Preplant Root Treatments to Reduce the Incidence of *Phytophthora* Species on Dormant Apple Rootstocks

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ABSTRACT

Jeffers, S. N. 1992. Preplant root treatments to reduce the incidence of *Phytophthora* species on dormant apple rootstocks. Plant Dis. 76:12-19.

In trials over a 6-yr period, roots of dormant MM.106 apple (*Malus domestica*) rootstocks were soaked in aqueous fungicide suspensions before planting to reduce the incidence of naturally occurring inoculum of *Phytophthora cactorum* and *P. cambivora*, causal agents of Phytophthora crown rot. Initially, roots were soaked for 10 min or 1 hr in 1,000 mg/L of captafol, copper hydroxide, mancozeb, or metalaxyl or in 1.05% sodium hypochlorite. In later trials, metalaxyl and copper hydroxide were compared with etridiazole, fosetyl-Al, and oxadixyl. The standard duration of soaking was 1 hr and the standard concentration of active ingredient was 1,000 mg/L, except that etridiazole was tested at 100 mg/L because the higher concentration was phytotoxic. After treatment, rootstocks were planted in sterilized or pasteurized soil and grown for 10–13 wk with periodic flooding to enhance disease development and pathogen proliferation. At the end of the trials, shoot and root growth were measured, and roots of plants were assayed for *Phytophthora* spp. with an apple-cotyledon baiting procedure. In addition, three and five times the standard concentration of active ingredients and a 3-hr soaking duration were evaluated for potential phytotoxicity. Preplant root-soak treatments significantly reduced the number of plants from which *Phytophthora* spp. were detected and decreased the severity of root and crown rot. Metalaxyl was the most effective treatment, although copper hydroxide also was effective. Both treatments consistently resulted in plants that were vigorous and appeared healthy. Phytotoxicity was not a significant problem with any of the fungicide treatments.

Phytophthora crown rot is one of the most important diseases affecting rootstocks of apple trees (*Malus domestica* Borkh.). The disease is caused by various species of *Phytophthora*, although *P. cactorum* (Lebert & Cohn) J. Schröt. is the species most frequently encountered worldwide (15). Unbudded apple rootstocks from propagation nurseries are known to be naturally infested with *Phytophthora* spp. (primarily *P. cactorum* and *P. cambivora* (Petri) Buisman) and, therefore, are a major source of primary inoculum (2,12,16,18,23). In a recent study (12), a high percentage of the apple rootstocks from all 11 of the nurseries sampled, including one in British Columbia, Canada, and two in the Netherlands, had *Phytophthora* spp. present in rhizosphere debris.

Pathogen-free planting stock is a basic component of effective plant disease management, even if the pathogen occurs naturally at the planting site (1). Ideally, pathogen-free plants should come from the propagator. However, clonal apple rootstocks, which comprise the majority of those being planted in commercial orchards, are produced in perennial propagation beds. Elimination of *Phytophthora* spp. from these beds or establishment and maintenance of pathogen-free propagation beds are likely to take considerable time. Consequently, a more immediate remedy is needed.

The elimination or reduction of primary inoculum can be an important component of a disease management strategy. Dipping or soaking infested roots in chemical suspensions or solutions before planting is one alternative to reducing the incidence of *Phytophthora* spp. on apple rootstocks. Although this approach was moderately successful in a previous study (2), phytotoxicity occurred with all treatments evaluated. Over the years, a variety of fungicides with different modes of action have been used to manage diseases caused by *Phytophthora* spp. (3,22), and some of these may be useful as preplant root-soak treatments. Of particular interest are the newer, systemic fungicides that are selectively active against oomycetes. The objective of this research was to develop a nonphytotoxic chemical treatment that could be applied before planting and would reduce naturally occurring inoculum of *Phytophthora* spp. on the roots of dormant, unbudded apple rootstocks. A preliminary report has been published (14).

This research was funded in part by the University of Wisconsin-Madison Graduate School (project numbers 0522 and 0663).

Accepted for publication 23 July 1991 (submitted for electronic processing).

MATERIALS AND METHODS

Detection of *Phytophthora* spp. An apple-cotyledon baiting bioassay (11) was used to detect *P. cactorum* and *P. cambivora* in rhizosphere soil or debris collected from apple rootstocks (12). All samples were baited both by direct and extended procedures. For direct baiting, a subsample of soil or debris was placed in a deep petri dish (100 × 20 mm) or a 475-ml glass jar (90 × 90 mm) and flooded with distilled or deionized water. Four to six apple cotyledons (open-pollinated cv. McIntosh) were floated on the surface, and containers with flooded soil were placed in a growth chamber at 20 C with a 12- to 16-hr photoperiod for 7 days. Cotyledons were examined microscopically after 4 and 7 days for the characteristic sporangia of *P. cactorum* or *P. cambivora* (12). For extended baiting, water was decanted from subsamples after 7 days, and subsamples were air-dried thoroughly for 5–10 days at room temperature (21–25 C) and then remoistened with enough distilled water to wet, but not saturate, them. Containers were covered with lids, placed back in the 20-C growth chamber for 3 days, and then flooded and baited as in the direct method. The number of subsamples assayed for each rootstock and specific volumes of soil and water used to bait each subsample varied among experiments.

General experimental methods. The methods described here apply to all trials unless noted otherwise. Unbudded MM.106 (or EMLA.106) clonal apple rootstocks, known to be susceptible to *Phytophthora* spp. (15), were used throughout the study. Over the course of this research, rootstocks were obtained from several nurseries located in Oregon and Washington that are common suppliers of rootstocks for the apple industry in the United States. All rootstocks used in a given year were from the same nursery. Before use each year, rootstocks were confirmed to be naturally infested with *P. cactorum* or *P. cambivora* by baiting the debris washed from one or two representative bundles (50–100 rootstocks per bundle).

All chemical suspensions or solutions (i.e., preparations) were prepared with tap water, and concentrations are expressed as amounts of active ingredient per liter of preparation. Rootstocks always were soaked in preparations while dor-

mant, because the concentrations used were likely to be phytotoxic to actively growing plants. Only the roots and lower stem of a rootstock were treated. After soaking for a specified duration, rootstocks were removed from chemical preparations, and excess liquid was allowed to drain passively from the roots. All rootstocks in a given treatment were then wrapped in paper (to prevent roots from desiccating) and held at ambient environmental conditions until planted. Planting commenced immediately after rootstocks were drained and wrapped. After planting, rootstocks were watered thoroughly and pruned to a uniform height. Except for the field trial conducted during 1984–1985, plants in all trials were arranged in a completely randomized design.

Treated plants in most trials were flooded periodically to enhance disease development and allow surviving pathogen propagules to proliferate. Plants were flooded by placing pots in plastic containers, each with a single drain hole that was plugged, and adding water to reach a level several centimeters above that of the soil in each pot. Rootstocks were flooded for continuous 48-hr periods at 2-wk intervals, beginning 2–3 wk after planting when shoots had begun to grow; there were four flooding episodes per trial, except in 1987 when there were five.

Plants were grown either outside, fully exposed to ambient environmental conditions, or in a greenhouse. As rootstocks broke dormancy, one to three shoots from the most distal buds were allowed to grow and all others were removed. Plants were watered and fertilized as needed in between flooding episodes. At the end of a trial, each plant was evaluated for root weight, shoot length (calculated as the sum of the lengths of individual shoots), and the presence of *Phytophthora* spp. in the rhizosphere (by baiting) and, occasionally, in roots (by direct isolation). Detection of *Phytophthora* spp. demonstrated that naturally occurring inoculum in or on the roots and lower stem of apple rootstocks at the onset of the trial had not been eliminated by the treatment.

Initial experiments. Initial experiments to compare chemicals with diverse modes of action and activity spectra were conducted at the New York State Agricultural Experiment Station at Geneva, NY, in 1983 and 1984. The chemicals evaluated were copper hydroxide (Kocide 101 77WP), captafol (Difolatan 4F), mancozeb (Dithane M-45 80WP), metalaxyl (Ridomil 2E), and sodium hypochlorite (Clorox, 5.25% a.i.). Based on preliminary experiments, concentrations (prepared with distilled water) of 1,000 mg/L for all four fungicides and 1.05% (10,500 mg/L) for sodium hypochlorite (i.e., 20% bleach) were evaluated.

In 1983, 11 dormant rootstocks were soaked in each preparation for 10 min, rinsed thoroughly in running tap water, drained, placed in polyethylene bags, and stored overnight at 2 C. The next day, each rootstock was planted individually in autoclaved coarse-textured vermiculite in a 15-L plastic pot. In addition, 22 rootstocks that were not treated were planted as controls. Rootstocks were grown outside for 13 wk (26 July–29 October). All plants, except 11 untreated controls, were flooded four times.

At the end of the experiment, each plant was uprooted gently, and the roots of all 11 rootstocks from the same treatment were washed collectively. Roots from each plant were removed, blotted dry, and weighed. If present, discolored, rotted roots or small pieces from stem cankers were removed and placed on PAR or PARH selective agar medium (13), which contained 5 mg of pimaricin, 250 mg of sodium ampicillin, 10 mg of rifampicin, and 50 mg of hymexazol (PARH only) per liter of Difco cornmeal agar. Culture dishes were placed in the dark at 20 C and were examined periodically for up to 14 days. The remaining roots from each rootstock were placed in 120-ml glass jars, covered with 50–75 ml of distilled water, and baited with two apple cotyledons per jar. Shoots from each rootstock were removed and lengths were measured.

In 1984, the experiment was repeated with some modifications. The same chemicals and concentrations were evaluated; however, six rootstocks were soaked in each aqueous preparation for 1 hr instead of 10 min. Rootstocks were not rinsed after soaking and were planted immediately. Twelve rootstocks that were soaked in water for 1 hr before planting served as controls. Plants were grown for 10 wk (9 May–17 July) in a greenhouse (22–27 C). All but six untreated controls were flooded four times. To detect *Phytophthora* spp., the root system of each rootstock was washed in 1 L of distilled water to dislodge adhering vermiculite, and this rhizosphere vermiculite was concentrated by sedimentation overnight at 2 C. Excess water then was decanted, and the sedimented vermiculite was divided among six jars and baited. Isolations also were made from roots and/or crown pieces of each plant as described previously.

A field trial to evaluate potential detrimental effects of preplant root soaks on apple tree growth was conducted during 1984–1985 at a commercial nursery near Geneva, NY. Seven treatments were evaluated, including an untreated control, metalaxyl at 500 or 1,000 mg/L for 30 or 60 min, and sodium hypochlorite

Table 1. Severity of Phytophthora root and crown rot and incidence of *Phytophthora* spp. on MM.106 apple rootstocks after soaking roots for 10 or 60 min in aqueous chemical preparations before planting[t]

Chemical	Concentration[u] (mg/L)	1983 (10-min soak)[v] Shoot length (mm)	Fresh root wt (g)	No. of plants infested[w]	1984 (60-min soak)[v] Shoot length (mm)	Fresh root wt. (g)	No. of plants infested[w]
Nonflooded control		774 c	12.94 c	8/11	1,558 c	20.00 c	6/6
Sodium hypochlorite	10,500[x]	586 bc	12.92 c	6/11	448 a	3.38 a	6/6
Metalaxyl	1,000	578 b	13.04 c	11/11	1,156 b	10.18 b	6/6
Copper hydroxide	1,000	307 a	10.53 bc	11/11	1,178 b	10.95 b	3/6
Captafol	1,000	322 a	5.76 ab	11/11	602 a	5.70 a	6/6
Mancozeb	1,000	264 a	3.55 a	11/11	443 a	3.35 a	6/6
Flooded control		169 a	1.84 a	11/11	306 a	2.75 a	6/6
P[y]		<0.001	<0.001		<0.001	<0.001	
LSD[z]		194	5.00		335	4.35	

[t] Rootstocks naturally infested with *Phytophthora* spp. were soaked or not soaked (controls) when dormant and then grown in coarse vermiculite in pots for 12 wk outside (1983) or for 10 wk in a greenhouse (1984). All treatments except nonflooded controls were flooded four times, for 48 hr each time, at 2-wk intervals.

[u] Concentration of active ingredients in aqueous suspensions or solution.

[v] Plants were rinsed immediately after soaking (1983) or were not rinsed (1984) before planting.

[w] Number of plants from which *Phytophthora* spp. were detected, either by isolation from root or stem pieces or by baiting rhizosphere soil, at the end of each trial out of the total number of plants treated.

[x] Equivalent to 1.05% NaOCl or 20% bleach.

[y] Probability of greater F statistics for treatments from one-way analyses of variance.

[z] Fisher's protected least significance difference with $P = 0.05$. Means within columns followed by the same letter are not significantly different.

at 0.53 or 1.05% (i.e., 10 or 20% bleach, respectively) for 60 min. Twenty-five rootstocks were used for each treatment. Immediately after soaking, all rootstocks from each treatment were planted together in a row (approximately 15–20 cm apart) to avoid contamination among treatments. Treatments were spaced 1 m apart within a row. Rootstocks were planted in June 1984 and were subjected to normal cultural practices. In August 1984, each rootstock was budded with the scion cv. Rogers McIntosh. To assess treatment effects on apple tree growth, trunk diameter, 3 cm above the bud union, and total shoot length were measured on 15 trees of each treatment in the autumn of 1985 before trees were dug and placed in cold storage; the five trees on either end of each treatment were not measured.

Experiments with fungicides selective for oomycetes. The more promising fungicides from initial experiments, metalaxyl and copper hydroxide, were then compared to several fungicides selectively active against oomycetes (3, 22). These experiments were conducted at the University of Wisconsin in Madison. In 1986, preliminary trials were conducted with rootstocks that were no longer fully dormant to initially evaluate concentrations of 1,000, 3,000, and 5,000 mg/L for metalaxyl, copper hydroxide, fosetyl-Al (Aliette 80WP), two formulations of etridiazole (Terrazole 2E or 35WP), and oxadixyl (Sandofan 25WP). Five rootstocks were soaked in each preparation for 1 hr and then were planted individually in an autoclaved mixture of field soil and sand (2:1, v/v) in 20-cm-diameter clay pots. Ten untreated rootstocks were planted as controls. Plants were grown outside for 10 wk (30 June–10 September). All but five controls were flooded four times.

At the end of the experiment, treatments were evaluated only for the presence of *Phytophthora* spp. Rhizosphere soil was washed from each plant and concentrated as described previously, and a 30-ml subsample of the sedimented soil was placed in each of five deep petri dishes. Each dish was flooded with 50–60 ml of distilled water, and five apple cotyledons were added. Five 30-ml subsamples of nonrhizosphere soil from each pot were baited similarly. The experiment was repeated with only the two higher concentrations, 3,000 and 5,000 mg/L, evaluated for each fungicide. In this trial, seven rootstocks were planted for each treatment, including the untreated control, and all plants were grown outside for 13 wk (25 July–22 October) without flooding.

In 1987, the effects of periodic flooding and no flooding were examined. Copper hydroxide, fosetyl-Al, metalaxyl, and oxadixyl were again evaluated at 1,000 mg/L, but the concentration of etridiazole was reduced to 100 mg/L because of phytotoxic effects observed in 1986. Twenty rootstocks were soaked in each fungicide preparation for 1 hr and then planted individually in a pasteurized (30 min at 60 C) mixture of field soil and medium-textured vermiculite (2:1, v/v) in 20-cm-diameter clay pots. Plants were grown outside for 13 wk (28 May–28 August). Ten plants from each treatment were flooded five times, and the other 10 plants were not flooded. To compare treatments, rhizosphere soil from flooded plants was collected and baited as described earlier. Three 30-ml subsamples per plant and four cotyledons per subsample were used. Weights of oven-dried (100 C for 48 hr) roots and total shoot lengths were measured for all plants. The experiment was repeated except that plants were grown in a greenhouse for 12 wk (8 June–28 August).

In 1987, a third trial was conducted at the Peninsular Agricultural Research Station in Sturgeon Bay, WI, to identify potential detrimental effects of preplant root soaks on subsequent shoot growth. Copper hydroxide, metalaxyl, and oxadixyl were tested at 1,000, 3,000, and 5,000 mg/L; etridiazole was tested at 100, 300, and 500 mg/L. Ten rootstocks were soaked for 1 hr in each fungicide preparation and then planted individually in untreated field soil in 4-L plastic pots. Ten rootstocks that received no preplant treatment were planted as controls. Plants were grown outside in a screenhouse for 13 wk (4 June–31 August) without flooding, and then total shoot growth was measured on each plant.

In 1988, the duration of soaking was examined. The same fungicides and rates were evaluated that had been tested in 1987. Ten rootstocks were soaked in each fungicide preparation for 1 or 3 hr and then planted as in 1987. In addition, 10 rootstocks were soaked in 0.26% (2,625 mg/L) sodium hypochlorite (i.e., 5% bleach), a fourfold dilution from initial experiments, for 1 hr before planting. Twenty untreated rootstocks were planted as controls. Plants were grown in a greenhouse for 11 wk (25 April–12 July). All plants except 10 untreated controls were flooded four times. Rhizosphere soil was collected from each plant by vigorously shaking the roots in a small polyethylene bag, and a single 60- to 70-ml subsample was placed in a glass jar and baited. Weight of oven-dried roots and total length of all shoots were measured for each plant. The experiment was repeated except that plants were grown outside for 13 wk (17 May–17 August).

In 1988, a third trial, similar to the one in 1987, was conducted at the research station in Sturgeon Bay to evaluate potential detrimental effects from preplant root soaks on subsequent shoot growth. The same chemicals, concentrations, and durations of soaking used in the first two trials in 1988 also were evaluated in this third trial. In addition, rootstocks were soaked in metalaxyl, copper hydroxide, or fosetyl-Al at 3,000 and 5,000 mg/ml; oxadixyl at 5,000 mg/L; or etridiazole at 300 and 500 mg/L for 1 hr before planting. Ten rootstocks per treatment were planted individually in untreated field soil in 4-L plastic pots and were grown outside in a screenhouse for 19 wk (25 May–6 October). Treatments were assessed by measuring total shoot length on each plant.

Data analysis. All data were analyzed with MINITAB (Version 6.2) statistical software (Minitab, Inc., State College, PA). To compare the five fungicides evaluated from 1986 to 1988 for overall

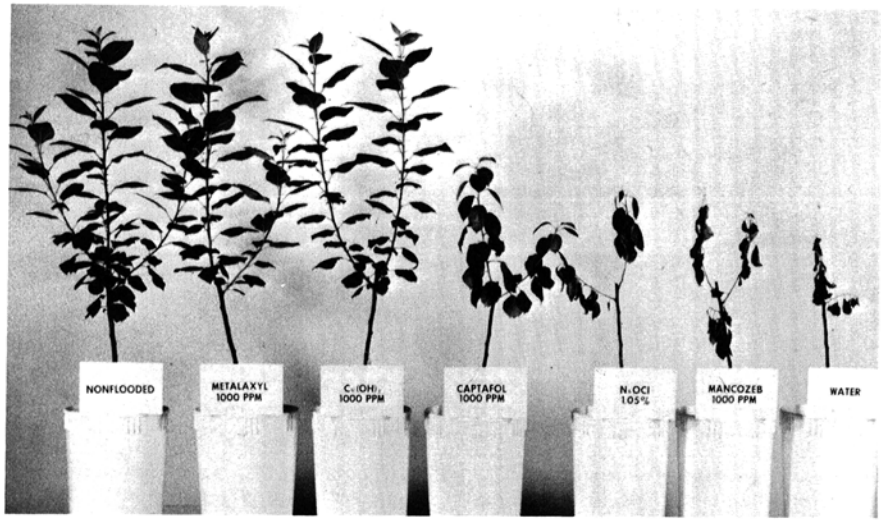

Fig. 1. Severity of Phytophthora root and crown rot on MM.106 apple rootstocks, naturally infested with *Phytophthora* spp., that received 1-hr root-soak treatments before planting in 1984. Roots of dormant rootstocks were (left to right) soaked in water and not flooded or soaked in metalaxyl, copper hydroxide, captafol, sodium hypochlorite, mancozeb, or water and then flooded periodically to enhance disease development. Concentrations of active ingredients of fungicides were 1,000 mg/L and of sodium hypochlorite was 10,500 mg/L (1.05%). Plants were grown in autoclaved vermiculite for 10 wk in a greenhouse.

efficacy at reducing the number of apple rootstocks infested, data for the 3-yr period were combined, using all concentrations and soaking durations for each fungicide. The proportions of rootstocks from which *Phytophthora* spp. were detected were compared in contingency tables by calculating chi-square statistics.

Root weights and shoot lengths were analyzed by one- or two-way analyses of variance (ANOVA). Occasionally, in trials analyzed by one-way ANOVA, a subset of treatments could be identified in which all levels of two factors were represented. Data for these treatments also were analyzed by two-way ANOVA to more critically examine the interaction and main effects of the two factors. When F statistics for treatments were significant ($P \leq 0.05$), treatment means were separated by Fisher's protected least significant difference (LSD) with $P = 0.05$.

RESULTS

Each year, both *P. cactorum* and *P. cambivora* were recovered from bundles of dormant rootstocks that were representative of those to be used in experiments that year. In 1987, inoculum density, particularly that of *P. cactorum*, appeared lower than in other years based on the number of cotyledons colonized when rhizosphere debris was baited. In 1988, *P. cambivora* was detected on only one of the two bundles assayed initially.

Initial experiments. In two experiments to evaluate chemicals with different modes of action and activity spectra during 1983 and 1984, untreated rootstocks that were not flooded (nonflooded controls) remained healthy and grew vigorously. These unflooded control plants had the greatest total shoot length and one of the two greatest root weights of all treatments, despite being infested with *Phytophthora* spp. (Table 1). These lengths and weights usually were significantly greater than those in other treatments. Rootstocks that were not treated but flooded (flooded controls) developed severe symptoms of Phytophthora root and crown rot and consistently had the poorest shoot and root growth (Table 1). Captafol and mancozeb were not effective as preplant root soaks for either 10 or 60 min. Plants soaked in these fungicides developed severe symptoms of Phytophthora root and crown rot, and shoot and root growth were not significantly different from the flooded controls (Table 1). All plants in these two treatments remained infested.

When rootstocks were soaked for 10 min and then rinsed immediately before planting, treatment with sodium hypochlorite or metalaxyl resulted in plants that were vigorous and appeared healthy; however, only sodium hypochlorite decreased the number of plants from which *Phytophthora* spp. were detected (Table 1). *Phytophthora* spp. were detected from five of these root systems by baiting and from one additional root system by isolation (Table 1). Among the chemical treatments, shoot growth was greatest with sodium hypochlorite, and root growth was greatest with metalaxyl (Table 1). However, these two treatments were not different from each other but were significantly greater than most of the other treatments.

When rootstocks were soaked for 60 min and not rinsed before planting, treatment with copper hydroxide or metalaxyl resulted in plants that were vigorous and appeared healthy; rootstocks receiving other treatments or that were untreated and flooded developed severe root and crown rot symptoms (Fig. 1). Both copper hydroxide and metalaxyl resulted in plants that had mean shoot lengths and root weights that were greater than those of plants from other treatments (Table 1). However, only copper hydroxide reduced the number of plants infested (Table 1). *Phytophthora* spp. were not detected on plants soaked in copper hydroxide by baiting but were detected only by direct isolation. Although *Phytophthora* spp. occasionally were detected by direct isolation and not by baiting, more often the converse was true, i.e., the pathogens were detected by baiting the vermiculite collected from rhizospheres of individual plants and not by direct isolation from root and stem pieces, which frequently had been colonized and rotted extensively by other organisms. Therefore, baiting was considered more reliable and effective of the two methods and was used exclusively to detect *Phytophthora* spp. on rootstocks in later experiments. In 1983 and 1984, *P. cambivora* was the predominant species detected on apple rootstocks.

In the field trial conducted in a commercial nursery, sodium hypochlorite treatments were phytotoxic, but metalaxyl treatments were not. All rootstocks soaked in 1.05% sodium hypochlorite for 1 hr died by the end of the first growing season. Mean trunk diameters were affected significantly by the other treatments ($P = 0.033$, one-way ANOVA) and, therefore, were compared by LSD. Plants from rootstocks that were soaked in 0.53% sodium hypochlorite had the only trunk diameter (9.6 mm) significantly less than that of plants in the untreated control (11.7 mm), which had the greatest trunk diameter. Trunk diameters of plants with rootstocks that had been soaked in the two metalaxyl concentrations for 30 or 60 min ranged from 11.6 mm (500 mg/L for 60 min) to 10.7 mm (1,000 mg/L for 30 min); all metalaxyl root-soak treatments, except the latter one, were significantly different from the 0.53% sodium hypochlorite root-soak treatment. However, there was no significant difference among treatments for total shoot length based on a one-way ANOVA ($P = 0.148$). Mean shoot lengths ranged from a maxi-

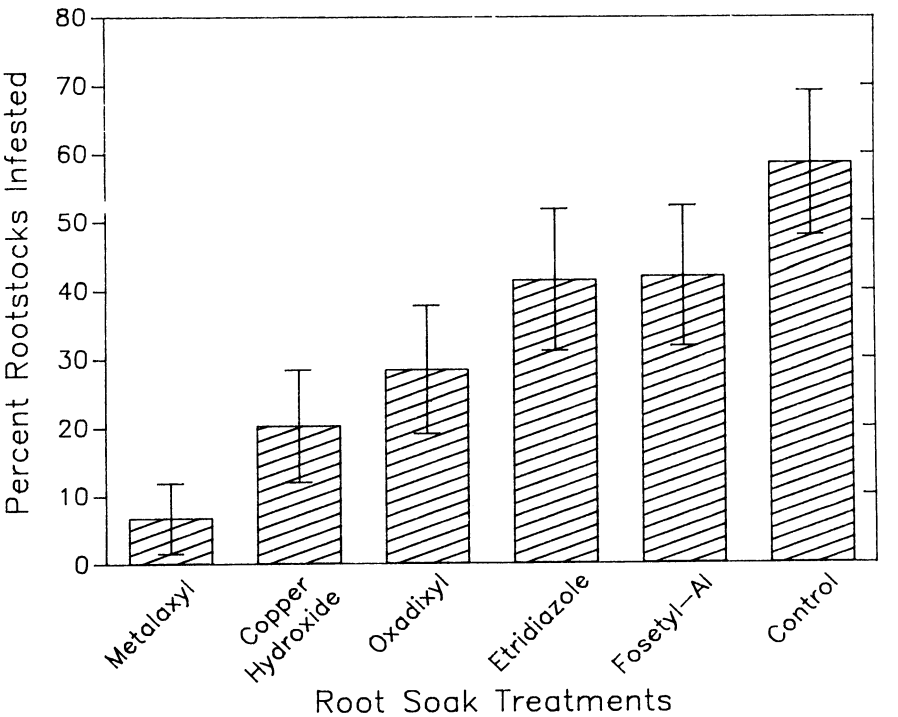

Fig. 2. Incidence of naturally occurring inoculum of *Phytophthora* spp. on MM.106 apple rootstocks that received preplant root-soak treatments in aqueous suspensions of fungicides or no treatment (control). Rootstocks were treated when dormant, planted in sterilized or pasteurized soil, and grown for 10–13 wk with periodic flooding before being assayed. Data are the summary of six trials conducted in a greenhouse or outside over 3 yr (1986–1988) in which each fungicide was used at several concentrations and the duration of soaking was 1 or 3 hr. The number of rootstocks per treatment ranged from 82 to 89; bars are 95% confidence intervals.

mum of 243 cm (1,000 mg/L metalaxyl for 30 min) to a minimum of 185 cm (0.53% sodium hypochlorite for 60 min).

Experiments with fungicides selective for oomycetes. During these experiments conducted between 1986 and 1988, the striking symptoms of root and crown rot did not develop as they had in 1983 and 1984 (e.g., Fig. 1) on untreated, flooded control plants or on plants receiving treatments that were ineffective even though *Phytophthora* spp. frequently were detected on individual rootstocks. Instead, more subtle differences in shoot and root growth occurred.

In preliminary trials conducted in 1986 with rootstocks that were not fully dormant, etridiazole root soaks were phytotoxic at all concentrations, and most plants in both trials died. Consequently, the standard etridiazole concentration was reduced from 1,000 to 100 mg/L for efficacy evaluations in later experiments. Some phytotoxicity also was observed on plants treated with metalaxyl and copper hydroxide, particularly in the first trial, when ambient air temperatures frequently exceeded 30 C during the days immediately after planting. *Phytophthora* spp. still were recovered readily from dead plants at the end of both trials. *P. cactorum* was the species most commonly detected on plants from all treatments in 1986.

Based on the total number of rootstocks treated with each of the five fungicides over the 3-yr period, *Phytophthora* spp. were detected least often on rootstocks that had been soaked in metalaxyl and most often on those not treated at all (Fig. 2). *Phytophthora* spp. were detected on six of 89, 18 of 89, 25 of 88, 36 of 87, and 37 of 88 rootstocks soaked in metalaxyl, copper hydroxide, oxadixyl, etridiazole, and fosetyl-Al, respectively, and were detected on 48 of 82 rootstocks not treated (controls). There were significant differences among all treatments ($\chi^2 = 65.8$, $P < 0.001$, df = 5); among the fungicide treatments ($\chi^2 = 39.2$, $P < 0.001$, df = 4); between metalaxyl and copper hydroxide, the two best treatments ($\chi^2 = 6.9$, $P = 0.008$, df = 1); and between fosetyl-Al, the least effective fungicide treatment, and the untreated control ($\chi^2 = 4.8$, $P = 0.032$, df = 1).

In trials conducted in 1987, treated rootstocks were both flooded and not flooded to determine if detrimental effects on plant growth occurred in the absence of flooding. The two trials produced similar results, so only data from the greenhouse trial are presented (Table 2). In a two-way ANOVA, the interaction between effects of flooding and fungicides was significant for shoot length (Table 2), which indicated that fungicides affected shoot growth differentially when plants were flooded compared with when they were not flooded. Consequently, individual treatment means were compared by LSD. Plants treated with fosetyl-Al and not flooded produced the greatest shoot length; however, plants in all other treatments that were not flooded as well as those that were flooded and treated with copper hydroxide or metalaxyl were not significantly different. Plants that were not treated but flooded had the least amount of shoot growth, and those that were flooded and treated with etridiazole or oxadixyl were not significantly different.

The flooding × fungicide interaction for dry root weight was not significant (Table 2), which indicated that fungicides affected root growth on flooded and nonflooded plants similarly. Therefore, main effects of flooding and fungicides were examined. Flooding had a significant effect ($P < 0.001$); flooded plants produced only 66% as much root weight as nonflooded plants (1.02 vs. 1.54 g, respectively). When fungicide main effects were compared (Table 2), root weight was greatest after treatment with copper hydroxide, but treatment with fosetyl-Al, metalaxyl, and oxadixyl produced similar weights. All fungicides resulted in greater root weights than the untreated control, which had the lowest weight of all. There was no deleterious effect from treatments on either shoot or root growth, and all fungicide treatments resulted in fewer infested rootstocks than the control treatment at the end of the trial. Only *P. cambivora* was detected on rootstocks in both trials in 1987.

In 1988, two trials were conducted to compare 1- and 3-hr soaking durations. Plants in the nonflooded control treatment usually had the greatest amounts of shoot and root growth, but this treatment was not included in statistical analyses because it was the only one that was not flooded periodically. As in 1987, the two trials produced similar results so only data from the greenhouse trial are presented (Table 3). Among the treatments that were flooded periodically, there was no significant difference in either shoot or root growth; however, growth usually was less on plants soaked for 3 hr compared with those soaked for 1 hr. When a subset of treatments, consisting of the five fungicides used for both 1- and 3-hr soaking durations (Table 3), was examined by two-way ANOVA, *F* statistics for the fungicide × soaking duration interaction and main effects of fungicides and soaking duration were not significant for either shoot length ($P = 0.662$, 0.258, and 0.630, respectively) or root weight ($P = 0.315$, 0.149, and 0.068, respectively). Therefore, increasing the soaking duration neither improved root soak efficacy nor caused adverse effects on plant growth.

All chemical treatments reduced the

Table 2. Effects of soaking roots of MM.106 apple rootstocks for 1 hr in aqueous fungicide suspensions before planting and periodic flooding after planting on plant growth and incidence of *Phytophthora* spp.[s]

Fungicide	Concentration[t] (mg/L)	Flooding[u]	Shoot length[v] (mm)	Dry root wt. (g)[w] Individual	Dry root wt. (g)[w] Combined	No. of plants infested[x]
Fosetyl-Al	1,000	NF	1,258 e	1.83		
	1,000	F	988 b–d	1.05	1.44 c	3
Copper hydroxide	1,000	NF	1,243 de	1.75		
	1,000	F	1,204 de	1.38	1.56 c	1
Metalaxyl	1,000	NF	1,090 c–e	1.57		
	1,000	F	1,175 de	1.37	1.47 c	1
Oxadixyl	1,000	NF	1,182 de	1.61		
	1,000	F	908 a–c	1.13	1.37 bc	1
Etridiazole	100	NF	1,131 c–e	1.52		
	100	F	765 ab	0.64	1.08 b	2
Control		NF	1,069 c–e	0.97		
		F	729 a	0.54	0.75 a	6
P[y]			0.031	0.656	<0.001	
LSD[z]			224		0.31	

[s] Results from a trial conducted in 1987. Rootstocks naturally infested with *Phytophthora* spp. were soaked or not soaked (control) when dormant and then grown in pasteurized soil in pots for 12 wk in a greenhouse.

[t] Concentration of active ingredients in aqueous suspensions.

[u] Plants were not flooded (NF) or were flooded (F) five times, for 48 hr each time, at 2-wk intervals.

[v] Means from a two-way analysis of variance (ANOVA) for simple effects of individual treatments (10 plants per treatment).

[w] Means from a two-way ANOVA for simple effects of individual treatments (10 plants per treatment) and those for main effects of fungicide treatments with nonflooded and flooded plants combined (20 plants per fungicide).

[x] Number of flooded plants out of 10 from which *Phytophthora* spp. were detected in rhizosphere soil at the end of the trial.

[y] Probability of greater *F* statistics for the fungicide × flooding interactions (shoot length and individual dry root weight) and for the fungicide main effect (combined dry root weight) from two-way ANOVAs.

[z] Fisher's protected least significant difference with $P = 0.05$. Means within columns followed by the same letter are not significantly different.

number of infested rootstocks compared with the flooded control (Table 3). In addition, 11 plants died in the greenhouse trial (Table 3), eight of which had been soaked for 3 hr. However, only two plants died in the trial conducted outside, one soaked in oxadixyl for 1 hr and one soaked in etridiazole for 1 hr. At the end of both trials, *P. cactorum* was the species detected most frequently on rootstocks.

Trials were conducted in both 1987 and 1988 to determine if there was potential for detrimental effects on shoot growth (i.e., phytotoxicity) to occur. In 1987, no significant difference among treatments occurred when total shoot lengths were compared by a one-way ANOVA (Table 4). To confirm that fungicide concentration did not affect shoot growth, a subset of treatments, consisting of the four fungicides used at all three concentrations (Table 4), was analyzed by two-way ANOVA. *F* statistics for the fungicide × concentration interaction and the concentration main effect were not significant ($P = 0.152$ and 0.118, respectively).

In 1988, 21 treatments were evaluated and shoot lengths varied significantly (Table 4). Shoot lengths on plants that received 16 of the treatments were not significantly different from that on untreated control plants. There were significant differences in shoot lengths among those treatments that used fosetyl-Al, oxadixyl, or etridiazole but not among those that used metalaxyl or copper hydroxide. To determine if either fungicide concentration or soaking duration was affecting shoot growth, two subsets of the treatments were analyzed by two-way ANOVAs. One subset consisted of the four fungicides used for 1-hr soaks at all three concentrations, and the other subset consisted of the five fungicides used for both 1- and 3-hr soaks at the standard concentration (1,000 or 100 mg/L) (Table 4). Neither the main effect of fungicide concentration ($P = 0.087$) nor that of duration of soaking ($P = 0.570$) was significant; in both ANOVAs, two-way interactions were not significant ($P = 0.541$ for the fungicide × concentration interaction and $P = 0.248$ for the fungicide × soaking duration interaction).

DISCUSSION

Soaking roots of dormant apple rootstocks in aqueous chemical preparations before planting resulted in increased shoot and root growth, particularly when plants were grown under conditions conducive to disease development, and reduced the number of plants from which naturally occurring inoculum of *Phytophthora* spp. was detected. Previously, others have had moderate success with preplant root dips or soaks for *Phytophthora* spp. on apple rootstocks. Brown and Hendrix (2) found captafol (a fungicide no longer commercially available in the United States) to be most effective of the six fungicides they evaluated, which were mostly broad-spectrum protectants. However, their experimental procedure differed from the ones used here, and the experiment was conducted only once with rootstocks from one nursery. They dipped rootstocks for 30 s in extremely concentrated fungicide preparations (e.g., captafol at 15,000 mg a.i./L), which resulted in some mortality with all treatments. Based on the preliminary results of Jeffers and Wilcox

Table 3. Effects of soaking roots of MM.106 apple rootstocks for 1 or 3 hr in aqueous chemical preparations before planting on plant growth and incidence of *Phytophthora* spp.[u]

Chemical	Concentration[v] (mg/L)	Soaking duration (hr)	Shoot length (mm)	Dry root wt. (g)	No. of plants	
					Infested[w]	Dead[x]
Fosetyl-Al	1,000	1	776	1.30	0	0
	1,000	3	647	0.78	0	1
Metalaxyl	1,000	1	683	1.21	0	0
	1,000	3	576	0.77	1	3
Copper hydroxide	1,000	1	637	1.12	0	2
	1,000	3	581	0.81	0	1
Oxadixyl	1,000	1	554	0.89	1	0
	1,000	3	712	1.10	1	0
Etridiazole	100	1	489	0.64	0	1
	100	3	465	0.57	1	3
Sodium hypochlorite	2,625[y]	1	446	0.73	1	0
Flooded control			510	0.70	3	0
P[z]			0.391	0.072		

[u] Results from a trial conducted in 1988. Rootstocks naturally infested with *Phytophthora* spp. were soaked or not soaked (control) when dormant and then grown in pasteurized soil in pots for 11 wk in a greenhouse. Plants were flooded four times, for 48 hr each time, at 2-wk intervals.
[v] Concentration of active ingredients in aqueous suspensions or solution.
[w] Number of plants out of 10 from which *Phytophthora* spp. were detected in rhizosphere soil at the end of the trial.
[x] Number of plants out of 10 that died during the trial.
[y] Equivalent to 0.26% NaOCl or 5% bleach.
[z] Probability of greater *F* statistics for treatments from one-way analyses of variance.

Table 4. Total shoot length from MM.106 apple rootstocks after soaking roots for 1 or 3 hr in various concentrations of aqueous chemical preparations before planting[u]

Chemical	Concentration[v] (mg/L)	Soaking duration (hr)	Shoot length (mm)	
			1987	1988
Metalaxyl	1,000	1	526	465 g
	1,000	3	...[w]	397 a–g
	3,000	1	718	418 c–g
	5,000	1	620	420 c–g
Copper hydroxide	1,000	1	594	357 a–d
	1,000	3	...	377 a–f
	3,000	1	663	401 a–g
	5,000	1	627	361 a–e
Fosetyl-Al	1,000	1	...	339 a–c
	1,000	3	...	399 a–g
	3,000	1	...	416 b–g
	5,000	1	...	328 a
Oxadixyl	1,000	1	551	446 fg
	1,000	3	...	427 d–g
	3,000	1	582	...
	5,000	1	621	333 ab
Etridiazole	100	1	559	412 a–g
	100	3	...	481 g
	300	1	491	444 e–g
	500	1	616	375 a–f
Sodium hypochlorite	2,625[x]	1	...	414 b–g
Control			637	444 e–g
P[y]			0.087	0.010
LSD[z]				84

[u] Rootstocks were soaked or not soaked (control) when dormant and then grown in untreated field soil in pots in a screenhouse for 13 wk in 1987 or 19 wk in 1988.
[v] Concentration of active ingredients in aqueous suspensions or solution.
[w] Treatments not evaluated.
[x] Equivalent to 0.26% NaOCl or 5% bleach.
[y] Probability of greater *F* statistics for treatments from one-way analyses of variance.
[z] Fisher's protected least significant difference with $P = 0.05$. Means followed by the same letter are not significantly different.

(14), Tidball and Linderman (23) found 10-min preplant root soaks, either before or after rootstocks were stored for 3 mo at 2 C, in metalaxyl (1,000–4,000 mg/L) and fosetyl-Al (6,000–24,000 mg/L) to be most effective of the fungicides they evaluated for reducing symptom severity on plants subsequently grown in a greenhouse.

In the present study, moderately concentrated aqueous suspensions or solutions of chemicals and relatively long soaking durations avoided phytotoxicity when fully dormant, apparently healthy rootstocks were treated. Three and five times the standard concentration evaluated in most trials (i.e., 1,000 mg/L for metalaxyl, copper hydroxide, oxadixyl, and fosetyl-Al; 100 mg/L for etridiazole) and three times the standard soaking duration of 1 hr did not adversely affect plant growth. However, results from the 1988 greenhouse trial suggest that longer soaking durations may have detrimental effects if poor-quality, unhealthy rootstocks are treated. Rootstocks used in the 1988 trials were inferior to those used in previous years (i.e., they had noticeably smaller root systems) and may have been severely infected by *Phytophthora* spp. In addition, rootstocks used for the greenhouse trial, in which 11 plants died, came from one of the bundles that initially was assayed for occurrence of inoculum. These plants, with their roots thoroughly wetted, were returned to 2 C for 10 days before being treated and planted, which may have allowed additional infection to occur. Although some treatments reduced shoot growth compared with the untreated control in another 1988 trial (Table 4), these results were not consistent among all treatments with a given fungicide in this trial or with similar treatments in other trials. In fact, at least one significant difference would be expected due to chance alone when 21 treatment means are compared by the LSD procedure with $P = 0.05$.

The phenylamide fungicide metalaxyl was the most effective fungicide evaluated. Over a 6-yr period, preplant root soaks in 1,000 mg/L of metalaxyl for 10 min, 1 hr, or 3 hr consistently produced vigorous, healthy plants even though plants were grown under conditions conducive to crown rot development. Between 1986 and 1988, metalaxyl root-soak treatments were most effective for reducing the incidence of *Phytophthora* spp. on naturally infested apple rootstocks. In an independent field experiment in New York (21), a preplant root soak in metalaxyl (600 mg/L for 15 min) combined with preplant soil fumigation with methyl bromide resulted in increased shoot growth and trunk diameter on 2-yr-old apple trees replanted in an established orchard, whereas soil fumigation alone did not increase growth. In other trials conducted by county extension agents and growers at eight commercial orchards in New York (21), the same metalaxyl preplant root-soak treatment resulted in no adverse effect on total shoot growth after one season in 16 of 18 trials but significantly reduced shoot growth in the other two trials, both conducted at the same orchard. When roots of 2-yr-old apple trees were soaked in 600 mg/L of metalaxyl for approximately 1 hr before planting at two orchard sites in Wisconsin, no deleterious effects were observed (S. N. Jeffers, *unpublished*). Previously, metalaxyl has demonstrated efficacy against *P. cactorum* on apple seedlings or trees when applied as a soil drench or trunk paint (6,7,10,20,24,25).

Copper hydroxide also was effective as a preplant root-soak treatment. Root and shoot growth from rootstocks soaked for 1 hr at 1,000 mg/L usually were similar to root and shoot growth of the best treatments and occasionally were superior to those of all other treatments. However, this fungicide was not as effective as metalaxyl at reducing the number of plants infested. Oxadixyl, fosetyl-Al, and etridiazole were less effective as root soaks at the concentrations and soaking durations used in this study. Oxadixyl and fosetyl-Al may be more efficacious at higher concentrations. In comparison with metalaxyl in other studies, higher rates of active ingredients of oxadixyl (8,9) and fosetyl-Al (10,20, 23–25) have been necessary to achieve similar levels of effectiveness in vitro and in vivo.

One potential problem with metalaxyl is the possibility that *Phytophthora* spp. could develop resistance (4,5,22), particularly if metalaxyl is used exclusively in multiple applications per season, when disease intensity is high, or at high concentrations in curative applications (5). Mixtures of metalaxyl and a fungicide with a different mode of action and, preferably, a broad spectrum of activity have been recommended to avoid or delay resistance development (5,22). Based on the research reported here, a mixture of metalaxyl and copper hydroxide would be a likely combination because both were effective as root soaks when used alone. Although this mixture has not been evaluated yet, the two products should be compatible because metalaxyl and copper oxychloride were available in a prepackaged mix in England (10), and a combination of metalaxyl and cuprous oxide was used effectively to manage black pod of cacao (*Theobroma cacao* L.) caused by *P. palmivora* (E. J. Butler) E. J. Butler (19). However, appropriate amounts of the two components in a mixture need to be determined and evaluated for both efficacy and potential phytotoxicity before such a mixture can be recommended. Another possible mixture would be metalaxyl and fosetyl-Al, two systemic fungicides selectively active against oomycetes but with different modes of action (4,5,22). The possibility of a synergistic interaction between two fungicides in a mixture also should be examined (9).

In trials conducted between 1986 and 1988, typical root and crown rot symptoms did not develop on plants in any treatment, including those that were untreated, despite the presence of *Phytophthora* spp. on apple rootstocks. This was contrary to results obtained in 1983 and 1984. *Phytophthora* spp. also were detected less often on untreated rootstocks at the end of trials during 1986–1988 compared with those in 1983 and 1984, which suggests that initial inoculum densities and, perhaps, disease incidences on rootstocks obtained during 1986–1988 were less than those on rootstocks obtained in 1983 and 1984. Increased use of both metalaxyl and fosetyl-Al in nursery propagation beds and more stringent grading of rootstocks have occurred since nursery personnel were informed, before publication of results, of the occurrence of *Phytophthora* spp. on nursery-grown apple rootstocks (12,23). Another possible reason for the lack of symptom development may be residual effects of metalaxyl or fosetyl-Al applied in the nursery. Both of these fungicides have been found to be somewhat active up to 15 mo after application to apple trees (17,20).

Soaking roots of dormant apple rootstocks or trees in an aqueous suspension of a fungicide or combination of fungicides before planting would provide an inexpensive, efficient use of the materials used and would be compatible with the integrated approach to crown rot management that has been suggested (6,15). In short, preplant root-soak treatments offer protection, at least temporarily, against Phytophthora crown rot at a critical period of plant growth, i.e., when roots are just beginning to grow and become established in soil. Fosetyl-Al already is labeled temporarily (i.e., a 2[ee] label was issued in several states for 1991) as a preplant root-soak treatment (2,876 mg a.i./L for 30–60 min) for nursery-grown apple trees based the success of research conducted to date. Unfortunately, however, the current label for metalaxyl (i.e., Ridomil 2E) specifically prohibits this method of application. I agree with Tidball and Linderman (23) that such a restriction does not appear to be justified based on the data from our combined studies. Perhaps in the near future, the metalaxyl label can be altered to permit root-soak applications.

ACKNOWLEDGMENTS

I thank CIBA-GEIGY Corp., Griffin Corp., Rhône-Poulenc Agrochimie Co., Uniroyal Chemical Co., and Sandoz Crop Protection Corp. for supplying fungicides; H. S. Aldwinckle for providing facilities at the New York State Agricultural Experiment Station; W. F. Wilcox for collecting data at the commercial nursery in New York; C. A.

Koschmann and R. W. Weidman for technical assistance; S. A. Vicen for preparing figures; and A. H. Ellingboe and W. R. Stevenson for reviewing the manuscript.

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