# Fungi Associated with Roots of Apple Seedlings Grown in Soil from an Apple Replant Site

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**ABSTRACT**

Jaffee, B. A., Abawi, G. S., and Mai, W. F. 1982. Fungi associated with roots of apple seedlings grown in soil from an apple replant site. Plant Disease 66:942-944.

Apple seedlings (Northern Spy) exhibited severe stunting and root discoloration when grown in steamed field soil amended with 5% (v/v) untreated field soil obtained from an orchard with a history of apple replant disease. Isolation of pathogenic fungi from discolored root segments harvested at different time intervals was variable. However, isolation made from roots of seedlings grown in steamed field soil amended with washed feeder roots obtained from seedlings previously grown in 5% untreated field soil consistently yielded *Cylindrocarpon lucidum* and *Pythium irregulare*. *C. lucidum* and *P. irregulare* were generally isolated from black and orange lesions, respectively. Both fungi were pathogenic to Northern Spy apple seedlings grown under growth chamber conditions.

Apple trees often grow poorly on sites previously planted with apple. This problem, termed apple replant disease (ARD), seriously suppresses growth and yield in most apple-growing regions of the world (1–3,6,9). Although ARD has been recognized for many years, its etiology is still not clear. Proposed agents include parasitic nematodes and fungi, nonparasitic rhizosphere microorganisms, and allelopathic compounds. The disease is difficult to diagnose because the stunting and root necrosis are neither distinctive nor always dramatic (3,10). Often the disease is recognized only when tree growth in fumigated and unfumigated soil is compared (3).

In Europe, pot tests are used to diagnose ARD (3). Young apple seedlings (two- to three-leaf stage) are grown in pots containing fumigated or unfumigated orchard soil. The grower is advised to fumigate when growth in fumigated soil is substantially greater. Results obtained from these pot tests correlate reasonably well with those obtained in the field (3).

In many New York orchards, the nematode *Pratylenchus penetrans* injures apple trees and is an important component of ARD (4,6). Jaffee et al (4) recently concluded that a biotic agent in addition to nematodes is involved in the disease. Apple seedlings grown in steamed soil amended with 5% (v/v) untreated field soil were stunted and had discolored feeder roots. The symptoms exhibited by seedlings grown in 5% untreated soil were similar to but less severe than those occurring in 100% untreated orchard soil. Because the 5% untreated soil contained few parasitic nematodes, Jaffee et al concluded that the stunting and root discoloration were caused by other agents. Prior to incorporation into steamed soil, treatment of the 5% orchard soil with certain biocides, including gamma radiation and chloropicrin, resulted in excellent control. Therefore, the unknown agent is probably a living organism and not a toxic compound that had accumulated in the soil. The purpose of the present investigation was to isolate and identify fungi that might be involved in the disease.

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## MATERIALS AND METHODS

Source and handling of soil and apple seedlings used in this study were identical to those reported previously (4). Briefly, soil (a well-drained, sandy loam, pH 6.8) was collected from an orchard with a history of ARD. Part of the soil was heated to 75 C for 30 min with aerated steam (SS = steamed soil). Ten-day-old apple seedlings (Northern Spy) were transplanted into 10-cm-diameter clay pots filled with SS or with SS amended with 5% (v/v) untreated ARD field soil (5% FS). Pots were placed in a growth chamber at 20 C with 14 hr of fluorescent and incandescent light (21 klux) per day and maintained for 6 wk until the seedlings were harvested. Unless otherwise indicated, all treatments were replicated eight times, and experiments were performed at least twice. Data were combined and subjected to analysis of variance and Duncan's multiple range test.

**Isolation and pathogenicity of fungi from roots.** Root systems of five seedlings growing in SS or 5% FS were collected at 3, 7, 14, 28, and 42 days after transplanting. After being washed in running tap water for 2 hr, 30 feeder root segments, approximately 3 mm in length, were placed on 2% water agar (WA) and cornmeal agar (CMA) plates. The remaining root systems were surface-sterilized in 0.5% sodium hypochlorite for 5 min and rinsed in sterile, distilled water. Thirty segments were then placed on acidified (0.1 ml of 10% lactic acid per plate) potato-dextrose agar (PDA). CMA plates were not used in the 28- and 42-day samples. The plates were incubated at 22 ± 2 C for 4–7 days.

Hyphal tips from the edge of advancing fungal colonies were transferred to PDA or CMA plates for identification. Two isolates of *Rhizoctonia* sp. and one of *Cylindrocarpon* sp. obtained in this test were tested for their pathogenicity to apple seedlings. *Rhizoctonia* inoculum was prepared according to the procedure of Ko and Hora (5) and was incorporated into SS at a rate of 1% (v/v). Ten-day-old seedlings were transplanted into the *Rhizoctonia*-infested soil or into noninfested soil. Inoculum of *Cylindrocarpon* sp. consisted of macroconidia and microconidia obtained from 10-day-old PDA cultures. Seedlings were dipped into an aqueous suspension of conidia for 5 min prior to transplanting. Controls were dipped in sterile, distilled water.

Isolations were also made from seedlings grown in SS amended with washed (2 hr running tap water) feeder roots. The feeder roots, obtained from seedlings grown in SS or 5% FS for 42 days, were cut into 2-mm pieces and incorporated into SS (2 g of fresh roots per 500 $cm^3$ of soil). Root systems of seedlings grown in root-amended soils for 42 days were removed from the soil and washed. Root segments were placed on WA, acidified PDA, or CMA containing pimaricin, vancomycin, and pentachloronitrobenzene (8). Ten segments from each root system were plated per medium. The two fungi recovered most frequently in this test, *Cylindrocarpon lucidum* Booth and *Pythium irregulare*

Buisman, were tested for their pathogenicity to Northern Spy apple seedlings. A macroconidial suspension obtained from a single-spore isolate of *C. lucidum* was added to SS at 0, 100, 500, 1,000, or 5,000 macroconidia per cubic centimeter of soil. Ten-day-old apple seedlings were then transplanted into infested or noninfested SS and were grown as previously described.

The isolate of *P. irregulare* was first separated from bacterial contaminants (11). Inoculum was prepared by growing *P. irregulare* on autoclaved oat seeds for 8 days in darkness at 22 ± 2 C. One or two *Pythium*-colonized oat seeds added to the planting hole or mixed in the soil served as inoculum sources in two tests. In a third test, the inoculum source consisted of 2 g of washed roots infected with *P. irregulare* obtained from a previous pathogenicity test.

**Table 1.** Pathogenicity of *Cylindrocarpon lucidum* to Northern Spy apple seedlings grown in artificially infested soil in a growth chamber

| Inoculum density[x] (conidia/cm³ of soil) | Shoot dry weight (g) | Root dry weight (g) | Root discoloration (%)[y] |
|---|---|---|---|
| 0 | 3.3 a[z] | 0.76 a | 5 a |
| 100 | 2.7 a | 0.72 a | 67 [illegible] |
| 500 | 1.1 b | 0.41 b | 92 c |
| 1,000 | 1.3 b | 0.40 b | 93 c |
| 5,000 | 0.8 b | 0.27 b | 93 c |

[x] Macroconidia obtained from 2-wk-old cultures grown on potato-dextrose agar were mixed into steamed soil. Ten-day-old apple seedlings were transplanted into the soil and grown for 6 additional weeks.
[y] Visually estimated.
[z] Means in a column followed by the same letter are not significantly different at $P = 0.05$.

**Table 2.** Pathogenicity of *Pythium irregulare* to Northern Spy apple seedlings grown in artificially infested soil in a growth chamber

| Inoculum source | Shoot dry weight (g) | Root dry weight (g) | Root discoloration (%)[v] |
|---|---|---|---|
| Oat seed[w] | | | |
| Uninoculated | 6.1*[x] | ...[y] | 5* |
| Inoculated | 3.2 | ... | 37 |
| Washed apple root[z] | | | |
| Uninoculated | 2.4* | 0.57* | 9* |
| Inoculated | 1.1 | 0.29 | 44 |

[v] Visually estimated.
[w] Ten-day-old apple seedlings were transplanted into pots containing 200 cm³ of steamed soil and grown for 1 mo. The root-soil ball was then carefully transferred to a 10-cm-diameter clay pot containing 300 cm³ of steamed soil plus two oat seeds colonized or not colonized by *P. irregulare*. The seedlings were grown for 5 additional weeks.
[x] Significantly different from inoculated treatment, $P = 0.05$.
[y] No data recorded.
[z] Ten-day-old apple seedlings were transplanted into 500 cm³ of steamed soil amended with 2 g of washed apple roots infected or not infected with *P. irregulare*. The seedlings were grown for 6 additional weeks.

## RESULTS

**Isolation and pathogenicity of fungi from roots.** Fungi of the following genera were isolated from seedlings grown in 5% FS: *Rhizoctonia, Cylindrocarpon, Fusarium, Alternaria, Trichoderma, Pythium*, and *Phytophthora. Alternaria, Gilmaniella*, and *Penicillium* spp. were isolated from seedlings grown in SS. Most fungi were isolated infrequently and in low numbers (ie, from less than 15% of the root segments placed on any medium at any time). Direct examination of squash mounts of stained or unstained fresh apple root tissues did not consistently reveal fungal structures during the first 3 wk after transplanting. However, stunting and root necrosis were evident by the third week after transplanting. By day 42, a *Rhizoctonia* sp. and a *Cylindrocarpon* sp. were recovered from 20 and 30%, respectively, of the surface-sterilized root segments plated on acidified PDA. Results of pathogenicity tests with both fungi were negative because neither organism induced stunting or root discoloration. Nevertheless, it was possible to reisolate these fungi readily by placing surface-sterilized root segments from inoculated seedlings on acidified PDA.

Seedlings grown in SS amended with

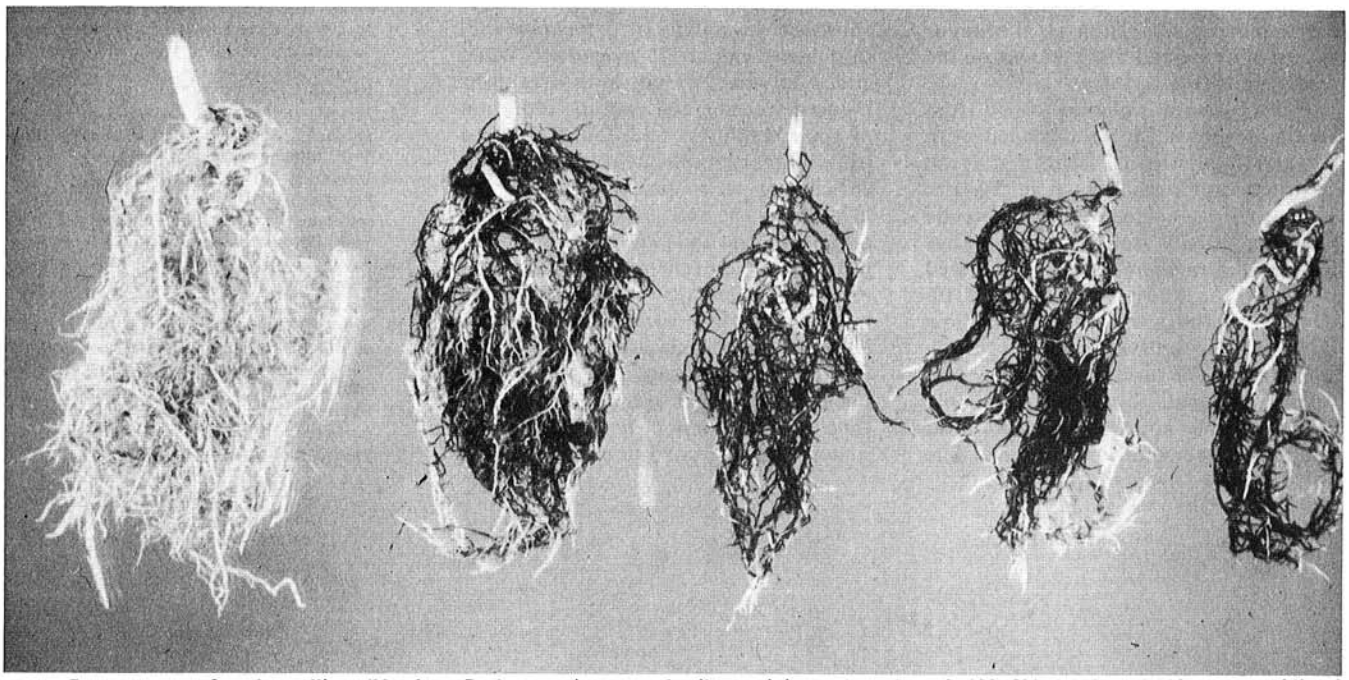

**Fig. 1.** Root systems of apple seedlings (Northern Spy) grown in steamed soil containing **(left to right)** 0, 100, 500, 1,000, or 5,000 macroconidia of *Cylindrocarpon lucidum* per cubic centimeter of soil.

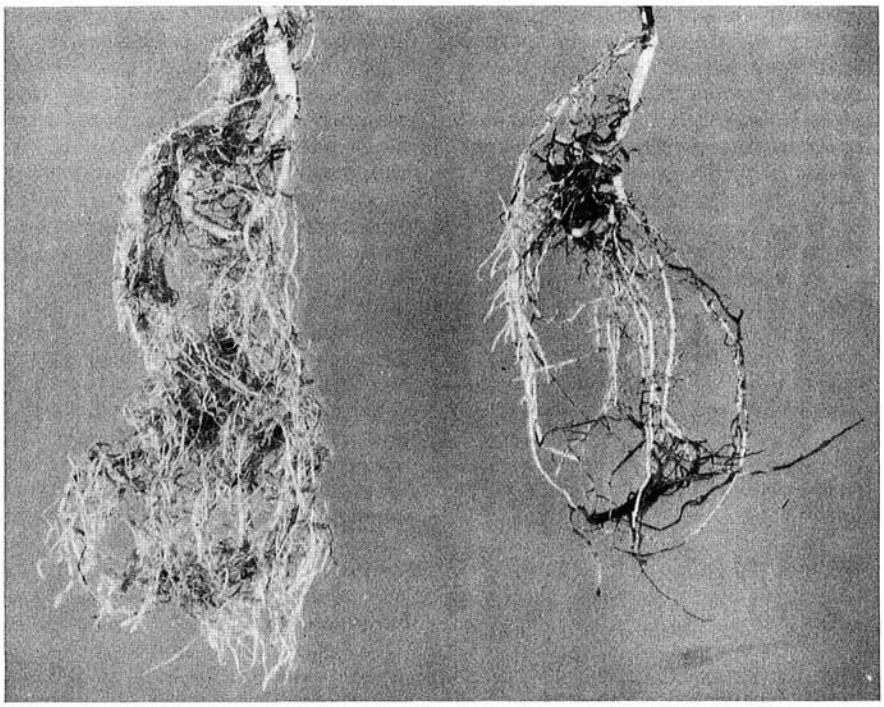

**Fig. 2.** Root systems of apple seedlings (Northern Spy) grown in steamed soil amended with 2 g of washed apple feeder roots that were **(left)** uninfected and **(right)** infected with *Pythium irregulare*.

washed feeder roots obtained from plants grown in 5% FS exhibited significant root discoloration that consisted predominantly of black lesions. Washed feeder roots obtained from plants grown in SS did not induce such root discoloration. When segments of roots with black lesions were plated on acidified PDA or WA, a *Cylindrocarpon* sp. that produced boat-shaped macroconidia and no microconidia was recovered from about 80% of the segments. This organism was tentatively identified as *C. lucidum* by Dr. A. Y. Rossman (New York Botanical Garden). *C. lucidum* was pathogenic to Northern Spy apple seedlings (Table 1). The fungus caused stunting and black lesions on the feeder roots (Fig. 1).

*P. irregulare* was also recovered from seedlings grown in SS amended with washed feeder roots obtained from seedlings previously grown in 5% FS. However, it was not recovered consistently and was usually isolated from orange lesions. *P. irregulare*-colonized oat seed placed in the transplanting hole of 10-day-old seedlings caused 25% seedling damping-off and subsequent death within 2 wk. The surviving seedlings were severely stunted. Similarly, 40-day-old apple seedlings transplanted into pots containing two colonized oat seeds were stunted and showed orange necrosis of feeder roots within 5 wk (Table 2, Fig. 2). Stunting and orange necrosis of the roots also occurred after 10-day-old apple seedlings were transplanted into SS amended with roots infected with *P. irregulare* (Table 2). *C. lucidum* and *P. irregulare* were readily reisolated from inoculated but not from uninoculated seedlings. Neither organism was isolated from seedlings grown in SS amended with washed feeder roots obtained from seedlings previously grown in SS.

## DISCUSSION

The addition of diseased roots enhanced the activity of *C. lucidum* and, to a lesser extent, *P. irregulare*. When added to steamed soil, both organisms induced stunting and root discoloration of apple seedlings. Addition of diseased roots to steamed soil might function as an enrichment technique resulting in the increase of the soil population of these organisms and thus an increase in their pathogenic potential. The reduced microbial competition in steamed soil might also have contributed to their increased pathogenic activities. Although the role of these fungi in ARD remains unclear, they might over a long period of time play an important role in ARD under orchard conditions. Species of *Pythium* and *Phytophthora* have been implicated in diseases of many fruit crops, including ARD (7,10). To our knowledge, this is the first report implicating *C. lucidum* as a pathogen of apple roots.

Apple seedlings have been widely used in studying ARD and are currently used in diagnosis of such diseases. Although the advantages of using seedlings rather than trees as assay plants are obvious, results obtained with seedlings should be considered preliminary until verified with trees in the field. Caution should also be used in the interpretation of results obtained from seedlings grown in 5% FS. The advantages of using 5% FS have been previously discussed (4). Although 5% FS presumably contains the entire soil microflora and is mixed thoroughly into the steamed soil, the soil system is clearly not in equilibrium. It is therefore possible that a fast-growing pathogen could take advantage of such a system and induce a disease different from that occurring in 100% FS. However, the disease occurring in 5% FS appeared to be identical, although less severe, than that occurring in 100% FS (4).

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