**MATERIALS AND METHODS**

Weevil infestation and damage assessment. Seed of Cleopatra mandarin and trifoliate orange 50-7 were obtained from registered seed source trees of the Florida Department of Agriculture and Consumer Services, Division of Plant Industry. Seed were sown in 150-cm³ containers (Stuewe & Sons Inc. Corvallis, OR) containing Metro Mix 500 (The Scotts Co., Marysville, OH) and seedlings were fertilized weekly with Peter’s 20-10-20 Peat Lite Special (The Scotts Co.). Nine-month-old seedlings of each rootstock were transplanted in Candler fine sand (Typic Quartzipsammets, pH 6.8, and 1% organic matter) in 150-cm³ containers and 180 of each rootstock were selected for uniform size and vigor for each experiment. Neonate larvae of *D. abbreviatus* (approximately 48 h old) were obtained from eggs laid by adult females collected from the field and confined to screen cages in a greenhouse at 27 ± 2°C. The larvae were counted in sets of two and five larvae into Eppendorf tubes. The soil in each container was moistened with water, then two or five larvae were added to each root system and stabbed into the surface of PARPH medium. If fewer than 20 root tips were present on a seedling, all available root tips were plated. After 4 days, the percentage of root tips positive for *P. nicotianae* of the total assayed was calculated as incidence of infection. Fibrous roots were separated from taproots and each tissue was dried and weighed (70°C for 48 h). Soil was assayed for populations of each Phytophthora sp. on PARPH medium and expressed as propagules/cm³ of soil.

**Statistical analysis.** The experimental design was a factorial with main effects of rootstock (R), *Phytophthora* sp. inoculation (P), larval damage class (L), and first and second order interactions. The analysis was performed with SAS PROC GLM (version 8.1; SAS Institute, Cary, NC). Data from the different years were pooled for the analyses after it was determined that there was no significant year-treatment interaction. Regression analysis was performed to obtain models that best described the relationship between initial larval damage and subsequent interactions of rootstocks with estimates of Phytophthora root rot damage. The root–soil leachate and larval interaction experiments

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Larvae (no./seedling)</th>
<th>Total larval weight (mg/seedling)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 larvae</td>
<td>5 larvae</td>
</tr>
<tr>
<td>Trifoliate orange</td>
<td>1.4 a</td>
<td>3.3 b</td>
</tr>
<tr>
<td>Cleopatra mandarin</td>
<td>1.3 a</td>
<td>2.9 c</td>
</tr>
</tbody>
</table>

*Significant difference between larval infestation levels and rootstocks at P < 0.05 (t test) is indicated by unlike letters in each row or column.*

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Results
Recovery of larvae, damage assessment, and root–soil leachates from rootstock seedlings. The number and weight of larvae recovered per seedling increased with level of infestation from two to five neonate larvae per seedling (Table 1). The recovery and weight of larvae per plant was slightly higher on trifoliate orange rootstock than on Cleopatra mandarin. The weight of larvae per plant decreased at the higher infestation level of neonates.

Larval damage and loss of fibrous root and taproot dry weight per seedling were similar for trifoliate orange and Cleopatra mandarin (Fig. 1A and B). Reducing sugars in root–soil leachates increased sharply as root ratings increased above 8 (≥75% of the root system damaged; Fig. 1C and D), and as fibrous root dry weight decreased below approximately 0.15 g per seedling of each rootstock (Fig. 1A and B). Taproot dry weight decreased slightly above a root rating of 8 (Fig. 1A and B).

Phytophthora infection of rootstocks with larval damage. The main effects and interaction of rootstocks and larval damage were highly significant for root infection and rhizosphere populations of *P. palmivora* and *P. nicotianae* (Table 2). Incidence of root infection and rhizosphere soil populations of *P. palmivora* were higher (*P* < 0.05) than those of *P. nicotianae* (47 versus 13.7% and 252 versus 47 propagules/cm³, respectively). *P. palmivora* had higher (*P* < 0.05) incidence of root infection and soil populations on trifoliate orange than on Cleopatra mandarin (70 versus 28% and 346 versus 160 propagules/cm³, respectively). Conversely, incidence and rhizo-

**Table 2.** Main effects and interactions of rootstocks (trifoliate orange and Cleopatra mandarin) and larval damage by *Diaprepes abbreviatus* for root infection and rhizosphere soil populations after inoculation of seedlings with *Phytophthora nicotianae* or *P. palmivora*

<table>
<thead>
<tr>
<th>Source</th>
<th>Rootstock (R)</th>
<th>Larval damage rating (L)</th>
<th>R × L</th>
<th>Root infection incidence (Probability &gt; F)</th>
<th>Rhizosphere populations (Probability &gt; F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td><em>P. nicotianae</em></td>
<td><em>P. palmivora</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rootstock (R)</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Larval damage rating (L)</td>
<td>10</td>
<td>0.069</td>
<td></td>
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</tr>
<tr>
<td>R × L</td>
<td>10</td>
<td>0.00002</td>
<td></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

**Table 3.** Main effects and interactions of rootstocks (trifoliate orange and Cleopatra mandarin) and larval damage by *Diaprepes abbreviatus* and inoculation with *Phytophthora nicotianae* or *P. palmivora* on root rot rating and fibrous root dry weight

<table>
<thead>
<tr>
<th>Source</th>
<th>Root rot rating (Probability &gt; F)</th>
<th>Fibrous root dry weight (Probability &gt; F)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. nicotianae</em></td>
<td><em>P. palmivora</em></td>
</tr>
<tr>
<td></td>
<td><em>P. nicotianae</em></td>
<td><em>P. palmivora</em></td>
</tr>
<tr>
<td>Rootstock (R)</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>Larval damage rating (L)</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>Phytophthora inoculation (P)</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>R × L</td>
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</tr>
<tr>
<td>R × P</td>
<td>1</td>
<td>0.22</td>
</tr>
<tr>
<td>P × L</td>
<td>10</td>
<td>0.96</td>
</tr>
<tr>
<td>R × L × P</td>
<td>10</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Fig. 1. Regressions of A and B, the relationships between fibrous root and taproot loss or C and D, concentration of reducing sugars in root–soil leachates after a range of damage feeding by *Diaprepes abbreviatus* larvae was established on seedlings of trifoliate orange and ‘Cleopatra’ mandarin. Larval damage ratings are based on the Horsfall-Baratt (H-B) system, where 1 = 0, 2 = 0 to 3, 3 = 3 to 6, 4 = 6 to 12, 5 = 12 to 25, 6 = 25 to 50, 7 = 50 to 75, 8 = 75 to 87, 9 = 87 to 94, 10 = 94 to 97, and 11 = 97 to 100% injury of roots. Regressions are significant at *P* < 0.0001.
Phytophthora root rot of rootstocks with larval damage. Rootstock, larval damage, and inoculation with Phytophthora spp. affected root rot rating and fibrous root dry weight as measures of root loss (Table 3). The rootstock–larval damage interaction was highly significant. As previously reported (3), seedlings of trifoliate orange regenerated fibrous roots more slowly than Cleopatra mandarin during 6 weeks of recovery from loss after larval feeding as measured by root rot rating or fibrous root dry weight (see responses of noninoculated rootstocks in Figs. 2 and 3).

P. palmivora was more damaging to trifoliate orange roots than the other Phytophthora spp.–rootstock combinations (Table 3). P. palmivora interacted with larval damage based on root rot rating but not on fibrous root dry weight. P. nicotianae did not interact with rootstocks or larval damage based on measures of root loss.

The relationship between larval damage and Phytophthora root rot was modeled. Inclusion of larval ratings from 1 to 11 resulted in polynomial curves for inoculated and noninoculated seedlings that converged at ratings ≥8 (analysis not shown). This interaction was noted in our previous study (17) as an inability to measure Phytophthora root rot when the quantity of fibrous roots was severely limited by larval feeding. Linear regressions of larval damage ratings from 1 to 7 provided the best fit for the relationships of larval damage with root rot rating and fibrous root loss (0.10 ≤ r² ≤ 0.97; Figs. 2 and 3).

After inoculation of trifoliate orange and Cleopatra mandarin seedlings with P. nicotianae, the slope and intercepts of the linear regressions for root rating and fibrous root loss with larval damage rating did not differ from those for noninoculated seedlings (Fig. 2A to D). The 95% confidence intervals for the linear regressions of root ratings and fibrous root loss due to P. nicotianae overlapped considerably with the noninoculated response for both rootstocks.

By contrast, P. palmivora interacted with larval damage (Fig. 3A to D). The increase in the root rot rating with larval damage rating was steeper for inoculated than for noninoculated seedlings (Fig. 3A and B). Little or no overlap in the 95% confidence intervals occurred for the regressions of root ratings for trifoliate orange and Cleopatra mandarin (Fig. 3A). Fibrous root loss of trifoliate orange inoculated with P. palmivora was greater than for the noninoculated seedlings but did not increase with level of larval damage (Fig. 3C and D). Fibrous roots of Cleopatra mandarin were much less affected by P. palmivora than were those of trifoliate orange.

Feeding on the structural roots of the seedlings predisposed each rootstock to infection of the taproot by P. palmivora (Fig. 4). Taproot infection was not observed after inoculation with P. nicotianae. Damage to the taproot by P. palmivora was not detected by measurement of fibrous roots. Taproot infection stimulated adventitious root growth (Fig. 4) that may have confounded assessment of fibrous root loss due to root rot by P. palmivora.

**DISCUSSION**

As previously reported (18), fibrous root and taproot damage of trifoliate orange and Cleopatra mandarin was severe 6 weeks after infestation of seedlings with neonate larvae. After larval feeding damage, trifoliate orange regenerated roots more slowly because this rootstock is slower growing and less vigorous than Cleopatra mandarin (3). Differential response to the Phytophthora–Diaprepes complex was not based on unique resistance of the rootstocks to larval feeding. Nevertheless, larval-damaged roots of trifoliate orange

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**Fig. 2.** Linear regressions of A and B, root rot rating and C and D, fibrous root weight on larval damage rating of seedlings of trifoliate orange and 'Cleopatra' mandarin after feeding damage by larvae of *Diaprepes abbreviatus* and inoculation with *Phytophthora nicotianae*. Root rot and larval damage ratings are based on the Horsfall-Baratt (H-B) scale, where 1 = 0, 2 = 0 to 3, 3 = 3 to 6, 4 = 6 to 12, 5 = 12 to 25, 6 = 25 to 50, 7 = 50 to 75, 8 = 75 to 87, 9 = 87 to 94, 10 = 94 to 97, and 11 = 97 to 100% injury of roots. Regressions are significant at P < 0.05, except the root rot rating curves for trifoliate orange and 'Cleopatra' mandarin in the noninoculated treatments (A and B) significant at P < 0.20. Dashed lines are the 95% confidence intervals for the predicted values.
inoculated with *P. nicotianae* were resistant to infection and reproduction by this pathogen; whereas, susceptible Cleopatra mandarin roots were more severely infected and rhizosphere populations higher compared with noninjured roots. Here and in a previous study (17), root loss from *P. nicotianae* did not increase measurably with larval damage. This lack of response to root infection may be due to the limited exposure (6 weeks) of the roots to *P. nicotianae* before the root assessments.

Root infection and rot of the two rootstocks were more severe when weevil-injured roots were challenged by *P. palmivora*. The greater severity of the complex of *D. abbreviatus* with *P. palmivora* than with *P. nicotianae* is further evidence that

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**Fig. 3.** Linear regressions of A and B, root rot rating and C and D, fibrous root weight on larval damage rating of seedlings of trifoliate orange and ‘Cleopatra’ mandarin after feeding damage by larvae of *Diaprepes abbreviatus* and inoculation with *Phytophthora palmivora*. Root rot and larval damage ratings are based on the Horsfall-Baratt (H-B) scale, where 1 = 0, 2 = 0 to 3, 3 = 3 to 6, 4 = 6 to 12, 5 = 12 to 25, 6 = 25 to 50, 7 = 50 to 75, 8 = 75 to 87, 9 = 87 to 94, 10 = 94 to 97, and 11 = 97 to 100% injury of roots. Regressions are significant at *P* < 0.05. Dashed lines are the 95% confidence intervals for the predicted values.

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**Fig. 4.** Taproot infection and adventitious root growth on seedlings of trifoliate orange and ‘Cleopatra’ mandarin after feeding damage by larvae of *Diaprepes abbreviatus* and inoculation with *Phytophthora palmivora*.
**P. palmivora** is a more aggressive and damaging root and fruit pathogen of citrus (7,24). In this study, infection and rot of the taproot of each rootstock by *P. palmivora* after larval feeding reproduced the rapid and extensive invasion of structural roots by *P. palmivora* in orchards (5).

Differential response of rootstocks to the *P. nicotianae–Diaprepes* sp. complex apparently was based on maintenance of tissue resistance to infection by this pathogen after larva attack. Larval feeding injured fibrous root and taproot tissues, increasing leakage of reducing sugars and probably other nutritional compounds from damaged root cells. These compounds serve as attractants and a food source for *Phytophthora* spp. (2,9). Substantial increase in leakage of compounds was detected only after root injury exceeded 75% and the response was similar for trifoliate orange and Cleopatra mandarin. Above this threshold, leachates probably stimulated the activity of *P. nicotianae* or *P. palmivora*, but seedling roots were severely reduced by larval feeding and provided limited substrate for *Phytophthora* infection. At lower levels of root injury, greater resistance of trifoliate orange to *P. nicotianae* and Cleopatra mandarin to *P. palmivora* was maintained despite the wounding. Fungal resistance compounds in roots have been postulated to reduce the rate of ingress of *Phytophthora* spp. into cortical and vascular tissues, allowing roots to heal and regenerate (3,23). In trifoliate orange, resistance compounds apparently were not as effective against *P. palmivora*, because roots were infected and damaged at a higher rate by this pathogen than by *P. nicotianae* (23). The greater susceptibility of trifoliate orange to *P. palmivora* was confirmed by a survey of orchards on Florida’s east coast where soil populations of this pathogen were higher on trifoliate orange hybrids, Swingle citrulmo, and Carrizo citrange than on Cleopatra mandarin and sour orange (1,4).

The significance of these findings in orchard management of the *Phytophthora–Diaprepes* complex depends on which *Phytophthora* sp. is present and whether the soil and water conditions are conducive to the fungus or to rootstock stress. In most situations, *P. nicotianae* is the predominant pathogen and populations of *P. palmivora*, although present, remain low due to soil conditions unfavorable for its activity as a root rot pathogen (7). Swingle citrulmo appears to perform acceptably as a replant in weevil-infested groves, provided soil conditions are suited for this rootstock (e.g., sandy soil texture, well-drained, favorable pH, calcium carbonate status, and so on). *P. palmivora* is damaging to citrus roots in fine-textured, poorly drained soils, high in clay, pH, and calcium carbonate. In these soils, Swingle citrulmo is rendered susceptible to the *Phytophthora–Diaprepes* complex and may decline rapidly (1,4). Thus, tolerance of Swingle citrulmo to the complex is restricted to certain locations and better soils. Under adverse soil conditions in Florida, new rootstocks of parentage other than trifoliate orange should be sought for resistance to *P. palmivora* and greater tolerance of the complex.

In this study, larvae were removed from roots before inoculation with *Phytophthora* spp. to demonstrate that increase in root rot resulted from feeding injury and not from larvae spreading infections to the roots through the soil. Other experiments involving interaction of soilborne pathogens with root insects have been performed as co-inoculations of the insect with the pathogen (11,12). Although the importance of vectoring of the pathogen cannot be estimated, this vectoring is likely secondary to the predisposition of roots by feeding damage. Our results here and previously (17) showed that greater infection resulted from root and bark injury, so it is likely that other citrus root and bark-feeding insects, such as subterranean termites (*Reticulitermes flavipes*) or fire ants (*Solenopsis invicta*), also predispose the roots and trunk to *Phytophthora* rots (20,21).

ACKNOWLEDGMENTS

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LITERATURE CITED