AN ABSTRACT OF THE THESIS OF

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Title: __The Biology and Control of Phytophthora Root and Stem Rot__

__of Apple Rootstocks from Stool Beds__ .

Abstract approved: Redacted for Privacy

Dr. Robert G. Linderman

The present studies were conducted to better understand the

etiology, epidemiology and potential control of the Phytophthora

stem root rot as it occurs in apple stool beds.

Symptoms of Phytophthora root and stem rot occurred as early

as May and continued to develop throughout the growing season.

Sunken brown-black lesions were visible on shoots, some of which

wilted, or exhibited rust-brown or purplish bronzed leaves.

Infected roots were red-brown. Some stem lesions were yellow-brown

with narrow dark brown margins forming concentric, wavy-margined

rings up the stem.

Phytophthora cactorum and P. cambivora were both recovered

from the stool beds and identified as the probable causal agents of

the disease. P. cactorum was more frequently isolated with peak

recovery in early September. P. cambivora recovery peaked in mid-

summer. Both species were pathogenic at either 15 or 24°C, but P.

cactorum was more virulent than P. cambivora at both temperatures

and is probably the more active of the two species in the field as
well.

Fosetyl-Al and metalaxyl applied to apple rootstocks in the
field reduced the severity of disease subsequently induced by P.
cactorum in an excised stem assay. These field treatments also
reduced the development of disease in trees graded as healthy and
held in cold storage for 3 to 9 months.

Root dipping pre-, post-, or pre- and post-cold storage of
trees graded either as healthy or mildly infected, with the
chemicals benalaxyl, etridiazol, fosetyl-Al or metalaxyl gave
variable results. Any chemical dip treatment reduced disease
development compared to the untreated controls, but fosetyl-Al and
metalaxyl at 12000 or 4000 ppm, respectively, applied pre-cold
storage gave the best disease control over all.

The development in apple stool beds of stem and root rot
caused by P. cactorum and P. cambivora was reduced in the field by
applications of fosetyl-Al and/or metalaxyl applied to trees during
the growing season, and reduced further by a root dip before cold
storage.

THE BIOLOGY AND CONTROL OF <u>PHYTOPHTHORA</u> ROOT AND STEM ROT OF

APPLE ROOTSTOCKS FROM STOOL BEDS

by

Carolyn Jean Tidball

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THE BIOLOGY AND CONTROL OF PHYTOPHTHORA ROOT AND STEM ROT OF

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INTRODUCTION

The world over, numerous workers have reported and studied the
Phytophthora crown or collar rot disease of orchard apple trees,
yet few have actually studied the disease as it relates to nursery
stock. Phytophthora disease of deciduous tree fruit nursery stock
was reported as early as 1915 (Smith), with the study of peach
nursery stock in California. Since then reports of Phytophthora
root or stem rot infections on apple nursery stock have been made
from England (Campbell & Byrde, 1969), South Africa (Van der Merwe
& Matthee, 1973), Canada (McIntosh, 1975), Romania (Rafaila &
Ciurea, 1981), and the United States (Young & Milbrath, 1959;
McIntosh, 1975; Julis et al., 1978; Brown & Hendrix, 1980; Jeffers
& Aldwinkle, 1984).

A number of Phytophthora spp. have been reported in
association with crown or root rot disease of apples in orchard
situations. However, among these species, P. cactorum (Leb. &
Cohn) Schroet. and P. cambivora (Petri) Buism. have been reported
most frequently as the causal organisms, and the only species
recovered besides P. syringae (Kleb.) Kleb. in isolations made from
commercial nursery stock.

Whether disease in the orchard is the result of planting of
contaminated nursery stock or whether the pathogen is indigenous to

the orchard site or already present as a result of a previously
infected crop remains controversial. Some studies have noted that
Phytophthora spp. may be indigenous in native habitats (Middleton &
Baxter, 1955; Jeffers, 1985), while others indicate that death of
trees in an apple orchard from Phytophthora disease may be due to
its presence as residue of a previously declining crop (McIntosh &
MacSwan, 1966) or from contaminated irrigation water (McIntosh,
1964 & 1966; Helton et al., 1984).

Regardless of the origin of Phytophthora in a particular
orchard, the recovery of Phytophthora spp. from some shipped apple
rootstocks (Julis et al., 1978; Brown & Hendrix, 1980; Jeffers &
Aldwinkle, 1984) indicates that Phytophthora may be present in the
stool beds, that it may be an important source of contamination in
young orchards, and that though trees are graded for quality,
health, and size before shipping, contaminated plant material which
appears healthy may still leave the production site.

The production of clonal apple rootstocks is achieved by
establishing a field of mother plants from which rooted shoots are
removed each year for 10-15 years. After the field has been
fumigated, the mother plant is established by planting 1 year-old
trees in a row at an angle of 30-40, 18-24 inches apart. Just
before growth begins the following spring, shoots are cut back,
then pegged flat on the bottom of a trench. Sawdust, used to
enhance root development, is added at intervals over the growing
season to cover the base of developing shoots. In the autumn when

the shoots are dormant and the leaves have dropped, the mounded sawdust is drawn away from the rooted shoots which are then cut off close to but above the parent plant (Hartman & Kester, 1975). The rooted stems are either kept at the nursery for grafting or shipped to orchardists. If machinery and irrigation water are clean, then the first Phytophthora infections probably originate from mother plants that were contaminated before they were planted. Once the infection is established in the mother plant, control is difficult.

Since a small number of nurseries produce the majority of the apple rootstocks used by orchardists in both Canada and the United States, the present study was conducted to better understand the etiology, epidemiology and potential control of the Phytophthora stem and root rot as it occurs in the apple stool beds. The objectives included: describing the disease as it occurs in the apple stool beds, elucidating the Phytophthora spp. present in the disease complex of an infected stool bed field, following the activity of the Phytophthora spp. involved in the disease over the growing season, comparing pathogenicity of Phytophthora spp. recovered from the stool bed, looking for reduction in disease on trees chemically treated in the field, observing the effects of field-applied fungicides on tree health after cold storage, and determining the efficacy of post harvest chemical root dip treatments to control Phytophthora root and stem rot of apple rootstocks.

Some researchers think (Helton, personal communication, 1987) that _Fusarium_ spp. are also part of the complex causing disease of apple stems and roots. For the purposes of this study, however, attention was given only to the study of _Phytophthora_ spp.

MATERIALS AND METHODS

Field Collections

Samples were selected from each experimental replicate block of the different chemical treatments applied to Emla 106 rootstocks in the stool bed field. Tissue samples were collected from visibly diseased roots and mother stocks, and shoot current year shoot growth that either appeared healthy or showed various characteristic symptoms of the Phytophthora disease. Diseased plant samples were collected every fortnight from June through November 1985. In 1986, samples were collected at monthly intervals from July through November. Samples were plated the same day as collected or were stored at 4°C for 1-2 days.

Sawdust for qualitative assays to detect propagules of Phytophthora was collected from the source pile, between rows, and within the row, sometimes directly adjacent to visibly diseased stems. Samples were stored in plastic bags at 4°C until they were analyzed by the bait trap method (Linderman and Zeitoun, 1977).

Diseased tissue was also collected from areas of the field which had been chemically treated by the grower. Chemical fungicides applied to Emla 106 rootstocks in an experimental area within stool bed fields during the summer of 1985 included fosetyl-Al applied twice during the season (FF) at 3.0 lb ai/A (3.4 kg ai/ha), fosetyl-Al applied thrice (FFF) at 3.0 lb ai/A (3.4 kg ai/ha), fosetyl-Al and metalaxyl (FM) applied once each to the same

plot at 3.0 lb ai/A (3.4 kg ai/ha) and 8.0 lb ai/A (8.9 kg ai/ha), respectively, and an untreated control (CTL).

Samples were also collected from field treated with fungicides by the grower in cooperation with one chemical company representative applied to Emla 106 rootstocks during the summer of 1986. The treatments included: a control receiving no chemical treatment (Trt 1); fosetyl-Al applied at 2.0 and 4.0 lb ai/A (2.2 and 4.5 kg ai/ha) every 2 months (Trts 2 and 3); metalaxyl applied at 8.0 lb ai/A (8.9 kg ai/ha) every 3 months (Trt 4); and fosetyl-Al applied at 1.0 and 2.0 lb ai/A (1.1 and 2.2 kg ai/ha) every month (Trts 5 and 6).

Isolation Techniques

Phytophthora was isolated from the margin between healthy and diseased tissue taken from beneath the periderm of apple stems or from root pieces. The tissue to be plated was surface sterilized for 10 min (stems) or 5 min (roots) in 6% sodium hypochlorite, or by dipping stem tissue in 70% ethanol and flaming. The tissues were then rinsed twice for 5 min in sterile water. Ten tissue pieces were taken from each plant sample, five were placed on a petri plate of each Phytophthora selective medium described below. The plates were stored in plastic bags at room temperature (20-23°C) or 15°C, without light, and examined for Phytophthora colonies at 3, 5, 10, and 15 days.

Attempts were made to isolate Phytophthora from roots, soil or sawdust from apple stool beds by the zoospore-trap assay of

Linderman and Zeitoun (1977). Five to ten _Eucalyptus_ leaf disks
(0.4 cm dia.) were used for each sample. After 24 hr the leaf
disks were removed from the baiting water, placed on selective
media, and incubated as mentioned above for other plant tissue
isolations.

The media used for isolating and growing _Phytophthora_ spp.
were based on clarified V8-juice or corn meal agar. Clarified V8-
juice concentrate was prepared by adding 1420 ml V-8® juice to 180
ml water and 23 g $CaCO_3$, autoclaving 10 min (1.05 kg/cm/115°C), and
filtering through 4 layers of cheese cloth. Either 150 ml or 200
ml (V8-200) of clarified concentrate and 20 g Difco agar were made
up to 1 L with distilled water, and then autoclaved (Pratt and
Mitchell, 1973). A variation included V8-200 with B sitosterol
added to enhance oospore production (Ribeiro, 1978). Isolation and
purification of isolates was achieved using Schmittenner's
selective V8-juice medium (Schmitthenner, 1973) and two different
corn meal agar media: CMPV containing 17 g Difco cornmeal agar, 20
ug/ml pimaricin and 20 ug/ml vancomycin for each liter of distilled
water (Helton, personal communication, 1985) and PVRPH, a modified
version of the medium described by Tsao and Ocana (1969) with
vancomycin increased to 250 ppm and rifampicin added at 10 ppm.
Stock cultures were maintained on V8-200 medium in screw top test
tubes, incubated without light at 8°C, and recultured every 8
months.

Identification of Phytophthora species

Isolates of Phytophthora recovered from various samples during the study were evaluated taxonomically using a variety of standard procedures as follows.

Colony morphology and growth rate. Colony morphology and growth rate of unknown isolates was observed on cultures transferred to Difco corn meal agar of uniform depth in plates incubated in the dark at 20°C.

Oospore production. Oospore production for species characterization was initially evaluated with single strain cultures grown on V8-200 and V8-200 with B-sitosterol media, incubated in the dark at room temperature, and observed for 1-3 weeks. If oospores did not form, then using the same cultural procedures, known isolates of heterothallic strains of different species were crossed (in dual culture plates) with the unknown isolate. The unknown isolate was also crossed with itself as an observational control.

Sporangia formation. The procedure used for sporangia formation was basically that described by Hamm and Hansen (1987). Sporangia were induced by transferring agar plugs of active mycelium of isolates and growing them for 3-5 days in pea broth made by adding 150 g split peas to 1 L of deionized water, autoclaving for 3 min and filtering through 2-4 layers of cheese cloth. The broth was autoclaved again for 15 min before being used. When the plug colonies were 1-3 cm in diameter, the pea broth was poured off, the colonies rinsed with deionized water,

flooded with soil extract, incubated in the dark at room temperature, and observed after 8-12 hr. The soil extract water was prepared by vigorously mixing equal parts of soil and water, and letting it stand overnight at room temperature. The following day the solution was decanted from the sediment, centrifuged at 5000 rpm for 10 min, and decanted again. The first flush of sporangia produced on the mycelium from the plugs were observed and measured on a compound microscope.

The final identification to species for cultures isolated from the field was based on the sum of both morphological and non-morphological characters available.

Inoculation Techniques

Pathogenicity and residual chemical tests were conducted using the excised stem piece assay of Jeffers et al. (1981). Glass canning jars (1 pt) were filled to a depth of 1 cm with cornmeal agar amended with 20 ppm pimaricin. The jars were inoculated with a mycelial plug of the test Phytophthora species or an uninoculated agar plug in the case of the control. After 6 days growth in darkness, 7.5 cm excised apple stem pieces were inserted into the colony and medium. The inoculation jars were incubated for various times under controlled temperature conditions, and then the severity of infection was determined by stripping the periderm from the stem pieces and measuring the length of the resultant lesion, beginning at the agar surface. Each jar contained 5 stem pieces, and each treatment was replicated 5 times.

Assay Variations

The pathogenicity tests were incubated at 15 or 24°C. For the
15°C test, the stem pieces (from dormant shoots) were inoculated
with 3 isolates of Phytophthora cactorum, 2 of P. cambivora and 1
of P. syringae, all originally recovered from apple stems taken
out of the stool beds. Harvesting and measuring of lesion length
was done after 4 wk of incubation. The stem pieces were dormant
when collected for this assay. For the test at 24°C the stem
pieces cut from actively-growing stool bed shoots were inoculated
with 2 isolates of P. cactorum, and 2 of P. cambivora. Harvesting
and measuring of lesion length was done after incubation for 10
days.

The inoculation trial to test for residual fungicide in apple
shoots after field application was conducted at 24°C using a P.
cactorum isolate. The stem pieces were taken from stool bed shoots
in each of six field fungicide treatments applied in 1986 which
included: Aliette 80 wp applied at 2.0 and 4.0 lb ai/A (2.2 and
4.5 kg ai/ha) every 2 months, or 1.0 and 2.0 lb ai/A (1.1 and 2.2
kg ai/ha) every month; Ridomil 2E applied at 8.0 lb ai/A (8.9 kg
ai/ha) every 3 months; and a control which received no chemical
applications in the field.

Chemical Root Dips

This experiment was conducted to study the efficacy of post-
harvest chemical dip treatments to eliminate Phytophthora on Emla

106 apple rootstocks. Each of four chemicals were used at three concentrations and compared with a tap water control. The chemical solutions were prepared at the following concentrations: benalaxyl and metalaxyl - 1000, 2000, and 4000 ppm; etridiazol - 155, 233, and 468 ppm; and fosetyl-Al - 6000, 12000, and 24000 ppm. Trees representing two health classes were dipped either pre- (1), post- (2), or both pre- and post- (3) cold storage. All trees were planted in the greenhouse after the post-cold storage chemical treatments had been applied. Trees in health class 1 exhibited no signs or symptoms of disease and were graded as apparently healthy. Trees in health class 2 exhibited one or two small lesions, generally located at one of the underground nodes of the stem, and were graded as mildly infected (normally discarded by the nurseryman).

Trees to be dipped for each treatment were tied with string in bundles of 10, and dipped for 10 min. Within each health class, the pre-, and pre-/post-cold storage trees were dipped simultaneously, the healthy trees being dipped before the mildly diseased trees. After dipping, each bundle of trees was allowed to drip until the excess solution had drained, and then was placed in a plastic bag containing 1 L of pasteurized (aerated steam 60°C/30 min) sawdust. After a second L of sawdust was added to cover the roots, each bag was tied with string and placed in a dark cold room at 2°C. The trees in the post-cold storage treatment were bagged with sawdust at the same time as the pre-cold storage chemical treatments, and after 3 months in cold storage, were removed and

the post-cold storage dip treatments applied in the same manner as
were the pre-cold storage treatments. Trees in all treatments were
then planted into containers and placed in the greenhouse.

Greenhouse Procedures

Trees treated with chemicals in the root dip trial or
collected from field trials were grown in the greenhouse in plastic
pots 9 cm square by 15 cm deep, each with its own saucer, and were
placed on the benches in a randomized treatment design. The
pasteurized (aerated steam, 60°C/30 min) soil mixture was composed
of equal volumes of sand, peat and soil. The plants were
maintained for 3 months during the spring and summer in the
greenhouse at 24:15°C (day:night) average temperatures without
supplemental lighting. The trees were flooded for 48 hr every 2 wk
to enhance any Phytophthora disease associated with the trees. The
trees were first flooded 2 wk after planting, and last flooded 2 wk
before harvest.

Disease Ratings

Trees grown in the greenhouse trials were rated for disease at
the time of harvest. The ratings were based on the percentage of
underground stem length covered by lesions as follows: 1 = no
visible lesions, 2 = > 0 up to 25%, 3 = > 25 to 50%, 4 = > 50 to
75%, and 5 = > 75 to 100%.

Statistical Methods

The statistical methods used to analyze the data for these experiments consisted of a one-way analysis of variance, except for the chemical root dip experiment which was analyzed by a three-way analysis of variance, and mean separation by Fisher's protected Bayes LSD test. One other variation from the previously mentioned methods was the Duncan's multiple range test used to determine significant differences between the means of trees graded for health/disease development after 9 mo of cold storage.

RESULTS

Disease Description and Phytophthora Recovery

Symptoms of the disease in the field. Symptoms of
Phytophthora root and stem rot in the apple stool beds was seen as
early as May and continued to develop throughout the growing season
which ended in October with the autumn frosts (see appendix).
Young shoots were infected at the base where black to brown lesions
were apparent. Such lesions often were contiguous with lesions on
the mother plant tissues. Infected shoots wilted and died with the
leaves still attached to the stem. These dead shoots were soon
masked by the flush of growth of the surrounding, apparently
healthy stems.

As the growing season progressed, additional shoots wilted and
died, but also seen were shoots with rust-brown bronzed leaves
which did not readily abscise, even when dead. Purplish bronzing
of leaves on otherwise healthy looking stems occurred in the autumn
as the temperature dropped; these leaves readily abscised when
touched. Visible lesions on the underground portion of shoots
showing various leaf symptoms were not always present.

Phytophthora infections appeared as sunken, moist, brown-black
cankers on stems localized around a root node, or extended over
part or all of the underground portion of the stem and in some
cases continued down to and included the clonal (mother) rootstock
itself. Occasionally white mycelium could be seen extending from
the lesion area. The upper limit of stem lesions often exhibited

an irregular margin. At infected nodes, roots were often absent,
or, if present were often infected and showed a red-brown
coloration in contrast with the white of healthy roots.

Some stem lesions were a yellow-brown color with narrow, dark
brown markings forming concentric, wavy-margined rings up the
stem. In some cases the bark split and peeled back at the edges of
the split. These lesions often produced a rust colored exudate.

The symptoms of the disease were similar between trees not
chemically treated and trees treated with metalaxyl during the
growing season. However, trees treated with fosetyl-Al showed
slightly different symptoms. In addition to the leaf browning, the
veins of leaves were also brown-bronze, but exhibited less purpling
of the leaves in the late season. They also had brightly colored
leaves of yellow- or red-orange not associated with autumn leaf
coloring and abscision.

A general mottling (red-, yellow-brown) of stems treated with
fosetyl-Al made lesions much less distinct. However, as with the
other treatments, Phytophthora was isolated from tissue of fosetyl-
Al treated shoots which didn't show distinct lesions.

Characteristically, shoots treated with fosetyl-Al, even those
with disease, showed much greater development of roots especially
at nodes away from obvious stem lesions compared with the untreated
shoots or shoots treated with metalaxyl.

By the end of the season there were bare patches in the low
areas of a field where the disease had spread along the row.

Though not restricted solely to low areas in a field, the disease did appear to be more widespread in those areas.

Phytophthora species recovered from apple stoolbeds. Recovery of Phytophthora, from a heavily infested field of Emla 106 rootstocks which contained some plots with fungicide treatments in 1985 was erratic (Fig. 1). P. cactorum and P. cambivora (A1 mating type) were the species most commonly isolated, with P. cactorum being isolated much more frequently than P. cambivora which was only occasionally recovered. Peak recovery was in early September for P. cactorum, but from mid-June to mid-August for P. cambivora.

Phytophthora was recovered from current year shoot growth, roots and mother stock. It was isolated from tissues appearing healthy on the surface as well as from tissues with visible lesions. The leaves on shoots in both cases either appeared healthy or showed light bronzing of the upper leaves and sometimes purpling of the lower leaves.

Phytophthora was not recovered by baiting from sawdust taken from within the rootstock row, between the rows or from the source piles used for stool bed production. No attempt was made in 1985 to bait from sawdust that was adjacent to infected stems.

P. cactorum was successfully recovered from all of the plots treated with fosetyl-Al and/or metalaxyl in 1985, while P. cambivora was isolated only from the untreated control and plots treated once with fosetyl-Al.

Phytophthora was generally less readily isolated from field samples in 1986 (Table 1) than in 1985. The peak recovery from

PHYTOPHTHORA RECOVERY
FROM APPLE STOOL BEDS
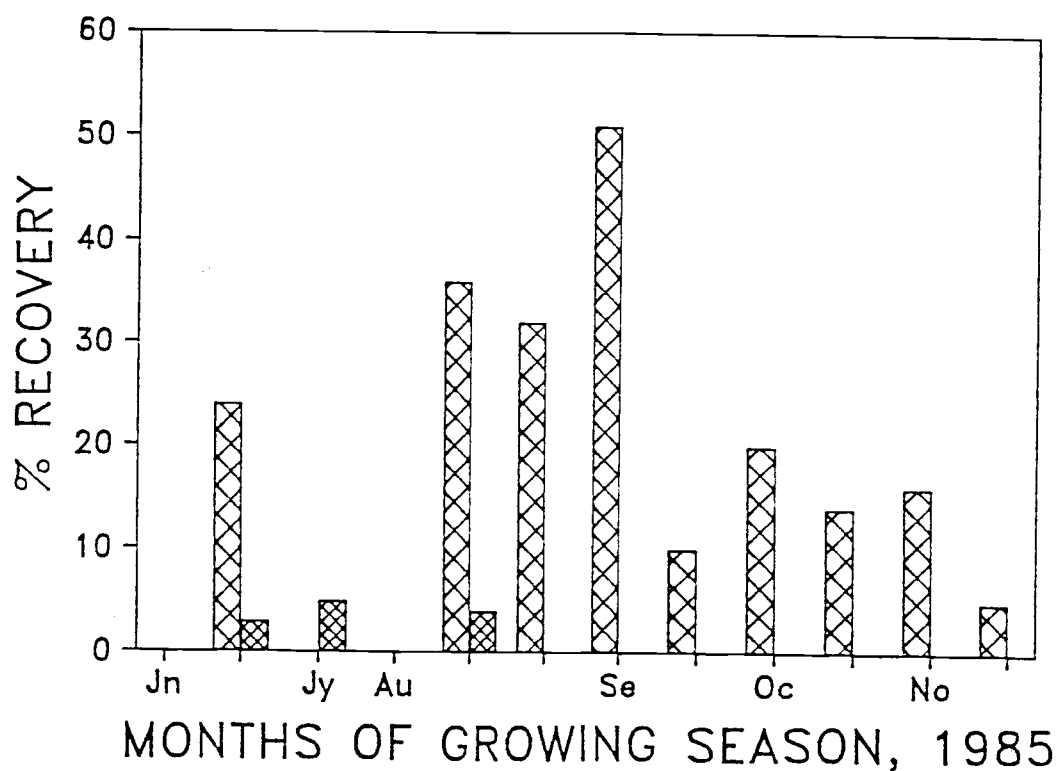
Figure 1. Percent of apple rootstock samples from the stool beds infected by each of the Phytophthora species. Sampling occurred at two week intervals over the growing season and included trees from each of the stool bed field plots. ⬚ = P. cactorum, ⬚ = P. cambivora.

Table 1. Percent recovery of <u>Phytophthora</u> from apple stool bed samples plated or baited in 1986.

	Percent Recovery			
Date	Plant Tissue		Sawdust*	
1986	<u>P</u>. cactorum	<u>P</u>. cambivora	<u>P</u>. cactorum	<u>P</u>. cambivora
July 1	6	0	-	-
August 5	0	0	-	-
September 8	11	0	-	-
October 6	67	0	60	13
November 20	52	0	40	45

* Values replaced by a dash indicate no samples were taken at the corresponding sampling date.

stems, roots, and mother stock tissue of P. cactorum occurred in October, while P. cambivora was undetectable in these field samples. Recovery of Phytophthora was particularly low in plant tissues not showing obvious symptoms of disease.

Baiting in 1986 from sawdust that had been adjacent to diseased stems with visible lesions yielded P. cactorum and P. cambivora (Al mating type). Some samples contained both species, while others contained only one.

Identification of Species

Species identification was based on the sum of all the available morphologic characters of the isolates and descriptions in published discussions of taxonomy (Ho et al., 1977; Newhook et al., 1978; Ribeiro, 1978; Gerrettson-Cornell, 1980; Ho, 1981; Gerrettson-Cornell and Lind, 1981; Hamm and Hansen, 1987) (see appendix).

The isolates were first classified as Phytophthora and distinguished from Pythium on the basis of their hyphae (larger size) and colony characteristics (slower growth rate) in addition to their method of zoospore release (liberated directly from the sporangium rather than from a vesicle).

Phytophthora cactorum was readily identified and separated from the majority of Phytophthora species by its sympodially branched sporangiophores; caducous, markedly papillate, limoniform or spherical to broadly ellipsoid sporangia; and its homothallic

capacity to produce abundant oogonia and paragynous antheridia on solid media.

Phytophthora cambivora was identified by its production of large, simply borne, internally proliferating, non-papillate sporangia; its absence of chlamydospores and botryose swellings; and its heterothallic nature which, when grown in paired culture with a compatible mating type, produced oogonia with bullate protuberances and large, often bicellular, antheridia. The bullate protuberances on the oogonial wall have been reported to be a particularly diagnostic characteristic for this species (Waterhouse and Waterston, 1966; Ho et al., 1977; Newhook et al., 1978; Gerrettson-Cornell, 1980; Gerrettson-Cornell and Lind, 1981).

Among the isolates that were classified as P. cambivora, there were two sub-groupings based on colony morphology. Group I had a radiant growth pattern with fairly straight mycelium and branching that made an angle just less than 90°. The side branches were short, straight, and narrow without any swelling, further side branching, or knobbiness. Occasionally there were circular projections attached to the side of a long mycelial strand rather than regular branching, but generally there was no swollen or bushy appearance to the mycelium. Group II had a more sweeping growth pattern with branching that occurred at right angles to the longer mycelial strand. The branches were short club shaped spurs, or were normal in diameter and had knobby appendages formed opposite each other on the branch. The swollen mycelium was abundant

particularly in the older part of a culture, and had a very
rounded, finger-like appearance.

Isolates in both morphology groups were the A1 mating type,
based on their production of oogonia only in paired cultures with
the A2 mating type of P. cinnamomi Rands and P. cambivora.
Although workers have observed the formation of oogonia in
interspecific crosses, and some have questioned whether such
represented true sexual reproduction, it appears that paired
cultures for some species can give helpful confirmation of isolate
identity (Hassis and Nelson, 1963; Savage et al., 1968, Shepherd,
1978). Based on the array of pairings made and the types of
oogonia that were produced, it seems likely that the unknowns being
analyzed were P. cambivora.

Although not a part of classical taxonomy, electrophoresis was
used (work done by Phil Hamm) to further characterize the potential
P. cambivora isolates. Protein bands from isolates representing
each of the two colony morphology types, as well as from known P.
cambivora isolates were compared. Isolates from both groups had
protein bands that closely matched the known isolates.

There is evidence from studies on other Phytophthora species
that different cultural types can develop from different single
zoospore sources (Erwin et al., 1963) while the other morphological
characters remain similar, or at least within the range for the
given species. Although there were differences in colony
morphology which distinguished the two groups, they were both

identified as P. cambivora based on other characters used in
Phytophthora classification.

Pathogenicity Tests

In the pathogenicity test using excised stem pieces inserted
into inoculated agar in jars held at 15°C all isolates had been
recovered from apple stems from apple stool beds and were
pathogenic (P < 0.05) compared with the uninoculated control (Fig.
2). Two of the P. cactorum isolates (P-91, P-115), one P.
cambivora (P-108) and the P. syringae (P-85) isolate were
significantly different from the control at the P = 0.01 level of
confidence. Measurements on the advance of external mycelium
extending beyond the lesion progression up the stem pieces
indicated that two P. cactorum (P-91, P-115) and both P. cambivora
isolates had more (P < 0.01) external mycelium than the remaining
P. cactorum (P-58) or the P. syringae (P-85) isolate. The two P.
cambivora isolates (P-108, P-112) produced an average length of
external mycelium 5-9 mm beyond the lesion, while the other
isolates (P-58, P-85, P-91 and P-115) developed external mycelium
on the stems of less than the full length of the lesion.

In the pathogenicity test run at 24°C all four isolates, two
P. cactorum (P-91, P-115) and two P. cambivora (P-108, P-112), were
pathogenic (P < 0.01) on excised apple rootstock stems compared to
an uninoculated control (Fig. 3). There were no significant
differences in virulence between isolates. There was little
external mycelium and no measurements were attempted.

LESION DEVELOPMENT AND MYCELIUM GROWTH AT 15C
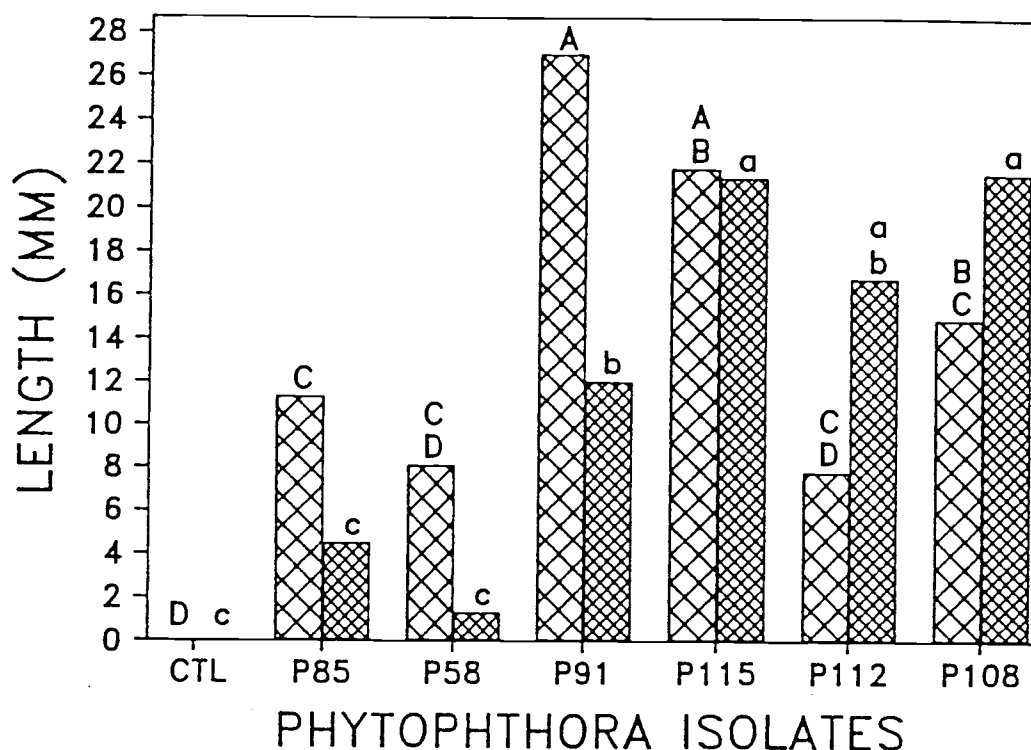
Figure 2. Lesion development and mycelium growth in the pathogenicity test using the excised stem assay, run at 15°C with P. syringae (P-85), P. cactorum (P-58, P-91, P-115), and P. cambivora (P-112 Group I, P-108 Group II) isolates recovered from apple stool beds. Means were separated independently for lesion and mycelium length by Fisher's protected Bayes LSD test. Values for lesion (upper case letter, P < 0.05) or mycelium (lower case letter, P < 0.01) length not followed by the same letter are significantly different. ▨ = stem lesion, ▨ = external mycelium

LESION DEVELOPMENT AT 15C AND 24C
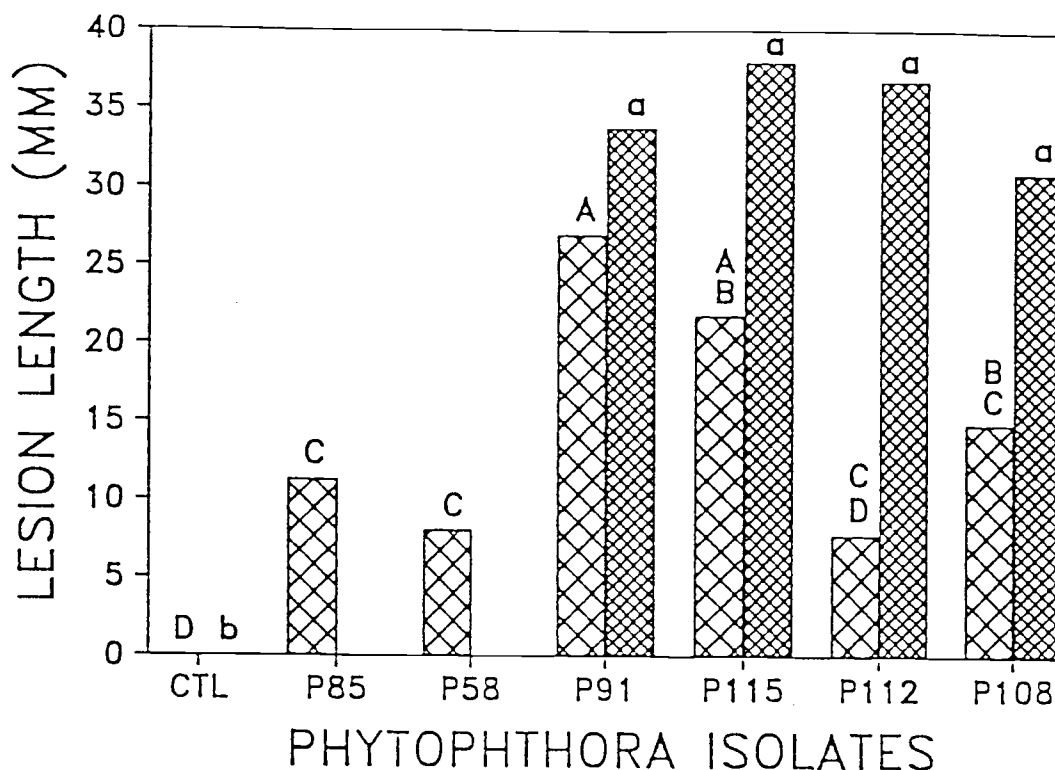
Figure 3. Comparison of lesion development between pathogenicity tests, run at 15°C and 24°C using excised stem assay, with P. syringae (P-85), P. cactorum (P-58, P-91, P-115) and P. cambivora (P-112 Group I, P-108 Group II) isolates recovered from apple stool beds. Isolates P-85 and P-58 were not included in the test run at 24°C. Means were separated (Fisher's protected Bayes LSD test) independently for the two temperatures; values not followed by the same letter (15°C, uppercase, P < 0.05; 24°C, lower case, P < 0.01) are significantly different, P<.01. ⊠ = 15°C, ⊠ = 24°C.

The two P. cactorum (P-91, P-115) isolates used in the pathogenicity test at both 15° and 24°C produced slightly longer lesions at 24°C. At the higher temperature, the two P. cambivora(P-108, P-112) isolates produced lesions that were twice (P-108) or four times (P-112) the length of those produced at the lower temperature.

Fungicide Residual and Disease Control

Bioassay of fungicide residual. Chemical fungicide treatments (1986) applied to apple rootstocks in the field 4 days to 3 wk before sampling, depending on the field treatment, affected the disease severity induced by a P. cactorum isolate used in an excised stem assay (Fig. 4). Using lesion length as a measure of disease severity, all chemical treatments significantly (P = 0.05) reduced the disease compared with the control stem pieces which received no chemical treatments in the field. There were no significant differences on disease development among the chemical treatments. However between chemical treatments, treatments 3 and 4 gave the lowest disease ratings overall.

Effect of fungicide carryover on tree health. Trees initially graded as healthy, planted in the greenhouse after field treatments applied in 1985 and a 3-month winter cold storage, showed significant (P = 0.01) differences in the development of Phytophthora disease at the end of a 3-month growing period (Fig. 5). A separation of means indicated field treatments 2 (FF) and 3 (FFF) gave better (P < 0.01) disease control, as measured by

RESIDUAL FUNGICIDE ACTIVITY
ON LESION DEVELOPMENT
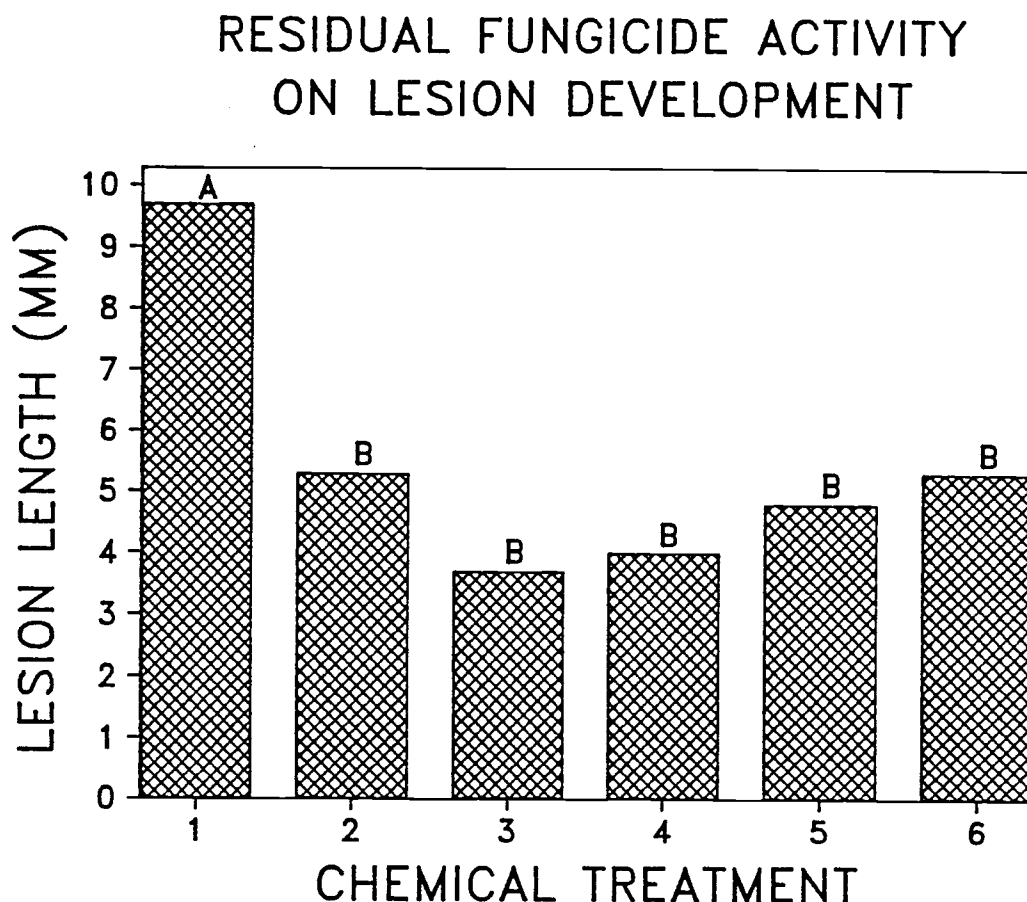
Figure 4. Effect of fungicide residual in apple rootstock stems on pathogenicity of a P. cactorum isolate. Treatments included: an untreated control (Trt 1), fosetyl-Al applied at 2.0 and 4.0 lb ai/A every 2 months (Trts 2 and 3), metalaxyl applied at 8.0 lb ai/A every 3 months (Trt 4), and fosetyl-Al applied at 1.0 and 2.0 lb ai/A every month (Trts 5 and 6). Means were separated by Fisher's protected Bayes LSD test. Values not followed by the same letter are significantly different at P = 0.05.

FUNGICIDE CARRYOVER ON TREE HEALTH
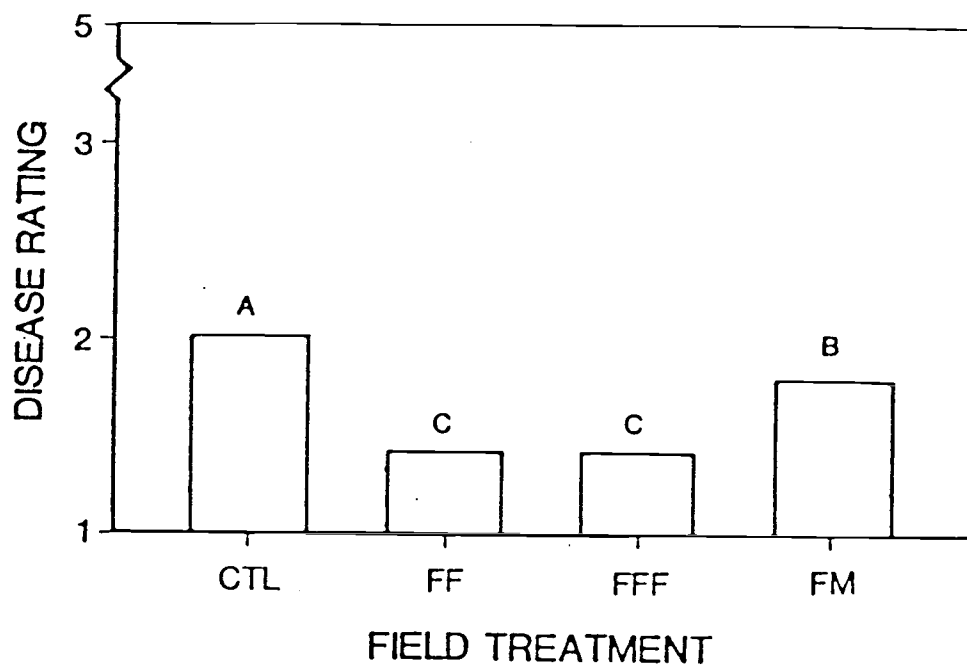
Figure 5. Effect of field applied fungicides on disease

development in apple rootstocks after 3 months in cold

storage, and a 3-month growing period. Means were separated

by Fisher's protected Bayes LSD test. Values followed by the

same letter are not significantly different at P = 0.05.

disease ratings, than treatments 1 (CTL), or 4 (FM). Treatment 4
also reduced disease more (P < 0.01) than the untreated control.
There were no significant differences between treatments 2 and 3.
Phytophthora cactorum and P. cambivora (Al) were successfully
isolated from both control and treated trees at the end of the 3-
month growing period.

Effect of fungicide residual and extended cold storage on tree
health. Trees harvested and graded initially as healthy, after
field treatments applied in 1985 were held in cold storage for nine
months, then observed. Disease development was evident in apple
rootstocks held in long term cold storage. Trees which had three
chemical field treatments (1985) showed significantly (P < 0.01)
less disease than the control, but there were no significant
differences in disease control between the chemical treatments
themselves (Table 2). P. cactorum and P. cambivora were isolated
from stem tissue or baited from root washings of trees held in cold
storage for nine months.

Chemical Root Dips

The chemical root dipping of Emla 106 rootstocks harvested
from the apple stoolbeds gave variable results. Some of the
treatments however did significantly reduce disease compared to the
untreated controls.

Table 2. Effect of extended cold storage (9 months) on health of
trees treated in the field with fungicides.

| | Trees | | |
Treatment	Healthy	Total	% Diseased*
1 control	66	157	58 A
2 fosetyl-Al (2x)	111	153	27 B
3 fosetyl-Al (3x)	125	162	22 B
4 fosetyl-Al/metalaxyl	125	155	19 B

* Means separated by Duncans multiple range test significant at P
< 0.01.
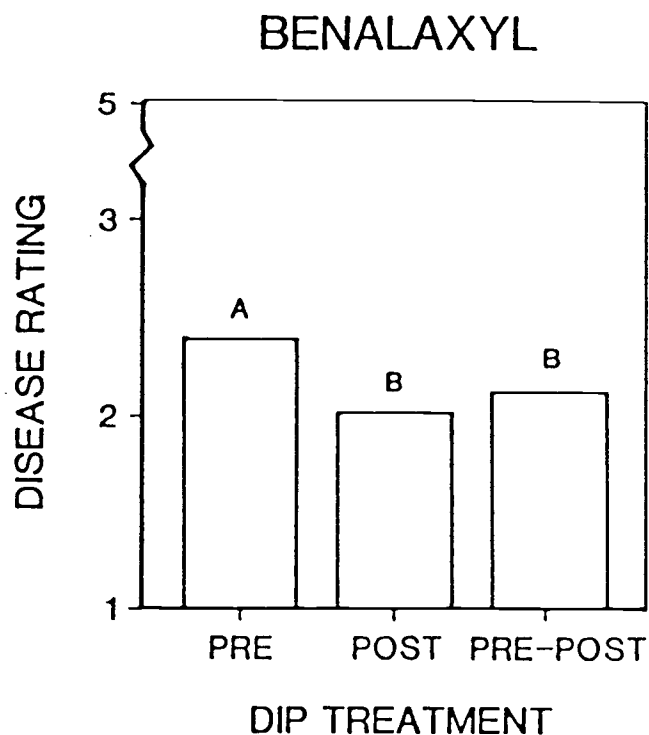
BENALAXYL

Figure 6. Effect on disease development of benalaxyl root dip treatments applied to apple rootstocks pre-, post- or pre- and post-cold storage. Means were separated by Fisher's protected Bayes LSD test. Values followed by the same letter are not significantly different, P < 0.05.
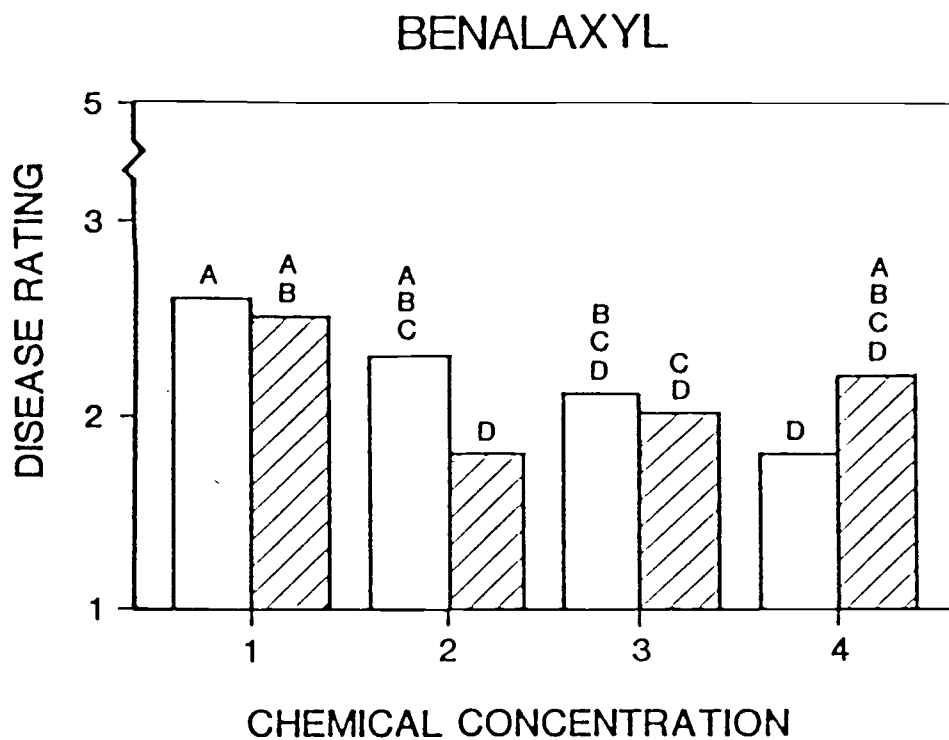
BENALAXYL

Health: ☐ = 1   ▨ = 2

Chemical Concentration:
$$1 = 0 \text{ ppm} \qquad 3 = 2000 \text{ ppm}$$
$$2 = 1000 \text{ ppm} \qquad 4 = 4000 \text{ ppm}$$

Figure 7. Effect of benalaxyl chemical concentrations on tree health as measured by disease ratings. Means were separated by Fisher's protected Bayes LSD test. Values followed by the same letter are not significantly different, $P < 0.05$.

Benalaxyl. Analysis of the disease ratings on trees treated with benalaxyl showed significant differences (P < 0.05) for the dip treatment (Fig. 6) and for the health x chemical concentration interaction (Fig. 7). Mean separation of the dip treatment showed dip treatments 2 (post-) and 3 (pre-/post-) to be better (P < 0.05) than treatment 1 (pre-) at reducing apple rootstock disease ratings.

Analysis of the interaction, tree health x chemical concentration, showed a gradual decrease in disease rating with an increase in chemical concentration for trees in health class 1. A separation of means showed disease ratings for chemical concentration 3 (2000 ppm) were significantly lower than those of the control at the 0.05 level, while ratings for concentration 4 (4000 ppm) were significantly less than those of the control at the 0.01 level. Trees in health class 2 had significantly lower disease ratings than the control for chemical concentration 2 (1000 ppm; P < 0.01) and 3 (P < 0.05). There was no evidence of phytotoxicity at any benalaxyl concentration.

Etridiazol. Disease ratings for the etridiazol treatment were significantly (P < 0.05) different between treatments in the health/dip/chemical concentration interaction (Fig. 8). After separating the means for trees in health class 1, only chemical concentration 4 (468 ppm) with dip treatments 1 (pre-) and 2 (post-) had significantly (P < 0.05) reduced disease ratings compared to the control. Trees in health class 2 were not significantly affected by the chemical x dip treatments compared to the

Figure 8. Effect of etridiazol chemical concentrations and dip

treatment on tree health (H1 = initially healthy, H2 = mildly

infected) as measured by disease ratings. Means were separated

independently for the 2 health classes by Fisher's protected Bayes

LSD test. Values followed by the same letter are not

significantly different, P < 0.05.

FIGURE 8

control. There was no evidence of phytotoxicity at any etridiazol concentration.

Fosetyl-Al. Fosetyl-Al treatments gave significant (P < 0.01) differences in disease ratings among treatments in the health/dip/chemical concentration interaction (Fig. 9). Mean separation for trees in health class 1 showed that all 3 chemical concentrations for dip treatments 2 (post-) and 3 (pre-/post-) significantly (P < 0.05) reduced disease ratings compared to the control. Post-cold storage treatment with concentration 4 (24000 ppm), and pre-/post-cold storage treatment with concentration 3 (12000 ppm) significantly reduced (P < 0.01) disease ratings compared to the control. There was no significant difference between any of the chemical concentrations for any dip treatment.

Among trees of health class 2, the disease ratings for dip treatment 1 (pre-) were progressively lower with increasing chemical concentration. However, only concentrations 3 and 4 reduced disease ratings below the control at either the 0.01 or 0.05 significance levels. The disease ratings for dip treatment 2 were significantly lower than the control for chemical concentrations 2 (6000 ppm) and 3 (P < 0.01) and for concentration 4 (P < 0.05). The pre-/post-cold storage treatment was not effective in disease control.

Among the trees treated with fosetyl-Al, there was no evidence of phytotoxicity at any chemical concentration.

Figure 9. Effect of fosetyl-Al chemical concentrations and dip

treatment on tree health (H1 = initially healthy, H2 = mildly

infected) as measured by disease ratings. Means were separated

independently for the 2 health classes by Fisher's protected Bayes

LSD test. Values followed by the same letter are not
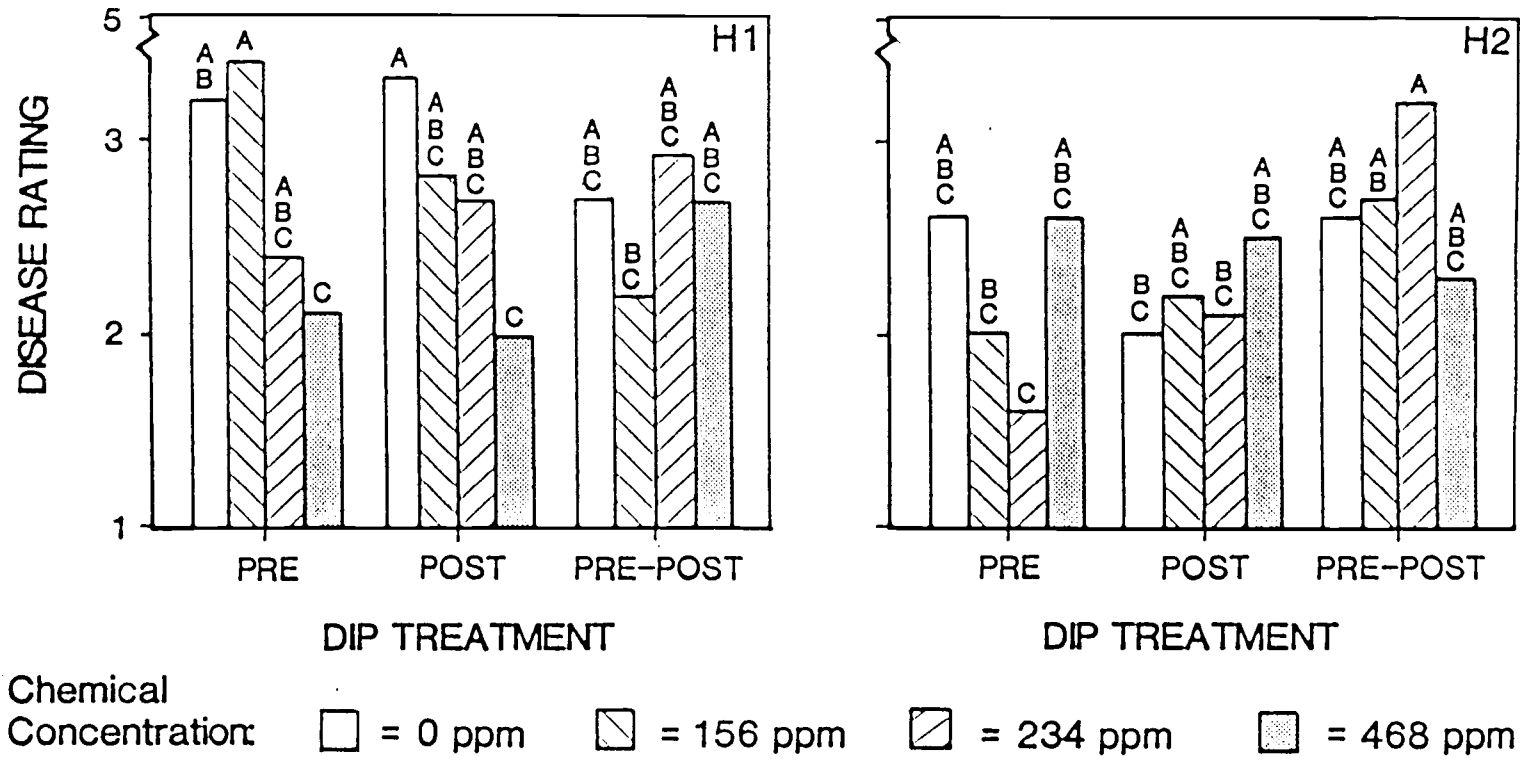
significantly different, P < 0.05.

FOSETYL ALUMINUM

FIGURE 9

Metalaxyl. The analysis of disease ratings for the metalaxyl treatment indicated significant (P < 0.05) differences between treatments for the health/dip/chemical concentration interaction Fig. 10).

Mean separation for the trees in health class 1 indicated the control trees had a higher (P < 0.01) disease rating than trees treated at chemical concentration 3 (2000 ppm) at any dip time, and concentration 4 (4000 ppm) at either the pre- (1) or post- (2) dip treatments. At the 0.05 significance level, trees treated at any chemical concentration with any dip treatment had a lower disease rating than the control. There was greater variation in the effect of the dip treatment with concentration 4. Trees given the post-cold storage treatment generally had lower disease rating with any chemical concentration. Trees in dip treatments 1 and 2 had lower disease ratings with increased chemical concentration.

Trees in health class 2 treated at any chemical concentration with the pre- or post-dip treatment had lower (P < 0.05) disease ratings than the control. However, of the chemical treatments, concentration 3 gave the best disease control with either dip treatment, and provided the greatest control with the pre-cold storage dip.

There was no evidence of phytotoxicity at any metalaxyl concentration.

Figure 10. Effect of metalaxyl chemical concentrations and dip treatment on tree health (H1 = initially healthy, H2 = mildly infected) as measured by disease ratings. Means were separated independently for the 2 health classes by Fisher's protected Bayes LSD test. Values followed by the same letter are not significantly different, P < 0.05.
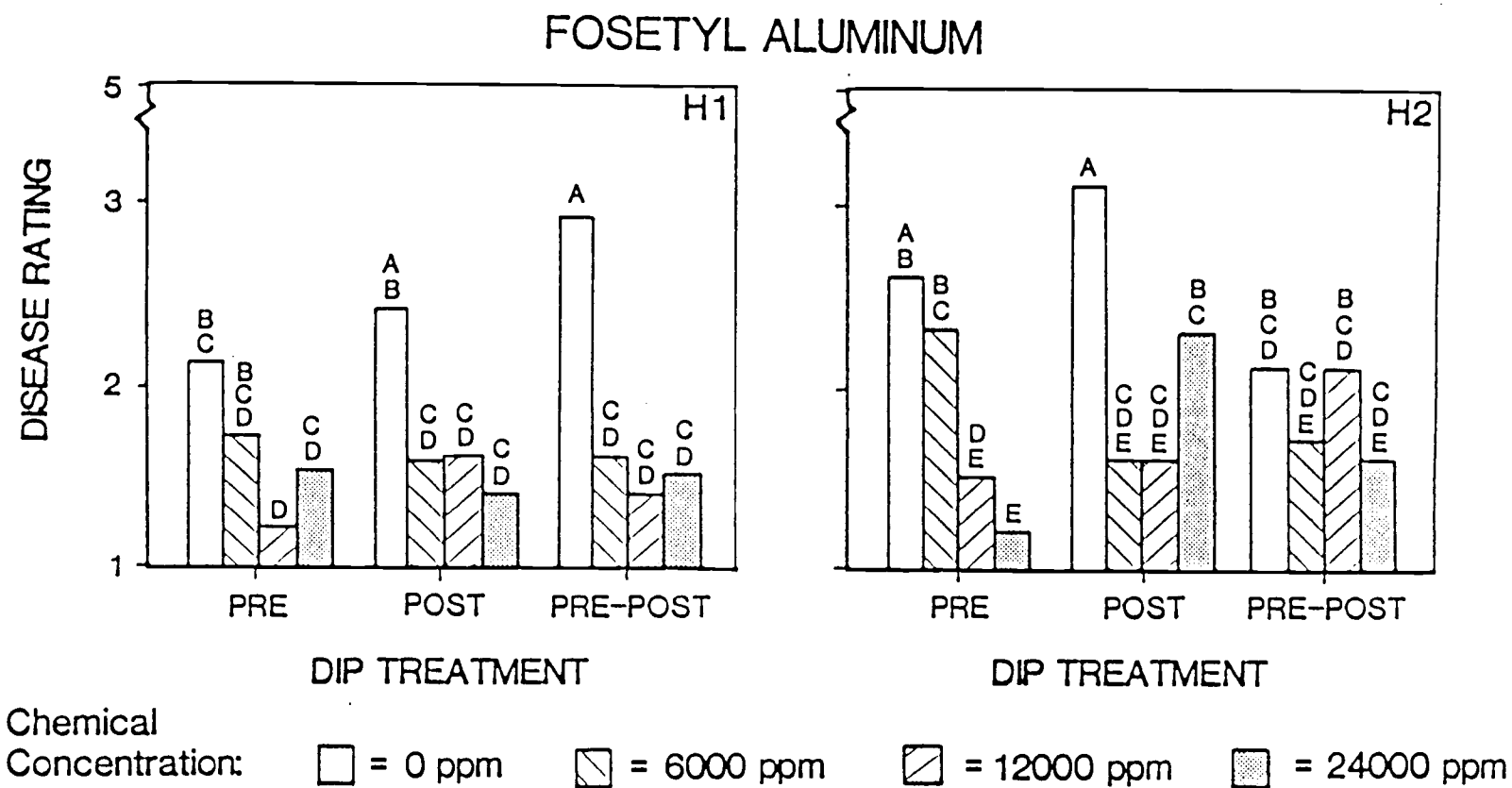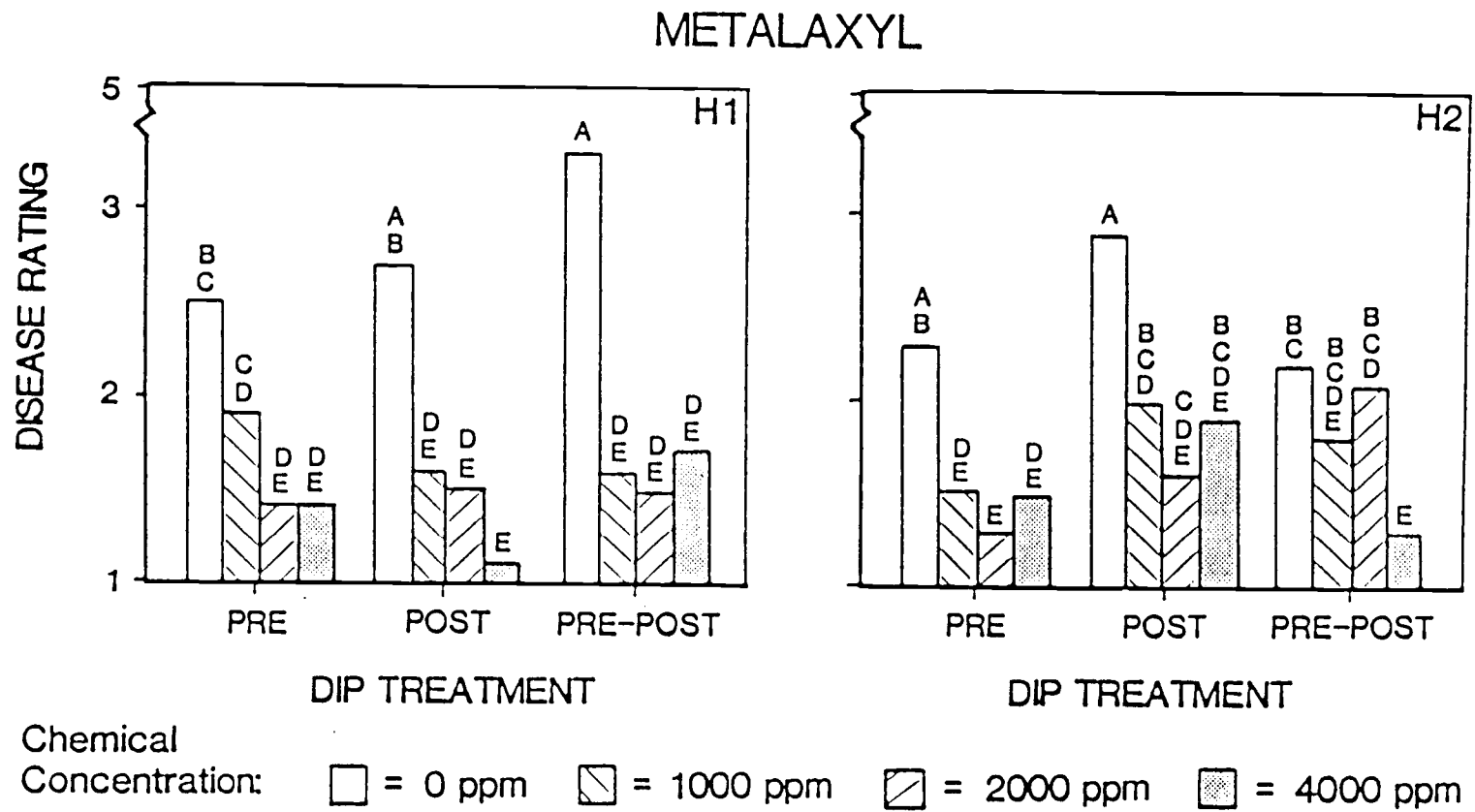
FIGURE 10

DISCUSSION

Disease Description and Phytophthora Recovery

Description. The site chosen for studying Phytophthora stem

and root rot in apple stool beds was a field of Emla 106 rootstocks

that was showing symptoms of the root and stem rot disease. As

mentioned by Julis et al. (1978), there are many plantings in a

stool bed nursery, and a Phytophthora problem in one field may not

be present elsewhere in a nursery. Since these studies focused on

one particular field, they do not represent a range of rootstock

varieties. Furthermore, the isolations do not summarize the

condition of either the whole nursery or nurseries of the Pacific

Northwest in general. Since dwarfing rootstock 106 is known to be

particularly susceptible to Phytophthora infection (McIntosh, 1975;

Julis et al., 1978; Helton et al., 1984; Utkhede, 1986), and the

site had trees visibly infected, it was an ideal location for

studying the disease complex and the effects of various control

measures.

The various symptoms of Phytophthora disease of rootstocks,

both recently shipped and in young orchard plantings, have been

described in part by some workers (McIntosh & MacSwan, 1966;

McIntosh Ed. 1975; Julis et al., 1978) and were similar for

rootstocks still within the stool bed production system.

Phytophthora recovery. When isolations were made from plant

tissue taken from the stool beds, it was considered that several

species might be involved in the disease complex, and that the activities of the different species would occur when temperature conditions were most conducive for the species involved. A number of workers (Gates & Millikan, 1973; Sewell & Wilson, 1963 & 1973; Bielenin, 1978; and Jeffers & Aldwinkle, 1986) have indicated that there are seasonal fluctuations in the susceptibility of apple trees to infection and colonization by Phytophthora species. Thus samples were taken periodically throughout the growing season to follow any seasonal fluctuations in Phytophthora activity.

P. cactorum, P. cambivora, and P. syringae have been previously isolated from shipped rootstocks (Julis et al., 1978; Brown & Hendrix, 1980; Jeffers, 1985) and were considered the most likely species causing infection. P. cactorum and P. cambivora were isolated from stool bed plant tissues; however, P. syringae, which was expected to be recovered in late October or November, was not recovered even from samples held in cold storage for 3 months. However, P. syringae may occur in apple stool bed fields not sampled, or it may have been in the samples assayed but was masked by faster growing Phytophthora species.

Why Phytophthora recovery differed so much between 1985 and 1986 is not known. Technically the sample preparation and isolation procedures were the same in both years. The visible symptoms appeared much later in 1986, possibly because the summer heat occurred later that year. P. cactorum was isolated most frequently from plant tissue one month after the hottest part of the summer in both years. However, temperatures aside, recovery

was generally poorer in 1986. The isolation of P. cambivora peaked
in mid-summer in 1985, the timing of which compares favorably with
its colonization patterns on apple rootstocks following artificial
inoculation (Jeffers & Aldwinkle 1986). As P. cambivora was not
recovered from plant tissue in 1986, no comparisons could be made
between recovery and weather data for the two years. Field
applications of fungicides was not the cause of poor recovery in
1986 because isolations were made from treated and untreated plots
in both years.

Isolations from shoots in chemically treated field plots
indicated that fosetyl-Al or metalaxyl alone, or a combination of
both, did not eradicate Phytophthora from the stool beds. However,
in every case, field treatment with these compounds significantly
reduced the disease compared with no field treatment at all. Based
on visual observations, plots treated in consecutive years had
overall less disease development than plots treated for only a
single year.

In the stool bed production system, sawdust is applied at
intervals throughout the growing season as a side dressing to hold
moisture around the apple stems to enhance rooting. Once applied
to the field, the sawdust is not removed for the following
production years and could potentially be an inoculum source for
consecutive crops. However, Phytophthora was recovered only from
sawdust samples taken directly adjacent to stems showing well
developed lesions. This indicates that Phytophthora is not
widespread in the rooting medium and that the sawdust itself is

probably not a major inoculum source for the spread of the disease in successive years. It is not known how long Phytophthora propagules would survive in the sawdust in the absence of host tissue. Survival could be longer if infected roots were mixed into the sawdust after harvest.

Pathogenicity Tests

It is evident from the inoculation test at 15°C that P. cactorum was more virulent than P. cambivora, and is probably more active both earlier and later in the growing season than P. cambivora. P. syringae, which was not isolated from the field plots of the current study but was used in the test, caused significant lesion development. This indicates that in some years P. cactorum and P. syringae might begin their activity in a stool bed as early as March and continue activity into the cool of autumn.

The differences in virulence seen among the isolates tested at 15°C largely disappeared in the 24°C test; and in all cases, where isolates were tested at both temperatures, they were more virulent at the warmer temperature. These results suggest that Phytophthora would cause more disease of apple rootstocks in mid-summer, an expectation that was confirmed by field isolations of Phytophthora from the apple stool beds.

It has also been shown in lab studies that colonization may depend partly on the change in rootstock susceptibility over the growing season (Jeffers & Aldwinckle, 1986). However, because lab

and field conditions are so different, extrapolations from the lab to the field must be done with caution. For example, Gupta and Singh (1979) reported that twigs from the same plant were equally susceptible throughout the growing season when inoculated in the lab, but plants inoculated in the field were differentially susceptible over the growing season. Furthermore, lesions on twigs inoculated in the lab were 3-4 times longer than those on field plants. Harris et al., (1984) also noted that, even with the presence of ample inoculum and wet conditions, disease development in field trials was not guaranteed even on a susceptible rootstock. Thus the results of the present pathogenicity tests, though corresponding favorably with the work of others, may vary if the tests were conducted in the field.

The development of external mycelium in the P. cambivora isolates of the excised stem assay conducted at 24°C is of potential significance. P. cambivora does not produce chlamydospores and only one mating type was identified among the isolates recovered from the field, essentially excluding oospores as a means of overwintering. Thus the development of external mycelium may be important for the persistance and movement of P. cambivora in the stool bed. Sporangia on mycelium extending into the sawdust but still attached to the food base may have provided the propagules of P. cambivora that account for the recovery achieved by baiting from sawdust directly adjacent to visibly diseased rootstock stems but not from the bulk of the sawdust.

Fungicide Residual and Disease Control

Bioassay of fungicide residual in apple stems. Results of the
study where stem pieces from trees field-treated (1986) with
metalaxyl or fosetyl-Al and inoculated with Phytophthora indicated
that there is significant chemical residual in stems after field
treatment to reduce infection by Phytophthora cactorum. All of the
fosetyl-Al treatments were last applied four days before the stems
were removed from the field for the bioassay, thus there was
probably insufficient time for differences in chemical rate to
become apparent. Further studies using this bioassay technique and
chemical applications to apple rootstocks in the stool beds might
be useful to determine residual effects at the different chemical
application rates as well as the residual chemical effect over
time. It would also be of value to test other Phytophthora spp.
under these conditions to determine differences in the effects of
these chemicals.

It is interesting to note that the metalaxyl treatment, which
was last applied three weeks prior to the tree sampling for the
bioassay, reduced lesion development to the same extent as fosetyl-
Al. This indicates that metalaxyl can effectively suppress
infection of apple rootstocks by P. cactorum, even though it was
less effective for disease control in the field than fosetyl-Al.
The method of field application, the mode of action, and pattern of
chemical distribution within the host tissues in the field may
explain this difference.

Effect of fungicide residual on tree health. Trees from
treated field plots in 1985, initially graded as healthy, showed
significant differences in disease development after 3 months in
cold storage and a 3 month growing period. While any chemical
treatment was better than no treatment at all, fosetyl-Al
treatments reduced disease development more than the metalaxyl
treatment. Though Phytophthora was not recovered from every sample
selected for isolation, recovery was correlated between treatments
with the visual symptoms present at the time the trees were
harvested and rated for disease. These results indicate that even
though field applied fungicides do carry their influence through a
commercial cold storage period and reduce disease development at
outplanting, disease development may continue to progress in cold
storage on trees appearing healthy before storage.

Observation of disease development on trees after a 9-month
cold storage period suggests that there is a long-term residual
effect of chemical treatments applied in the field during the
growing season. The differences in effect between chemicals
disappeared over the 9-month period. Whether the effect is a
result of less disease or contamination associated with the trees
going into cold storage, or whether it is a direct effect of
chemical residual in the stored trees protecting them from disease
development, cannot be determined from this information alone.
Though it has been reported that both fosetyl-Al and metalaxyl
remain active in root tissue for at least 2 months (Menge, 1986),
and it is known that metalaxyl is readily translocated upward from

the roots into woody tissue after application as a soil drench (Ellis et al., 1982), and that injection of avocado trees every 6 months with fosetyl-Al is an effective treatment for controlling P. cinnamomi (Darvas, et al., 1984), it is not known just how these chemicals remain active in woody stem tissue. However, it seems reasonable to suspect that by 9 months tree health reflects the condition of the rootstocks before they were put into cold storage rather than a difference in ability of the compounds to control disease development in long-term storage. Field work by others (Burr, personal communication, 1986) further supports this by indicating that, at least with treatments of fosetyl-Al, monthly applications gave better control than applications applied bimonthly. Regardless of the mechanism, it is evident that field treatments reduce disease development in the field, that treated trees maintain their health advantage over untreated controls in long-term storage, and that the effect between chemical treatments is moderated over time.

Chemical Root Dips

The chemical dip experiment was conducted to see if rootstock plants that were contaminated or only slightly infected, but symptomless, could be made or maintained pathogen and/or disease free prior to storage and shipping to orchardists or to nurserymen for grafting. The chemicals chosen all had known activity against Phytophthora, although their modes of activity were different.

Benalaxyl is an experimental compound which functions systemically similar to metalaxyl and has showed significant inhibitory effects on oomycete fungi. It was included as a prospective product on the market.

Etridiazol is a fungicidal compound used widely to control Pythium and Phytophthora diseases in nursery crops. It was chosen as a contact material which could prevent disease development in cold storage by eradicating surface contamination on the rootstocks.

Fosetyl-Al is both a xylem and a phloem transported compound. Though it has been proposed that fosetyl-Al may act indirectly by triggering a host resistance response, further work has indicated that one of the breakdown products (H_3PO_3) of fosetyl-Al in the plant, is highly active against Phytophthora and that the primary effect of these products is on the pathogen and the secondary effect is a host response (Fenn and Coffey, 1984). Whatever the mode of action of fosetyl-Al, its activity in suppressing sporulation of Phytophthora spp. (Farih et al., 1981a) and its effectiveness against Phytophthora when used as trunk paints or injections (Darvis et al., 1984; Taylor and Washington, 1984; Edney et al., 1984; Long Ashton, 1983) without indication of phytotoxicity, suggested that this compound might also be effective as a root dip.

Metalaxyl has been shown to be highly inhibitory to mycelial growth, sporangia production and zoospore germination of Phytophthora spp. (Farih et al., 1981; Ellis et al., 1982). It is

translocated best laterally and upward from the site of application
(Zaki et al., 1981) in sufficient quantities to prevent growth of
Phytophthora above the application site (Ellis et al., 1982), and
has been shown to be active in woody tissue for about 6 months
(Benson, 1979; Timmer, 1979). These characteristics suggested this
compound also, as an effective candidate for root dip treatments.

It was difficult to interpret the results of the dip
experiment which compared the four chemicals at four
concentrations, applied at three time regimes associated with cold
storage after harvest. The intent was to identify the best
chemical, concentration, and time of application for Phytophthora
disease control. The results were generally too erratic to draw
those conclusions or to even theorize about how they worked.
Benalaxyl, an experimental compound supposedly similar to
metalaxyl, gave erratic results and was much less efficacious than
metalaxyl. Etridiazol did not significantly effect disease
development, even though it has contact fungicidal properties, and
it was apparent that disease progressed from no symptoms at harvest
to significant disease after cold storage. This may suggest that
internal infections had already begun and could not be eradicated
by etridiazol.

Fosetyl-Al and metalaxyl gave the best results as post-harvest
treatments. While they did not totally eliminate the Phytophthora
disease, they greatly reduced it, especially if applied before cold
storage. Fosetyl-Al appeared to be slightly better than metalaxyl
as a post-harvest dip, and this relationship paralleled the results

observed with field applications. Because these two compounds have different modes of action, it may be useful to study their efficacy when used in combination or in succession, both in the field as well as in post-harvest dips.

It has been cautioned that both fosetyl-Al and metalaxyl can suppress without eradicating the pathogen population and are recommended as preventive rather than curative treatments in nurseries (Menge, 1986). The ideal application of these compounds as root dips is not intended to be the sole means of disease control, but rather, used in conjunction with other field and storage management practices to encourage and ensure the sale of healthy apple rootstocks.

CONCLUSIONS

While this study has left many questions unanswered about the Phytophthora stem and root rot disease of apple rootstocks growing in stool beds, it has led to some useful information and suggestions for control. The following conclusions seem reasonable from the data available:

(1) Phytophthora cactorum and P. cambivora are the species primarily involved in the stool beds, with P. cactorum being the most frequently encountered.

(2) The disease begins each season in May as new shoots emerge from the clonal mother plants and progresses during the entire season.

(3) Infections occur primarily in vegetative growth from the mother plants up into the new shoots rather than from new infections begun by inoculum that may be present in the sawdust used to mulch the plants for root initiation.

(4) P. cactorum functions in a wide temperature range, being pathogenic at both 15 and 24°C, while P. cambivora is pathogenic primarily at 24°C. P. syringae was not encountered in this study, but an isolate was pathogenic at 15°C.

(5) Applications of metalaxyl and/or fosetyl-Al in the field effectively reduced disease incidence in the field and these effects carried over to the cold storage stage of production.

(6) Post harvest dips with chemicals having activity against Phytophthora effectively reduced disease that developed after harvest. Fosetyl-Al and metalaxyl gave the best control when applied as a pre-cold storage treatment.

Considering the data presented here and that which is known in the literature, it seems possible to devise a comprehensive control program to reduce the Phytophthora stem and root rot disease of apple rootstocks. The program should begin when the stool beds are prepared by planting only known disease-free stocks in fumigated soil. To keep the stocks disease free or to minimize disease that may occur, an early season drench with metalaxyl (before new shoots appear and sawdust is added for root development), followed by several in-season sprays with fosetyl-Al would reduce the disease during the season. After harvest, a pre-cold storage treatment with metalaxyl or fosetyl-Al would further control any disease that was not detected by the grading procedure immediately following harvest. Root dips with the same chemicals before orchard planting would be the last opportunity to prevent in the orchard the Phytophthora disease known as collar rot.

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APPENDIX

LIST OF PLATES

A-1. Phytophthora root and stem rot - field views. A. Bare areas
in hedge rows where disease development has eliminated rootstock
development. B. Effect of chemical treatment in field plots -
control vs. fosetyl-Al. C. Dead trees in an orchard, the probable
result of planting Phytophthora infected rootstocks.

A

B

C

A-1

A-2. _Phytophthora_ root and stem rot - leaf symptoms. A. Rust-
brown leaf bronzing. B. Shoot tip wilting and die-back.

A-3. <u>Phytophthora</u> root and stem rot - lesions. A. Infected roots showing red-brown discoloration and dark brown lesions. B. Brown-black canker localized around the root node. C. Lesions extending from infected mother stock.

A

B

C

A-4. Morphology - <u>Phytophthora cactorum</u>. A. Limoniform, strongly papillate sporangium. B. Oogonia with paragynous antheridia.
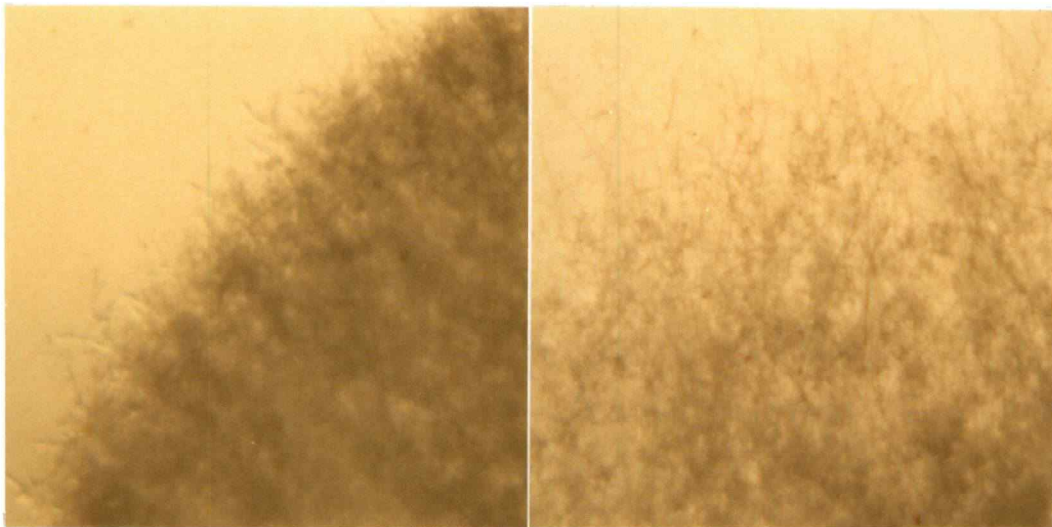
A-5. Morphology - _Phytophthora cambivora_. A. Internally proliferating, non-papillate sporangium. B. Oogonium with bullate protuberances and large antheridia. C. Colony morphology group 1. D. Colony morphology group 2.