# Role of Soil Microflora and *Pratylenchus penetrans* in an Apple Replant Disease

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## ABSTRACT

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Consistent reproduction of apple replant disease (ARD) was developed under controlled conditions. A small quantity of untreated field soil (FS) obtained from an orchard with a history of ARD was incorporated into a steamed (75 C for 30 min) portion of the same soil (SS). Ten-day-old apple seedlings (Northern Spy) were transplanted into SS or 5% FS (5 parts FS + 95 parts SS) and maintained for 6 wk in a growth chamber. The addition of 5% FS reduced plant dry weight by 50% and induced orange and black root discoloration. Treatment of the FS (prior to incorporation in SS) with gamma radiation, broad-spectrum fumigants, and heating (60 C or higher for 30 min) eliminated the stunting and root discoloration. *Pratylenchus penetrans* was the most abundant parasitic nematode, but its highest density in 5% FS was only four per 100 $cm^3$ soil. This density was considered to be below the damaging level. Addition of 140 or more *P. penetrans* per 100 $cm^3$ SS resulted in significant stunting and root necrosis. Our data suggest that at least two agents contribute to the ARD occurring in the field: an unknown organism(s) inducing stunting and root discoloration in 5% FS, and *P. penetrans*.

Apple replant disease (ARD) refers to the poor growth of replanted apples on old orchard sites. Although many researchers have studied ARD, its etiology is still not clear. The disease is common and widely distributed throughout the apple growing regions of the world. In the United Kingdom, approximately 50% of all orchard soils surveyed were considered as potential ARD soils (21). A recent study in Washington state indicated that 14 of the 17 orchard soils examined were ARD soils (5). In both cases, a soil was considered an ARD soil if substantially greater growth of apple was obtained as a result of soil fumigation. In New York, fumigation of an ARD soil increased yield by as much as 100% (1).

Oostenbrink and Hoestra (17) recognized two general types of ARD; a specific apple replant disease (SARD) and a nonspecific replant disease (NSARD). Both types of ARD involve stunting and root necrosis, but these symptoms are not diagnostic. NSARD affects other fruit crops as well as apple and is caused, at least in part, by plant parasitic nematodes (6,8,13,14). The poor growth usually has a nonuniform distribution in the orchard and is accompanied by high counts of parasitic nematodes, primarily *Pratylenchus* spp. In contrast, SARD is specific (only apples are affected) and its occurrence does not correlate with the presence of plant parasitic nematodes. The stunting is usually distributed more evenly throughout the orchard and may therefore go unnoticed unless the condition is severe. Both types of ARD are controlled by preplant application of several soil fumigants. However, a broad-spectrum biocide is required to control SARD, whereas a nematicidal fumigant such as dichloropropene-dichloropropane (DD) is sufficient to control NSARD (7,20).

Although SARD was the subject of numerous investigations, its exact etiology remains unknown. The causal agent is generally considered to be biotic because SARD is controlled with soil fumigation and heat treatment. Because parasitic pathogens could not be observed or recovered consistently from diseased roots, both Savory (20) and Hoestra (7) suggested that the causal agent does not penetrate the roots. Savory (20) proposed that rhizosphere bacteria may be responsible. Hoestra (7) hypothesized that rhizosphere bacteria or actinomycetes were responsible because partial control of ARD was obtained by lowering soil pH. Others (2,16) suggested that apple root exudates or decomposition products are important factors. Sewell (22) has recently provided evidence indicating the involvement of pythiacious fungi.

In a recent review, Sewell (21) stated that much of the specificity attributed to SARD was based on observational rather than experimental evidence. He noted that apple growth is often suppressed in nonreplant sites and that the growth of unrelated crops is also affected on old apple sites. Mai and Abawi (12) described an ARD resembling the nonspecific type in that apple, pear, and cherry were affected and the soil contained high counts of *Pratylenchus penetrans* (Cobb) Filipjev and Schuurmans-Stekhoven. However, the disease also resembled SARD in that treatment with a broad-spectrum biocide (chloropicrin) resulted in considerably greater growth response than did treatment with the nematicide DD. Because both chloropicrin and DD gave excellent nematode control, Mai and Abawi suggested that organisms in addition to nematodes were involved in the disease.

The purpose of the present investigation was to identify and assess the importance of factors contributing to an ARD in a New York orchard soil.

## MATERIALS AND METHODS

**General.** Soil was collected from the drip-line of 12-yr-old apple trees on MM-106 rootstocks growing in an orchard with a known history of apple replant disease located in Wayne County, NY. This was the same source of apple orchard soil used by Mai and Abawi (12). The soil was passed through a 6-mm screen and was mixed thoroughly. Part of the soil was heated in 15-L lots to 75 C for 30 min with aerated steam. This soil will be referred to as SS (steamed soil). Following steaming, the soil was spread on a greenhouse bench for 3 days for aeration and drying. It was stored at 5 C for at least 3 wk prior to use. Part of the soil was not steamed and was stored at 5 C. This soil will be referred to as FS (field soil).

Seeds of cultivar Northern Spy apples were surface sterilized in 1% NaOCl for 30 min, rinsed with water, dried, and stored at 4 C. To achieve stratification, dried seeds were placed in 0.5% NaOCl for 5 min, rinsed, dusted with captan 50% WP, placed in moist, autoclaved vermiculite, and incubated at 4 C. Seedlings with radicles approximately 5 mm in length were planted in a flat containing autoclaved vermiculite. Ten days after emergence, seedlings were transplanted into 10-cm diameter clay pots (one seedling per pot) containing 500 $cm^3$ soil. All experiments were conducted in a 20 C growth chamber with 14 hr of 21 klux light per

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day. Plants were watered as needed and fertilized every 10 days with 0.15 g RA-PID-GRO fertilizer (23-19-17 plus micronutrients, RA-PID-GRO Corp., Dansville, NY 14437) in 50 ml of water per plant. Experiments were terminated 6 wk after the seedlings were transplanted. Root discoloration was visually rated from 0 to 100%. Shoots and roots were dried at 95 C for 2 days prior to weighing.

Unless otherwise indicated, all experiments consisted of eight replications per treatment and were performed at least twice. Data were combined and analyzed by analysis of variance and, where appropriate, by least significant difference and Duncan's multiple-range tests.

In a preliminary experiment, apple seedlings were transplanted into SS amended with various percentages (v/v) of FS. Shoot dry weights of seedlings growing in 0, 1, 2, 5, 10, 50, or 100% FS were 3.9, 2.3, 1.7, 1.2, 0.9, and 0.5 g, respectively (Fig. 1). To reproduce and study the disease, 5% FS (5 parts FS + 95 parts SS) was generally used in subsequent experiments. The advantages of using 5% FS rather than 100% FS are presented in the discussion.

**Effect of initial population densities of *P. penetrans* on seedling growth and root discoloration.** Apple seedlings were transplanted into SS previously amended with 0, 2, or 5% (v/v) FS. One day later, *P. penetrans* in aqueous suspension (juveniles and adults from alfalfa callus) were pipetted into four holes about 3 cm from the stem. Initial population densities tested were 0, $10^3$, $3.5 \times 10^3$, or $7.0 \times 10^3$ nematodes per pot. Nematodes were extracted from the soil with a pie-pan modification of the Baermann funnel. A shaker technique was used to extract nematodes from roots (12).

**Effect of biocides on the incidence of apple replant disease.** Apple seedlings were transplanted into SS to which FS (5%) had been added. Prior to incorporation, the FS was exposed to gamma radiation, fumigation, air-drying, heat-treatment, or treatment with selected fungicides as follows: FS in glass test tubes (2 cm in diameter) was exposed to 2.1–2.7 Mrad gamma radiation at the Ward Laboratory of Nuclear Science and Engineering, Cornell University, Ithaca, New York (test tubes that were kept outside the gamma cell served as controls); 400 $cm^3$ FS was placed in 600-ml beakers and fumigated with chloropicrin (0.5 ml/L) or DD (0.25 and 2.0 ml/L) and the beakers were immediately sealed in plastic bags, incubated for 3 days, opened, and the samples were aired for 2 days prior to incorporation into SS (FS treated in the same manner minus fumigation served as a control); FS (approximately 15% moisture) was spread (approximately 5-mm-thick layer) on a growth chamber bench and air-dried for 5 days with frequent mixing (final moisture content of the soil was 1%); FS was heated to 40, 50, 60, and 70 C for 30 min with aerated steam (one sample was not heated and served as a control); fungicides were applied as a drench treatment in 100 ml water per 400 $cm^3$ FS and the amount of each fungicide used was adjusted to give application rates in the planting medium (95% SS + 5% FS) of 1, 5, 10, and 50 mg benomyl per liter; 1, 5, 10, 50, and 100 mg fenaminsulf per liter; 75 mg mancozeb per liter; 1, 10, 50 mg metalaxyl per liter; 5 mg etridiazol per liter; and 200 mg terrachlor per liter. Seven days later, the treated FS was incorporated into SS. The effects of mancozeb and terrachlor were tested once.

**Effect of fertilization on severity of disease.** Seedlings were transplanted into SS or 5% FS. They were fertilized with 0.15 g of RA-PID-GRO fertilizer per pot every 5 days or every 10 days. One treatment was not fertilized. This experiment was performed once.

**Persistence of apple replant disease agent in soil.** Apple seedlings were transplanted into 5% FS as previously described (first planting). The first planting was terminated 6 wk later, the roots were carefully removed from the soil, and the soil for all replicates was pooled and mixed thoroughly. For the second planting, apple seedlings were transplanted into newly steamed soil amended with 5% (v/v) of the 5% FS saved from the first planting. Likewise, for the third planting, apple seedlings were transplanted into newly steamed soil amended with 5% (v/v) of the soil saved from the second planting. Therefore, the concentration of FS in the first, second, and third plantings ("FS series") was 5, 0.25, and 0.01%, respectively. A similar procedure was used for the control ("SS series") beginning with unamended steamed soil. For the third planting, an additional control of seedlings grown in unamended SS was included. All seedlings were fertilized every 10 days as previously described.

## RESULTS

**Effect of *P. penetrans*.** The addition of nematodes and/or FS to steamed apple orchard soil resulted in significant reduction in dry weight of seedlings (Fig. 2). However, the nematode-field soil interaction was not significant ($P = 0.05$). Similar results were obtained for root dry weight and percent root discoloration. At the

**Fig. 1.** Apple seedlings (cultivar Northern Spy) grown in steamed soil amended with (left to right) 0, 1, 2, 5, 10, 50, or 100% (v/v) untreated field soil.

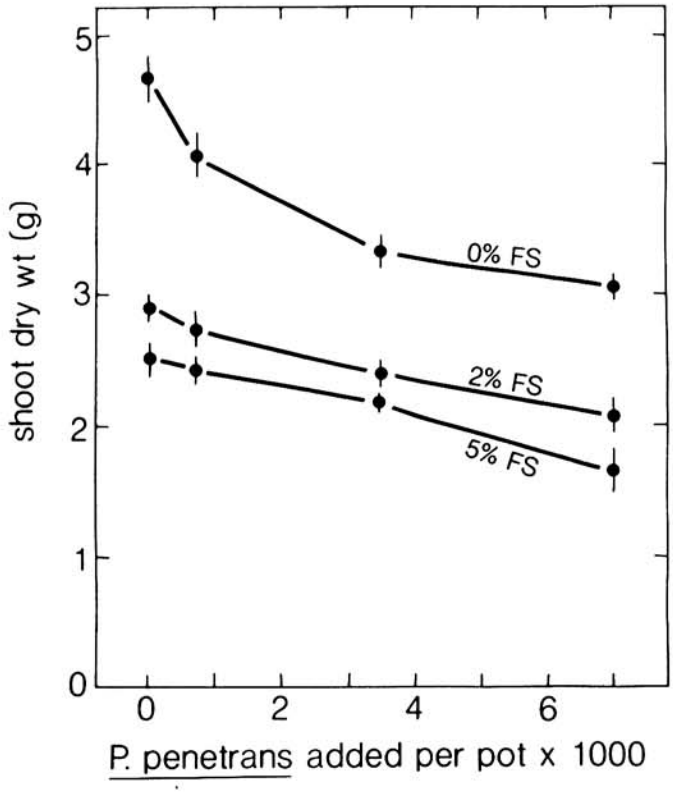


**Fig. 2.** Effect of four levels of *Pratylenchus penetrans* and three levels of field soil on shoot dry weight of apple seedlings (cultivar Northern Spy). Seedlings were transplanted into steamed soil amended with 0, 2, or 5% (v/v) untreated field soil (FS). Nematodes obtained from alfalfa callus were added the next day. Vertical bars indicate the standard error.

time of transplanting, the 5% FS contained about four *P. penetrans* per 100 $cm^3$ soil and less than one per 100 $cm^3$ soil of other species of plant parasitic nematodes. At termination of the experiment, less than three and no *P. penetrans* were recovered per gram of root and per 100 $cm^3$ soil, respectively, from the 5% FS treatment. Other plant parasitic species were recovered in even lower numbers.

Shoots of seedlings growing in 5% FS were stunted, but showed no obvious symptoms of nutritional deficiency. Feeder roots were stunted, pruned, and had orange and black lesions (approximately 50% discoloration) (Fig. 3). The black lesions were relatively distinct and were often limited to the cortex, whereas the orange lesions were less distinct and involved the cortical and stelar tissues. The tap roots were stunted but were not discolored. These symptoms, although less severe, were similar to those occurring on seedlings grown in 100% FS.

**Effect of biocides.** Treatment of FS with gamma radiation or chloropicrin prior to its incorporation into SS resulted in effective control of ARD as measured by shoot dry weight and percent root discoloration (Table 1). Similar results were obtained with root dry weights. DD applied at a rate of 2.0, but not 0.25, ml/L of soil resulted in moderate disease control. Although air-drying reduced the number of *P. penetrans* recovered at transplanting time from four per 100 $cm^3$ to less than one per 100 $cm^3$ soil, it had no effect on disease severity. Heat treatment with aerated steam, however, gave partial control at 50 C and total control at 60 and 70 C as measured by dry weight and percent root discoloration (Fig. 4). Benomyl, fenaminsulf, metalaxyl, etridiazol, mancozeb, and terrachlor at the rates tested were ineffective against the causal agent of the ARD. Fenaminsulf at rates of 50 and 100 mg/L and metalaxyl at a rate of 50 mg/L were phytotoxic.

**Effect of three levels of fertilization.** Level of fertilization affected shoot dry weight in both SS and 5% FS (Fig. 5). Regardless of fertilization rate, growth of apple seedlings in SS was significantly higher than that in 5% FS. The FS-fertilizer interaction was not significant at $P = 0.05$. Similar results were obtained with root dry weight. Root discoloration was not affected by fertilization.

**Persistence of the apple replant disease agent in soil.** The agent responsible for stunting and root discoloration in 5% FS could not be reduced to a less than damaging level by dilution of the original FS through time from 5 to 0.25 and 0.01%. In the third planting SS, which contained small quantities of previously used SS also induced stunting, but did not induce root discoloration (Table 2). The mean shoot dry weight of seedlings transplanted into unamended SS was 3.1 g in the third planting. This was significantly greater ($P = 0.05$) than the other treatments.

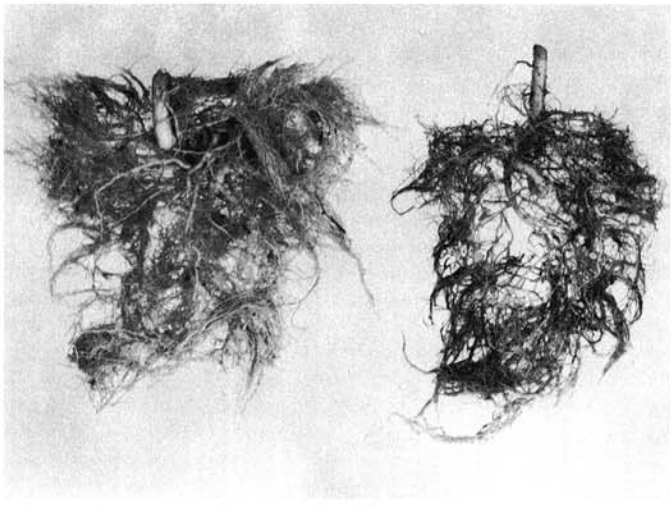

**Fig. 3.** Roots of apple seedlings (cultivar Northern Spy) grown in steamed soil (left) and 5% field soil (right).

## DISCUSSION

Proposed agents of ARD include parasitic nematodes and fungi, nonparasitic rhizosphere microorganisms, and allelopathic compounds. Our previous observations and published data suggested that the plant parasitic nematode, *P. penetrans,* is an important factor affecting growth and yield of apple in the orchard soil used in this study (6,8,10,13,14,19). The pathogenicity of *P. penetrans* to apple and other fruit trees is well documented and its population density in this soil is substantial. Mai and Abawi (12) recovered approximately 450 *P. penetrans* per 100 $cm^3$ soil whereas in the present investigation we recovered approximately 100 *P. penetrans* per 100 $cm^3$ soil. Hoestra and Oostenbrink (8) found that 35 *P. penetrans* per 100 $cm^3$ soil was the minimum damaging level in the orchard soil they studied. The nonspecific nature of the disease (12) and the uneven distribution of stunting in the young orchard (W. F. Mai, *observation*) also point to *P. penetrans* as a primary causal agent. However, the results of the present investigation strongly suggest the involvement of another pathogenic factor that appears to be at least as important as *P. penetrans* in ARD. Seedlings growing in 5% FS consistently exhibited severe stunting and root discoloration even though the number of *P. penetrans* present was far below any published

TABLE 1. Effect of several biocides on the severity of apple replant disease

| Treatment[w] | Shoot dry wt. (g) | Root discoloration[x] (%) |
|---|---|---|
| Steamed soil[y] | 3.1 a[z] | 4 a |
| Gamma radiation (2.4 Mrad) | 3.4 a | 6 a |
| Chloropicrin (0.5 ml/L) | 3.4 a | 6 a |
| DD (2 ml/L) | 2.8 b | 29 b |
| DD (0.25 ml/L) | 1.9 c | 51 c |
| Air dried | 1.6 c | 47 c |
| 5% Field soil (nontreated) | 1.5 c | 43 c |

[w] Apple seedlings (cultivar Northern Spy) were transplanted into steamed soil (SS), 5% field soil (five parts nontreated field soil + 95 parts SS), or 5% field soil in which the field soil was exposed to gamma radiation, fumigation, or air drying prior to incorporation into SS.

[x] Visually estimated.

[y] Soil was heated in 15-L lots to 75 C for 30 min with aerated steam.

[z] Means in a column followed by the same letter are not significantly different at $P = 0.05$.

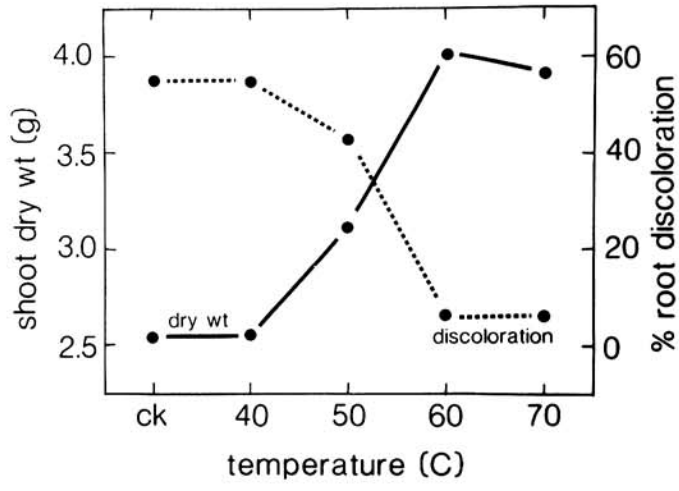


**Fig. 4.** Effect of soil steaming on the severity of apple replant disease. Apple seedlings (cultivar Northern Spy) were transplanted into steamed soil amended with 5% field soil. Prior to incorporation, the field soil was untreated (ck) or treated with aerated steam for 30 min at 40, 50, 60, or 70 C. Root discoloration was visually estimated. LSD ($P = 0.05$) was 0.5 g and 10% for shoot dry weight and percent root discoloration, respectively.

TABLE 2. Shoot dry weight and percent root discoloration of apple seedlings (cultivar Northern Spy) from three consecutive plantings in amended and unamended steamed soil[x]

| Treatments | First planting | | Second planting | | Third planting | |
|---|---|---|---|---|---|---|
| | Shoot dry weight (g) | Root discoloration (%)[y] | Shoot dry weight (g) | Root discoloration (%) | Shoot dry weight (g) | Root discoloration (%) |
| SS series | 3.3*[z] | 6* | 2.5* | 10* | 1.5 a | 11 a |
| FS series | 1.7 | 52 | 1.3 | 51 | 1.2 a | 39 b |
| SS (unamended) | ... | ... | ... | ... | 3.1 b | 7 a |

[x] Apple seedlings were transplanted into steamed soil (first planting, SS series) or 5% field soil (first planting, FS series). The first planting was terminated 6 wk later, the roots removed and the soil of each treatment was pooled and mixed thoroughly. For successive plantings, newly steamed soil was amended with an increment of soil (5%, v/v) from the previous planting in the series. In the third planting, an additional treatment consisted of seedlings grown in unamended steamed soil. Seedlings were fertilized every 10 days.

[y] Visually estimated.

[z] Means in a column for shoot weight or root discoloration followed by asterisk or different letter are significantly different at $P = 0.05$.

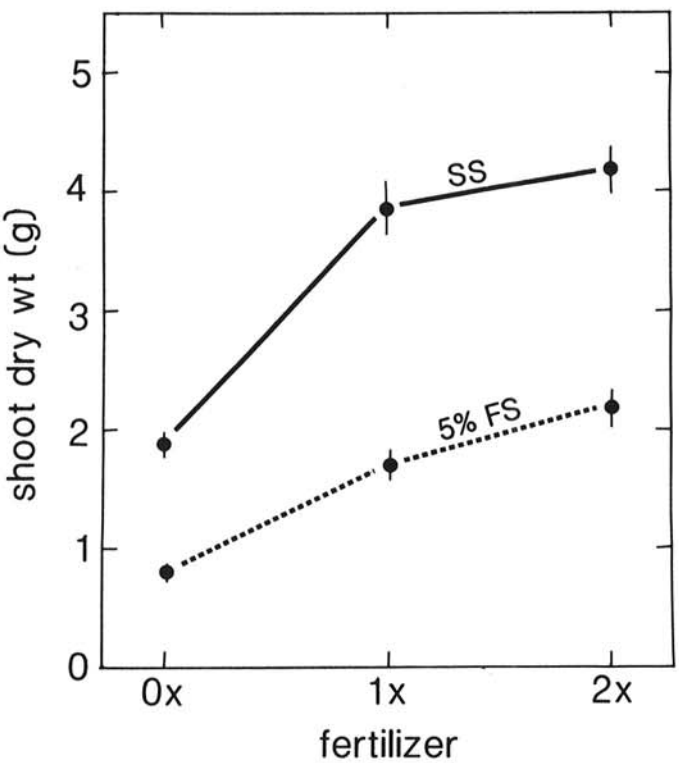


**Fig. 5.** Effect of the rate of fertilization on the severity of apple replant disease. 0× = no fertilization. 1× = normal rate of 0.15 g of 23-19-17 plus micronutrients per plant every 10 days. 2× = same as 1× but every 5 days. Apple seedlings (cultivar Northern Spy) were transplanted into steamed soil (SS, solid line) or 5% field soil (5% FS, broken line). Vertical bars indicate standard error.

damaging level. The generation time for this species ranges from 5 to 12 wk and no increase in population density occurred during the 6 wk of seedling growth. Furthermore, air-drying the soil greatly reduced the population density of all nematodes present, but had no effect on the disease. Treatment with aldicarb also greatly reduced the nematode density, but had no effect on the disease (B. A. Jaffee, *unpublished*).

The disease control obtained with gamma radiation, relatively mild heat treatment (60 C for 30 min), chloropicrin, and high rates of DD, strongly suggests that the agent(s) inducing stunting and root discoloration in 5% FS, although not a nematode, was biotic. Gamma radiation, in particular, is recognized as a biocide with few chemical and physical side effects (11). Our results confirm those of Otto (18) and Hoestra (7) who also reported that 60 or 70 C treatment of SARD-conducive soils resulted in significant disease control. Chloropicrin and high rates of DD are biocides with broad spectrums of activity (7,15,23). The inability to dilute the agent from the soil (Table 2) also indicated an agent that could reproduce. Jaffee (9) isolated several pathogenic, parasitic fungi from apple seedlings growing in an ARD-conducive soil. However, after comparing the growth of apple seedlings in SS amended with washed feeder roots or with small quantities of rhizosphere soil, he hypothesized that the primary causal agent was a nonparasitic rhizosphere organism. Hoestra (7) and Savory (20) suggested a similar hypothesis.

Microbe-plant competition for nutrients is one way in which rhizosphere organisms can induce stunting of plants (3). In the present investigation, the addition of small amounts of FS may have introduced specific organisms that utilized certain nutrients at the expense of the plant. However, the absence of deficiency symptoms and the failure to reduce disease severity with increased fertilization rates argue against this hypothesis.

Several papers have indicated that a "non-SARD soil" could become a "SARD soil" after relatively brief cropping with apple (4,7,20). It has also been suggested that apple roots release compounds which, perhaps after microbial degradation, are toxic to apple trees (2,16). The results reported in Table 2 are particularly interesting in this respect. By the third planting, apple seedlings growing in SS amended with small amounts of previously used SS were as stunted as those growing in SS amended with FS. One possible explanation for this result might be that an allelopathic compound emanating from apple roots had accumulated in the soil. However, the dilution factor and those data that indicate a biotic pathogen argue against this possibility. Another possible explanation is that the steamed soil was recontaminated with the causal organism or that the aerated steam greatly reduced but did not eliminate the organism(s) involved. With time and availability of the required substrates, the organism may have multiplied and again reached a damaging level.

This study made extensive use of a 95% aerated steamed + 5% field soil system. There are several advantages to this system. First, because there were so few nematodes in 5% FS, the effect of other agents could be studied with minimal influence by nematodes. Second, only a small amount of soil required treatment and thus the soil could be treated thoroughly, safely, and in nonconventional ways (eg, with gamma radiation). Finally, the side-effects associated with certain soil treatments (eg, fumigation and steaming) were minimized when only 5% of the soil was actually treated.

The findings reported here are derived from one site in New York state. Considering the importance of ARD in this state (1,12), a survey of other sites is certainly warranted. If one soil is used as the steamed base soil, the disease induced by adding small quantities of soil obtained from many different orchards could be tested relatively easily. It would also be valuable to test nonorchard soils and to assay crops other than apple.

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