# Infection of Apple Roots by Actinomycetes Associated with Soils Conducive to Apple Replant Disease

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**ABSTRACT**

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Apple seedling roots became infected by actinomycetes when grown in five soils collected from apple and pear nurseries in New York. Steam treatment of these soils (about 60 C, 30 min) eliminated actinomycete infection of roots and controlled symptoms of apple replant disease. Neither symptoms of apple replant disease nor root infections by actinomycetes were observed on seedlings planted in three additional soils collected from the root zones of apple trees. Soils collected from the same nurseries, but from sites not associated with previous planting of either apple or pear, contained low or undetectable levels of infectious actinomycetes. Seedling growth was not inhibited in four of these soils compared with growth in steamed samples of each soil. Inhibition of seedling growth in the other two soils appeared to be related to nutrient deficiencies. Concentrations of nitrogen, boron, and several other nutrients were significantly higher in shoots of seedlings grown in steamed vs. unsteamed samples from these two soils. Therefore, all six nonapple soils were judged not conducive to apple replant disease. Our evidence supports an association between infectious actinomycetes and the apple replant disease as proposed by several others. Actinomycetes may be important in the etiology of the apple replant disease.

Apple replant disease (ARD) is characterized by delayed establishment of apple transplants at sites previously planted to apple. Its etiology is only partially understood. Most early work on the disease was reviewed by Hoestra (2) in the Netherlands and Savory (15) in England. Both authors agreed that *Pratylenchus* spp. were involved in some, but not all, ARD situations. In those instances where nematodes were not involved, the causes were not verified; however, two hypotheses concerning the etiology of ARD have been presented with substantial evidence. One suggests that species of *Pythium* cause most of the root damage associated with ARD (10,16). Another hypothesis assumes that actinomycetes, found invading apple roots, are responsible for ARD (12,13).

Mulder (10) indicated that various *Pythium* spp. cause root injury similar to that observed for ARD in the Netherlands. Sewell (16) found several *Pythium* spp. that infected apple roots, particularly *Pythium salvaticum* Campbell & Hendrix; he proposed that they are major causes of ARD in England. Although several fungicides affecting species of *Pythium* (14) have not controlled ARD (2), Sewell (16) suggested rapid recolonization of soils after fungicide treatment allowed these fungi to appear as if unaffected by the fungicides.

Hoestra (2) suggested that bacteria or actinomycetes were involved in the etiology of ARD in the Netherlands based on the failure of nematicides and fungicides to control ARD or inhibit procaryotes. He also observed lower incidence of ARD in acidic soils, a relationship that has been noted for diseases caused by *Streptomyces scabies* (Thaxt.) Waksman & Henrici (1). In the Netherlands, Mulder (11) reported that ARD could be controlled with streptomycin, which inhibits some actinomycetes (19). Otto and Winkler (12) provided direct evidence for the involvement of actinomycetes in ARD. They stained roots collected from sites considered highly, moderately, and minimally conducive to ARD in East Germany and found that colonization of the outer tissues of the roots by actinomycetelike organisms was positively correlated with severity of ARD at these sites. Subsequently, selected pesticides effective against actinomycetes also were effective against ARD (13). Unfortunately, they did not isolate or determine which actinomycetes actually invaded the apple roots (G. Otto and H. Winkler, *personal communication*, 1984).

We have studied actinomycetes in roots of apple seedlings grown in soil conducive to ARD and concluded that at least one actinomycete was pathogenic based on histological studies (20,21). In addition, the degree of infection of roots by an actinomycete that resided in the soil around apple roots was negatively correlated with the number of lateral roots and plant weight in a 2-wk seedling bioassay (22; S. W. Westcott, *unpublished*). Three biocides that controlled infection of apple seedling roots by actinomycetes also controlled ARD in a rapid seedling bioassay (20).

Our previous studies and those of Jaffee and coworkers (4–6) used soil from a single site. To determine the extent of association between actinomycetes and ARD, we sampled soils from several fruit tree nurseries and orchards in New York. Actinomycetes infected roots of apple seedlings planted in 18 of 23 apple orchard soils sampled, but actinomycetes were not commonly found in 10 other soils not associated with apple or pear plantings (S. W. Westcott, *unpublished*). Because symptoms of the ARD are not distinct from those caused by nematodes and some nutrient deficiencies, both of these factors were monitored in subsequent analyses of soils from six nursery sites. Frequent replanting of apple trees in nurseries provided evidence for identification of sites conducive to ARD. Soils conducive to ARD as well as nearby soils that had no recent history of apple planting were included to provide both positive and negative associations.

## MATERIALS AND METHODS

Samples of soils were collected from five commercial nurseries in four counties in western New York State (Table 1). Soil was collected from the root zones of apple or pear trees and from nearby sites having no recent history of either crop. Portions of each soil were steamed (60–70 C, 30 min), then aired for at least 3 days. Steamed and untreated soils were placed in 11-cm-diameter plastic pots and planted with germinated seeds from Northern Spy apples (six seedlings per pot, three pots per treatment). Plants were watered daily and fertilized weekly with 150 mg of Ra-Pid-Gro (23-19-17) per container (Ra-Pid-Gro Corp., Dansville, NY). After 4 wk in a controlled-environment chamber (21 C, 40–75% relative humidity, 14 hr of light), seedlings were harvested and shoots dried at 90 C for 3 days and then weighed. Roots were cleared in 10% (w/v) potassium hydroxide and stained with trypan blue in lactic acid/glycerol/water (100:7:7, v/v) (8). Actinomycete hyphae appeared dark blue and could be scored at 15×. Levels of infection were

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determined subjectively and reported as none, low (scattered spots), or high (many spots and areas of contiguous spots on the taproot) based on the number and extent of roots observed that were colonized by actinomycetes.

Dried shoots were separated into three subsamples of six shoots each for analysis of 20 elements (N, K, P, Ca, Mg, Mn, Fe, Cu, B, Zn, Mo, Al, Na, Co, Cd, Cr, Ni, Pb, Se, and As) by the Pomology Department, Cornell University. Nitrogen concentration was determined by micro-Kjeldahl methods, and the concentrations of all other elements were measured with an inductively coupled argon plasma atomic emission spectrometer. Bulked soil samples from each site were analyzed for content of P, K, Ca, and Mg and for pH by the Department of Agronomy, Cornell University. Elements were extracted from soil with sodium acetate buffer (pH 4.8), and their concentrations were determined with standard colorimetric or atomic absorption techniques. Water extracts were used for pH determinations. Means of shoot and root weights and nutrient levels for each test were compared with a protected least significant difference (LSD) test (18).

Roots from three replicates of six roots for each treatment were rinsed in water, weighed, and then shaken in flasks with water for 3 days to extract nematodes (9). Suspensions were passed three times through screens with 1,940-$\mu m^2$ openings (325-mesh), and nematodes were transferred to small dishes for counting after each passage. Because few other plant-parasitic nematodes were recovered, only populations of *Pratylenchus* spp. in roots are reported.

**Table 1.** Location, planting history, and soil type of soils collected from fruit tree nurseries in New York in 1984

| Township/county | Site code | Soil type | Crop history | |
|---|---|---|---|---|
| | | | Previous | Current |
| Dansville/Livingston | Aa | Clay loam | Apple (5 yr) | Apple[a] |
| | Ab | Clay loam | Maple (10 yr) | Maple |
| Stanley/Ontario | Ba | Clay loam | Apple in past[a] | Fallow |
| | Bb | Clay loam | Corn in past | Fallow |
| Huron/Wayne | Ca | Silt loam | Meadow | Meadow |
| | Cb | Silt loam | Apple orchard | Apple (new) |
| | Cc | Silt loam | Apple nursery | Apple (new) |
| Lyons/Wayne | Da | Loam | Nursery | Oak |
| | Db | Loam | Nursery | Apple |
| Lyons/Wayne | Ea | Loam | Nursery | Cherry |
| | Eb | Loam | Nursery | Pear (4 yr) |
| Wolcott/Wayne | Fa | Silt loam | Nursery | Alfalfa |
| | Fb | Silt loam | Uncultivated | Apple |
| | Fc | Silt loam | Cover/pear[a] | Fallow |

[a] Grower had noticed that apples planted on the site were not growing as rapidly as expected.

**Table 2.** Seedling growth, actinomycete infection, and population densities of *Pratylenchus* spp. recovered from seedling roots after growth for 4 wk in unsteamed or steamed soils collected from six fruit tree nurseries in New York

| Site code | Previous crop | Final weight (mg)[a] (unsteamed/steamed) | | Actinomycete infection[b] | *Pratylenchus* spp. (no./g root)[c] |
|---|---|---|---|---|---|
| | | Dry shoot | Fresh root | | |
| Aa | Maple | 220/240 | 460/440 | None | 0 |
| Ab | Apple | 120/250** | 190/510** | High | 0 |
| Ba | Corn | 340/300 | 630/420** | Low | ...[d] |
| Bb | Apple | 180/270** | 170/310* | High | ...[d] |
| Ca | Grass | 210/240** | 420/460 | None | 3 ± 1 |
| Cb | Apple | 190/200 | 350/360 | None | 18 ± 4 |
| Cc | Apple | 220/310** | 460/510 | Low | 8 ± 5 |
| Da | Oak | 220/230 | 370/300* | None | 21 ± 4 |
| Db | Apple | 190/250** | 310/370* | High | 9 ± 2 |
| Ea | Cherry | 190/200 | 320/320 | None | 0 |
| Eb | Pear | 130/200** | 160/300** | High | 432 ± 206 |
| Fa | Alfalfa | 240/280* | 380/590** | None | 21 ± 8 |
| Fb | Apple | 200/220 | 310/530** | None | 30 ± 19 |
| Fc | Pear | 190/280** | 390/570** | High | 0.4 ± 0.6 |

[a] Significant differences between unsteamed and steamed treatments were determined using a protected LSD. Levels of probability are indicated as * = $P = 0.05$ and ** = $P = 0.01$.
[b] Stained roots were rated for infection as follows: none, low (scattered spots), and high (many spots and areas of contiguous spots).
[c] Mean and standard deviation of nematode densities in roots.
[d] All soil sampled collected from sites Ba and Bb were air-dried before planting; no extraction of nematodes was attempted.

## RESULTS

Steam treatment of nine soils collected from apple nurseries resulted in enhanced growth of the apple seedlings planted in them. These responses were associated with reductions in the number of actinomycete-infected roots, lower densities of *Pratylenchus* spp. (Table 2), or increased levels of nutrients in the shoots (Table 3). In one instance (soil "Da"), steaming resulted in poorer growth of the apple seedlings. Roots were small and deformed at harvest and appeared to have been affected by a phytotoxic substance. Moderate to high levels of infection of roots by actinomycetes were associated with natural field soils previously planted to apple or pear (Table 2), except in three soils (Cb, Cc, and Fb) with low soil pH (Table 4). Steam treatment of soils eliminated infection of roots by actinomycetes in all but one soil (Ba) that likely became contaminated with actinomycetes after a previous experiment with the same soil samples. Results from the previous experiment indicated no trace of actinomycete infection and no effect of steaming on root growth of seedlings grown for 4 wk in this soil. In four of five soils (Ab, Db, Eb, and Fc) in which control of actinomycete infection was associated with improved plant growth after steaming (Table 2), the concentration of nutrients in the foliage decreased or remained unchanged (Table 3). In the other similar soil (Bb), only the potassium concentration was significantly higher in shoots from the steamed than the unsteamed soil.

Densities of *Pratylenchus* spp. were relatively low in roots grown in many of the natural soils (Table 2). Soil samples from two nurseries (A and B) had been air-dried before use in experiments. This treatment may have killed plant-parasitic nematodes in the samples and was an advantage in that nematodes did not have to be considered for these trials. A much larger population of *Pratylenchus* spp. was recovered from roots grown in one soil (Eb). Steam treatment of all infested soils reduced plant-parasitic nematodes to undetectable levels.

There was no consistent direct relationship between the changes in levels of nutrients extracted from the shoots resulting from steaming soils (Table 3) and the changes in the same nutrients extracted from soils for the same treatments (Table 4). In only two of 14 instances, the concentrations of K, Mg, and Ca changed in the same direction for levels of nutrients extracted from the soil

and from shoots. Therefore, nutrient levels detected in soil were not useful for predicting the effects of steaming on the availability of these nutrients to plants.

Steaming soils affected the concentration of some nutrients detected in shoots and was associated with enhanced growth of seedlings compared with unsteamed treatments of four soils (Ca, Cc, Fa, and Fb) (Table 3). Nitrogen and boron increased most frequently in association with a measured growth response. Changes in the concentrations of P, Mg, Mn, and Cu also were associated with growth responses.

## DISCUSSION

We have found actinomycetes infecting roots of apple seedlings grown in five of eight nursery soils previously planted to apple or pear. Seedlings grown in those five soils had symptoms typical of ARD. Some of these soils had been identified by growers as "problem soils" (Table 1). Steam treatment of the five soils improved seedling growth and eliminated infection of roots by actinomycetes. Little or no actinomycete infection was detected on roots grown in soils not previously planted to apple or pear. Although plant growth in two of these soils was improved by steaming, the effects were associated with control of low densities of nematodes and enhanced uptake of some nutrients. This evidence supports our hypothesis that one or more actinomycetes play a role in the etiology of ARD.

For the three soils previously planted to apple that did not produce typical symptoms of ARD, the pH was between 4.8 and 5.3. Hoestra (2) found that ARD was rarely severe in soils with a pH below about 5.5. In another experiment (2), the effects of ARD were substantially diminished by acidifying a soil conducive to ARD with hydrochloric acid. Perhaps, a similar situation applies to ARD in New York.

Nematodes, especially *Pratylenchus penetrans*, can inhibit growth of young apple seedlings more severely than older seedlings (7). Therefore, damaging levels for our experiments must be determined from studies involving seedlings of similar age. Jaffee (4) found that *P. penetrans* population densities of 150 nematodes per gram of root were associated with detectable stunting of 5-wk-old seedlings. Apple seedlings grown in all but one soil (Eb) tested in our study had population densities of *Pratylenchus* spp. well below this damage threshold (Table 2). This does not mean that the lower populations detected in roots did not inhibit plant growth to some degree. Interactions between the nematodes and actinomycetes cannot be determined from these experiments but could be involved in some cases of ARD.

Determination of the exact effects of nutrient availability on seedling growth within these experiments would require a separate study. We considered only those nutrients that were found to be in significantly higher concentration in shoots from plants grown in steamed soils than those grown in unsteamed soils (Table 3). From published (17) and unpublished sources, we estimated critical concentrations of these nutrients below which effects on seedling growth might be detected. From this analysis, there was evidence for release of additional nutrients after steaming that could have affected the growth of seedlings in five soils (Bb, Ca, Cc, Fa, and Fb). Nitrogen and boron were identified as candidates in most of these soils. Phosphorus, magnesium, and copper

**Table 3.** Concentration of elements in shoots of apple seedlings grown in unsteamed and steamed soils[a]

| Site code | Extractable element from shoots in unsteamed/steamed soil[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Ca | Mg | Zn | Cr | Se | Mn | K | N |
| Aa | 1.10/0.90** | ... | ... | ... | ... | ... | ... | ... |
| Ab | 1.00/0.79** | 0.39/0.33** | 14.9/11.2* | 2.10/1.76** | 5.52/4.75* | ... | ... | ... |
| Ba | ... | ... | ... | ... | ... | 33/66** | ... | ... |
| Bb | ... | ... | ... | ... | ... | ... | 0.65/0.90* | ... |
| Ca | 0.82/0.62** | ... | 44/31** | ... | ... | 65/105** | ... | 1.98/2.25* |
| Cb | 0.83/0.64** | ... | ... | ... | ... | 76/205** | 1.04/0.83** | ... |
| Cc | 0.74/0.57** | ... | ... | ... | ... | ... | ... | 1.82/2.12* |
| Da | 0.94/0.66** | ... | 13/9 | ... | ... | ... | 0.96/0.69** | 1.64/2.20** |
| Db | 1.02/0.79** | ... | 16/12** | ... | ... | 46/37** | 0.99/0.75** | ... |
| Ea | 0.94/0.75** | ... | 10/8* | ... | ... | ... | 0.96/0.83** | 1.58/2.36** |
| Eb | 1.01/0.66** | 0.37/0.31** | 11/10* | ... | 5.36/4.06* | ... | 0.76/0.59** | ... |
| Fa | ... | 0.28/0.32* | ... | ... | ... | ... | ... | 2.02/2.60** |
| Fb | ... | 0.27/0.32* | ... | ... | ... | ... | 1.03/0.78* | 1.83/2.32** |
| Fc | 1.12/0.84** | ... | ... | ... | ... | ... | 1.37/1.11* | ... |
| | Ni | B | Co | As | P | Na | Pb | Cu |
| Aa | ... | ... | ... | ... | ... | ... | ... | ... |
| Ab | ... | ... | ... | ... | ... | ... | ... | ... |
| Ba | ... | ... | ... | ... | ... | ... | ... | ... |
| Bb | ... | ... | ... | ... | ... | ... | ... | ... |
| Ca | 1.45/1.02* | 30/33* | ... | ... | ... | ... | ... | ... |
| Cb | ... | 27/33** | 0.62/1.09** | 4.89/6.05* | ... | ... | ... | ... |
| Cc | ... | 22/26** | ... | ... | ... | ... | ... | ... |
| Da | ... | 21/17** | 13/9** | ... | ... | ... | 1.99/1.63* | ... |
| Db | ... | 24/20* | ... | ... | 0.11/0.09* | 164/110* | ... | ... |
| Ea | ... | ... | ... | ... | ... | ... | ... | ... |
| Eb | ... | 19/15** | ... | ... | 0.14/0.10** | 206/94** | 1.98/1.61* | ... |
| Fa | ... | 25/33* | ... | ... | 0.11/0.15** | ... | ... | 7.21/9.83** |
| Fb | ... | ... | ... | ... | ... | ... | ... | 5.65/8.04** |
| Fc | ... | ... | ... | ... | ... | ... | ... | ... |

[a] Only those elements that changed significantly when soils were steamed are reported.
[b] N, K, P, Ca, and Mg expressed in g/100 g; all others expressed in μg/g. Significant differences between unsteamed and steamed soil samples were determined using a protected LSD. Levels of probability are indicated as * = $P = 0.05$ and ** = $P = 0.01$.

**Table 4.** Concentration of elements extracted from and pH of soil samples from six fruit tree nurseries before and after samples were treated with steam (60 C, 30 min)[a]

| Site code | Previous crop | Treatment | pH | Extractable element (μg/g) | | | |
|---|---|---|---|---|---|---|---|
| | | | | P | K | Mg | Ca |
| Aa | Maple | Unsteamed | 7.5 | 29 | 95 | 375 | 5,000 |
| | | Steamed | 7.8 | 37 | 120 | 350 | 5,000 |
| Ab | Apple | Unsteamed | 7.1 | 33 | 90 | 525 | 2,700 |
| | | Steamed | 7.8 | 33 | 105 | 475 | 2,600 |
| Ba | Corn | Unsteamed | 6.6 | 59 | 197 | 155 | 1,600 |
| | | Steamed | 6.3 | 45 | 200 | 148 | 1,500 |
| Bb | Apple | Unsteamed | 7.1 | 25 | 108 | 285 | 1,750 |
| | | Steamed | 7.0 | 27 | 114 | 265 | 1,700 |
| Ca | Grass | Unsteamed | 4.5 | 6 | 110 | 85 | 700 |
| | | Steamed | 5.5 | 6 | 120 | 85 | 800 |
| Cb | Apple | Unsteamed | 4.6 | 7 | 150 | 50 | 600 |
| | | Steamed | 4.6 | 6 | 145 | 45 | 500 |
| Cc | Apple | Unsteamed | 5.3 | 10 | 225 | 250 | 1,200 |
| | | Steamed | 6.3 | 15 | 275 | 275 | 1,200 |
| Da | Oak | Unsteamed | 7.0 | 22 | 125 | 300 | 1,800 |
| | | Steamed | 7.3 | 31 | 160 | 275 | 1,800 |
| Db | Apple | Unsteamed | 6.4 | 14 | 75 | 165 | 1,800 |
| | | Steamed | 7.2 | 16 | 105 | 155 | 1,800 |
| Ea | Cherry | Unsteamed | 6.7 | 10 | 195 | 135 | 1,000 |
| | | Steamed | 7.0 | 11 | 195 | 125 | 1,000 |
| Eb | Pear | Unsteamed | 6.7 | 15 | 95 | 185 | 1,200 |
| | | Steamed | 7.4 | 16 | 100 | 165 | 1,200 |
| Fa | Alfalfa | Unsteamed | 4.9 | 10 | 200 | 135 | 700 |
| | | Steamed | 5.8 | 10 | 200 | 135 | 700 |
| Fb | Apple | Unsteamed | 4.8 | 9 | 110 | 90 | 500 |
| | | Steamed | 5.6 | 10 | 110 | 80 | 500 |
| Fc | Pear | Unsteamed | 5.8 | 18 | 185 | 170 | 1,300 |
| | | Steamed | 6.5 | 25 | 200 | 170 | 1,300 |

[a] Values represent single determinations.

also may have contributed to growth of seedlings in some of the steamed soils. Manganese levels were considered adequate for both treatments in the soils that resulted in significant changes, so this element was not considered as a cause of differences in plant growth. Ammonium-nitrogen, manganese, calcium, magnesium, and potassium have been shown to increase in some soils after steaming (15). In another study, Hoyt and Neilsen (3) correlated the levels of magnesium from soil with growth of McIntosh apple trees. Thus, these nutrients could have contributed to the stimulation of plant growth in steamed soil. We had no information on which to base a prediction of the amount of plant growth that should be expected from each identified nutrient, nor was it possible to evaluate effects of any interactions between nutrients.

Moderate to high levels of infection of roots by actinomycetes were always associated with significant inhibition of shoot and root growth. In two soils, there were other factors that could have contributed to inhibition of growth. In one soil (Bb), less uptake of potassium from the unsteamed than the steamed soil may have contributed to effects on growth, but we did not feel that the additional potassium uptake could explain the degree of growth improvement observed. In another soil (Eb), *Pratylenchus* spp. were probably an important factor because the population density was well above the damage threshold (4). Jaffee et al (5) found no evidence of interaction between *P. penetrans* and ARD in inoculation studies. Therefore, the damage caused by this nematode would be approximately additive to that caused by ARD; additional experiments are required to estimate the relative effects attributable to each factor in soil "Eb." Although the number of sites evaluated in this study was small, the data support a previous report (12) that actinomycete infection of roots was associated with orchard soils conducive to ARD.

Information derived from work with seedlings, as in our studies, should be supported by studies with grafted transplants in the field and at various locations. However, before meaningful field studies can be developed, the pathogenic actinomycetes must be isolated and identified and their role as pathogens confirmed. Isolation of actinomycetes from infected seedling roots has been attempted, but only saprophytic species have been collected (21). Successful isolation may require special techniques, because the pathogenic actinomycetes appear sensitive to several surface disinfestants and they do not compete well in vitro with saprophytic bacteria.


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