Effect of the Ring Nematode Upon Growth and Physiology of Peach Rootstocks 
Under Greenhouse Conditions

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ABSTRACT


Seedlings and herbaceous cuttings of cultivars Lovell and Nemaguard peach were grown for 8-13 mo in soil with or without ring nematodes, Criconomella xenopla (Cx). In seedlings and rooted herbaceous cuttings, the presence of Cx resulted in reduced root fresh and dry weights and reduced free amino acids of shoots and roots as measured by levels of ninhydrin-reactive compounds. The proportions of specific amino acids were changed. Molar percentage of proline, glycine, and alanine increased, whereas arginine decreased in roots of both seedlings and herbaceous cuttings in the presence of Cx. Levels of the cyanogenic glucoside prunasin decreased in stem tissue of cuttings and seedlings in the presence of Cx, but levels in the roots were increased.

Additional key words: cyanoide, ectoparasite, peach tree short life.

Peach tree short life (PTS) syndrome continues to limit peach production in the southeastern United States despite more than 30 yr of research on the problem (23). “Predisposing” factors are now thought to include the ring nematode [Criconomella xenopla (Raski) Luc & Raski], rootstock, cultural practices, and fluctuating winter temperatures (16, 17, 23, 28). Little is known of the physiological and biochemical basis for this predisposition although changes in auxin levels have been suggested (2). Tree death, which is often limited to the aboveground portion, is apparently caused by cold injury or bacterial canker caused by Pseudomonas syringae van Hall.

As early as 1949, a ring nematode [C. simile (Cobb) Chitwood] (= C. xenopla) was found associated with declining orchards in Maryland and North Carolina (3). Growth reduction of peach by C. xenopla (Cx) was demonstrated only after potted trees were kept in infested soil for 2.3 yr (11). Subsequent research in California indicated 16 mo of exposure to Cx could reduce fresh weight and increase susceptibility to bacterial canker of trees in pots (12). That C. xenopla can produce a heat-labile auxin inactivating enzyme has been reported (24). When 1,2-dibromo-3-chloropropene (DBCP) was available, regular pre- and postplant soil fumigation kept Cx at low levels and prolonged peach tree life in the southeastern United States (23, 28). This circumstantial evidence and the results of recent work (17) indicate that Cx plays a key role in tree death. Resistant rootstocks would be an attractive alternative to chemical fumigation, especially since the nematocides currently available are less effective than DBCP.

Lovell and Nemaguard cultivars are the most common rootstocks for peach in the United States. At one time, Lovell was the predominant drying peach in California, so seed was readily available for nursery use. Although trees on Lovell generally outlive those on Nemaguard in the southeastern United States, particularly where Cx is prevalent, all are nonetheless subject to cold injury and tree death. Nemaguard, which was released by the USDA in 1961, is resistant to root-knot nematode [Meloidogyne incognita (Kofoid & White) Chitwood and M. javanica (Treub) Chitwood] whereas Lovell is susceptible to root knot. Although Nemaguard was originally developed in Georgia, poor survival limits its use in the southeastern United States.

Although trees on Lovell may be longer-lived than those on Nemaguard, data on the tolerance or resistance of Lovell rootstock to Cx are inconclusive. Apparent differences in rate of population buildup on Lovell as compared to Nemaguard (28) may be more related to the higher growth rate of Nemaguard (27) than to inherent resistance or susceptibility. It is not clear whether the ideal rootstock would i) resist or inhibit buildup of Cx on its roots, ii) tolerate high levels of Cx without growth reduction, iii) simply not predispose a scion to injury from cold or P. syringae (unrelated to i, ii, or iv) have some combination of these characteristics. Screening procedures for resistance and tolerance are relatively straightforward and currently underway. Screening for longevity requires an understanding of the physiology of predisposition so that it can be induced and measured under controlled conditions in a reasonable length of time. Although it is difficult to simulate fluctuating weather conditions and field cultural practices under greenhouse conditions, a comparison of Lovell and Nemaguard may provide clues to why Nemaguard is shorter-lived and how to screen for survival potential. One measure of differences in plant metabolism is the level of free amino acids that appear as ninhydrin-reactive compounds (NRC). Both NRC levels and specific amino acids have been related to cold hardiness (4, 8, 10) and nematode injury (5, 7, 14, 20). Cyanide has been suggested to have a role in replant problems (13, 25). This paper describes the effect of Cx on growth parameters, free amino acids, and prunasin in peach. Preliminary work has been reported (19, 22).

MATERIALS AND METHODS

Plant materials. Experiment 1 consisted of 1-mo-old seedlings of Lovell and Nemaguard planted in September 1981 and harvested in October 1982. Experiment 2 was initiated in May 1983 and harvested in January 1984. It consisted of 1-mo-old Nemaguard seedlings and herbaceous cuttings of Lovell and Nemaguard seedlings rooted in April 1983 as previously described (18).
Seedlings and cuttings were transplanted into individual 3-L (experiment 1) or 1.5-L (experiment 2) clay pots containing steam-pasteurized Faceville loam soil from a site planted to peaches from 1970 to 1975, and again in 1981. Half of the trees were infested by adding 2,300 Cx in 500 ml (experiment 1) or 2,000 Cx in 400 ml (experiment 2) of a similar, but infested, soil from greenhouse cultures. Each pot was initially fertilized with 10 g of Osmocote 18-6-12 slow-release fertilizer; after that, Peters 20-20-20 soluble fertilizer was added as needed. Pots were arranged in a split-plot design with cultivars as main plots, and nematode treatments as subplots. There were eight randomized complete blocks for experiment 1, and six blocks for experiment 2 with one pot per treatment per block. All plants were grown in the greenhouse under ambient light at 25 ± 5°C. Stem diameter and root fresh and dry weights were measured. Percent dry matter was calculated as dry weight/fresh weight × 100.

**NRC analysis.** About 1 g of fibrous roots and a 1-to-2-cm section of the stem 5 cm above the soil line were collected from each plant and analyzed for NRC by using the technique of Moore and Stein (15). Samples were extracted by autoclaving for 15 min in 10 ml of water and held in a cold room overnight. Duplicate 0.05-ml aliquots of the aqueous extracts were reacted with ninhydrin. Absorption was converted to millimolar glycine equivalents on a root dry weight basis by using a standard curve generated by analyzing known amounts of glycine. After removal of the entire soil mass from the pot, nematodes were extracted from 150 cm² of soil by centrifugal flotation (9,26) and counted under a dissecting microscope.

**Amino acid analysis.** Analysis of roots from experiment 2 was conducted as described in Product Data Bulletin 9702-2 (Alltech Associates, Deerfield, IL). Specifically, triplicate samples from each treatment were obtained by combining two replicates of the treatment and pulverizing the entire root systems with a Wiley mill. Pulverized roots were mixed well to ensure uniform sampling and 3-g samples were removed for analysis. Samples were extracted for 24 hr at 60°C in 20 ml of 80%...
ethanol. Residue was removed from the ethanol by filtration with Whatman #1 filter paper, washed with an additional 20 ml of 80% ethanol, and refiltered. The combined filtrate containing the amino acids was taken to dryness at 60°C under vacuum. The residue was dissolved in 5 ml of deionized water and adjusted to pH 2.5 with glacial acetic acid. Ten micromoles of norleucine was added as the internal standard and the extract was subjected to ion exchange chromatography and derivatization as described in Product Data Bulletin 9702-2. The N-acetyl amino acid n-propyl esters were detected and quantitated with gas liquid chromatography (GLC). Amino acid separation was attained with a 0.61 m (2 ft) × 3.2 mm (⅛ in.) stainless steel amino acid analysis column (Alltech Associates, Deerfield, IL); carrier gas, helium, 12 ml/min; injector temperature, 250°C; detector temperature F.I.D., 300°C; oven temperature program 110–182°C at 8°C/min, 182–275°C at 31°C/min.

Prunasin analysis. From plants in experiment 2, 0.5-g samples of fresh root and stem tissue were taken and placed in 80% ethanol and crushed with a Polytron operated at a control setting of 6 for 15 sec. Samples were then extracted at 75°C for 30 min, filtered through Whatman #1 filter paper, and concentrated to 1–2 ml under a stream of N2 at 80°C. Concentrated samples were adjusted to 4.0 ml with distilled H2O and reacted with 1 ml of 1 M β-glucosidase (Sigma Chemical Co.) for 30 min at 22°C. Benzaldehyde was extracted from the reaction mixture with 2 ml of ethyl acetate. The concentration of prunasin was calculated from the amount of benzaldehyde measured by GLC. A 1.83 m (6 ft) × 3.2 mm (⅛ in.) stainless steel column containing 10% Alltech AT-1000 on 80/100 chromasorb H-AW (Alltech Associates) was used to achieve separation. Chromatography conditions were: oven, 150°C; injector, 225°C; detector F.I.D., 250°C; and carrier gas helium, 20 ml/min. Authentic prunasin (Sigma Chemical Co.) was used as an external standard and processed in the same manner as the experimental tissue.

RESULTS

Experiment 1. Three check pots were contaminated with C. xenoplasia and were not included in the analysis. The presence of Cx significantly reduced root fresh and dry weight on Lovell and Nemaquad seedlings (Table 1). NRC of roots decreased 47 and 41% while shoot NRC decreased 38 and 48% with nematodes for Lovell and Nemaquad, respectively. Rootstock effect differed only for stem diameter and root NRC (Table 1). No treatment × rootstock effects were significant.

Experiment 2. The presence of Cx on rooted cuttings of Lovell and Nemaquad significantly reduced root fresh and dry weight, and percent dry matter (Table 1). NRC of roots decreased 35 and 23% for Lovell and Nemaquad cuttings, respectively. Shoot NRC remained the same for Lovell but in Nemaquad decreased 31% in the presence of nematodes. Rootstocks differed in root dry weight and dry matter content.

Table 2. The effect of 8 mo of exposure to Criconemella xenoplasia (Cx) on the cyanogenic glucoside prunasin in peach seedlings and rooted herbaceous cuttings

<table>
<thead>
<tr>
<th>Rootstock</th>
<th>Cx Stem</th>
<th>Cx Root</th>
<th>Lovell Stem</th>
<th>Lovell Root</th>
<th>Nemaquad Stem</th>
<th>Nemaquad Root</th>
<th>Nemaquad Stem</th>
<th>Nemaquad Root</th>
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</thead>
<tbody>
<tr>
<td>Herbaceous</td>
<td></td>
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<tr>
<td>Lovell</td>
<td>–</td>
<td>6.7 ± 1.0</td>
<td>21.5 ± 2.7</td>
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<tr>
<td>Nemaquad</td>
<td>+</td>
<td>6.0 ± 1.0</td>
<td>26.2 ± 1.9</td>
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<tr>
<td>Seedlings</td>
<td></td>
<td>5.2 ± 0.5</td>
<td>16.8 ± 4.0</td>
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</tr>
<tr>
<td>Nemaquad</td>
<td>+</td>
<td>3.1 ± 0.3</td>
<td>23.8 ± 2.5</td>
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</table>
| Significant effects
| Cx         | *       | **      |             |             |               |               |               |               |
| Rootstock   | +       |         |             |             |               |               |               |               |

* Mean of six determinations per treatment.
** F-test significant at P = 0.10 (*), 0.05 (**), or 0.01 (**).
although absolute levels differ. Mizutani (13) has suggested that breakdown of prunasin to release cyanide may be involved in peach tree decline and PTSL. Prunasin is present in relatively high concentrations in bark and roots of peach trees, and has been found to be a potent inhibitor of nitrate reduc-tate (21) upon breakdown to mandelonitrle and cyanide.

These results demonstrate that an ectoparasitic nematode Cx can cause biochemical changes not only in the roots where they feed, but also in the shoot. Additional work is needed to clarify the nature of the predisposition of peach trees to PTSL, determine the relationship of changes in amino acid and prunasin levels and predisposition, and develop an efficient rootstock screening technique for long-term survival.

LITERATURE CITED