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COMPARISON OF FIVE FUNGICIDES USED FOR CONTROL OF PYTHIUM BLIGHT OF FESTUCA RUBRA

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COMPARISON OF FIVE FUNGICIDES USED FOR CONTROL
OF PYTHIUM BLIGHT OF FESTUCA RUBRA

DISSERTATION

Presented in Partial Fulfillment of the Requirements for
the Degree Doctor of Philosophy in the Graduate
School of The Ohio State University

By
Freda Machelle Ashbaugh, B.S., M.S.

The Ohio State University
1984

Reading Committee:
Dr. L. H. Rhodes
Dr. G. S. Taylor
Dr. A. F. Schmitthenner
Dr. J. R. Street
Dr. P. O. Larsen

Approved by

Advisor
Department of Plant Pathology
To Debbie for her empathy, Ramona for her objectivity, and Tim for his tenacity.
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VITA

September 12, 1956.............. Born - Bremen, Ohio

1977............................... B.A., Biology, Northland College, Ashland, Wisconsin

1980............................... M.S., Department of Plant Pathology, The Ohio State University, Columbus, Ohio

1980-1984.......................... Research Associate, Department of Plant Pathology, The Ohio State University, Columbus, Ohio

PUBLICATIONS


VITA (cont.)

FIELDS OF STUDY

Major Field: Plant Pathology

Studies in conjugation of drug resistant plasmids.
Professor D. L. Coplin

Studies in uptake of systemic fungicides.
Professor P. O. Larsen
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INTRODUCTION

Pythium blight is a common and potentially severe disease of turfgrasses caused by several species of Pythium (53), including P. graminicola, P. ultimum and P. aphanidermatum. Due to its potential destructiveness, fungicides are often used to control this disease.

Chloroneb and ethazole are standard fungicides for control of Pythium blight. Two other fungicides, metalaxyl and propamocarb, have recently been registered on turfgrass. A fifth fungicide, fosetyl aluminum, is registered for control of diseases on ornamentals caused by Pythiaceous fungi. Although many researchers have examined different properties of these fungicides for control of Pythium blight and Pythiaceous diseases, a comprehensive, comparative study of these five fungicides has never been published.

Reports have documented systemic movement of all five fungicides in various crops. Chloroneb, metalaxyl and propamocarb have also been reported systemic in turfgrass. Metalaxyl demonstrated systemic activity in ryegrass and bentgrass (56). A report by Wells and Littrell (40) on the translocation of chloroneb in ryegrass contradicted an earlier paper from the same laboratory (78). Unpublished reports by P. O. Larsen and P. Sanders (The Upjohn Co., internal report) found evidence for the systemic activity of
propamocarb in red fescue and ryegrass, respectively.

Several researchers have published evidence indicating systemic movement in crops other than turfgrass (1, 14, 18, 37, 45, 52, 72). Fosetyl aluminum may be translocated basipetally in citrus (18, 37). However, in a recent report by Sanders et al. (58), application of fosetyl aluminum to the roots of ryegrass did not control Pythium blight when the foliage was inoculated with P. aphanidermatum, suggesting that the fungicide was not translocated acropetally.

The following research was undertaken for the purpose of comparing various properties of chloroneb, ethazole, fosetyl aluminum, metalaxyl and propamocarb, and their relationship to the control of Pythium blight of Festuca rubra. The specific properties studied were:

1) minimum inhibitory concentration in vitro; 2) residual activity of foliar applications, and efficacy of preventive and curative control; 3) systemic activity of fungicides applied to foliage or soil; and 4) effect of temperature and soil moisture on uptake of fungicides.

In addition, it is hoped that the assays used in these experiments will provide a system to further study the relationship of systemicity to duration of preventive and curative disease control. A better understanding of the nature of fungicidal translocation in plants may lead to methods of application which increase systemic activity, thereby increasing effectiveness in the field, and reducing the amount of fungicide applied.

Portions of this research have been reported elsewhere (3, 4).
Systemic fungicides, strictly defined, refer to those compounds which exhibit both fungicidal activity in vitro and protective or therapeutic activity in the host at a point distal to the point of application (65, 75). Most discussions of systemic fungicides, however, also include those compounds which are not fungitoxic in vitro, but instead act indirectly through their effects within the host.

Widespread use of systemics for disease control did not come about until the late 1960's with the introduction of the benzimidazoles, of which benomyl is the most important example. (The history of systemic compounds prior to this era has been reviewed by Wain and Carter (76).) Because of its early and widespread use, benomyl has been studied more thoroughly than any other systemic fungicide. Comparisons suggest that uptake and distribution of benomyl are similar to that of most other fungicides (16, 23).

Crowdy (16) and Erwin (23) have reviewed the translocation of systemic fungicides. Systemic movement in plants occurs via the apoplast, the non-living portions of the plant, or the symplast, the living portions of the plant (54). Most fungicides use the apoplast, following the flow of water in the xylem and accumulating in those
portions of the plant where the transpiration and respiration rates are both high (21, 22, 32, 48).

Very little uptake of fungicides in aqueous solution is achieved through the plant cuticle, due to its hydrophobic nature (54). The application of fungicides in combination with nonphytotoxic oils has been found to increase absorption through the cuticle, presumably by increasing the permeability of cutin (68, 81). However, when fungicides are foliarly absorbed, they are translocated in the apoplast to the edge of the leaf and do not afford protection to the crown or roots (11). In order for significant uptake of these chemicals to occur, they must reach the roots (52).

In the soil, fungicides are subject to microbial degradation, decomposition by sunlight or chemicals, and leaching (74). When a fungicide enters the rhizosphere, it may diffuse through cell walls into the intercellular spaces (51, 54). The fungicide moves with the water in the intercellular spaces until it reaches the endodermis. Here it must cross living protoplasm to traverse the Casparian strips, but once through, it is transported in the xylem vessels upward to areas of high transpiration (11). The fungicide may then be deposited upon the leaf surface where it acts as a protectant or washes back into the soil to recycle (5, 16). Some of the chemical may be bound in the leaf tissue and deactivated or it may remain unbound and act as a chemotherapeutant (54).

The movement of chemicals in the symplast requires penetration of the leaf cuticle. The cuticle represents a gradient which is
highly non-polar on the outer surface, and increasingly polar toward the epidermal cell wall (5). As mentioned above, chemicals which are lipoidal easily penetrate the outer matrix of cuticle and may enter the cell wall via channels in the cuticular wax (5, 16). Once through the cuticle, the fungicide is transported across the cell membrane by active transport. Movement then proceeds from cell to cell via the plasmodesmata until the phloem is reached. Transport in the phloem continues from the "source", (mature leaves) to the "sink", (fruits, tubers, roots, immature leaves), through the sieve tube elements (42, 54).

The advantage of symplastic transport is that movement may proceed basipetally, i.e., downward from the point of application. This is an especially important attribute in the control of root diseases on perennial crops, because it implies that control can be achieved by foliar application of a fungicide. Fosetyl aluminum (18, 37), metalaxyl (11, 82) and pyroxychlor (71), among others, have shown evidence of basipetal movement. The amount of fungicide transported, however, was not always in quantities sufficient to achieve disease control (33, 82). Overall, very few fungicides are translocated in this manner.

Several review articles have discussed the advantages and disadvantages of systemic fungicides (21, 23, 65, 75). Edgington (21) states that systemics may "compensate for poor coverage because of redistribution within the plant". When compared to non-systemic fungicides, both the amount of active ingredient required and the
frequency of application are often reduced (21). Systemic fungicides are transported within the plant, requiring selective toxicity against the fungus with little or no damage to the plant host. But selectivity has resulted in chemicals which usually exhibit a very narrow target range of fungicidal activity and must be combined with other fungicides in order to achieve disease control on any particular crop (52). Another disadvantage is that systemics usually have only one or two modes of action, and fungi may develop resistance (19).

Very little research has been done on factors which modify uptake and translocation of systemics. In its 1979 report on "Contemporary Control of Plant Diseases with Chemicals" (2) the American Phytopathological Society recommended that "basic studies on systemic chemicals used in disease control should be carried out in several areas. These include the effect of structure on uptake, systemicity, and target organism activity. The effect of adjuvants, host predisposition and other factors on systemic action should be investigated."

The degree of systemicity of a fungicide depends upon properties of the chemical and its formulation. Edgington and Petersen (22) found that the ability to cross the plasmalemma of cells was a factor of the oil-water partition coefficients of fungicides. Thiabendazole, carbendazim and carboxin were very soluble in lipids, whereas prothiocarb and metalaxyl were very water soluble, indicating that a fairly wide range of coefficients exist among systemic chemicals.
Erwin (23) has reviewed various factors affecting the uptake of benzimidazoles, oxanthiins and pyrimidines. Evidence suggests that adjuvants may increase uptake of fungicides by increasing penetration through the leaf (68, 81). Edgington and Martin (21) conclude that the formulation of fungicides as emulsifiable concentrates may improve uptake in a similar fashion.

Uptake may also be affected by the soil environment. Adsorption and absorption of fungicides to soil particles varies considerably among soil types, thus affecting the amount of fungicide available to the plant.

Cayley and Hide found that uptake of iprodione by potato plants was affected by soil composition (12). Drenching the chemical controlled infection by *Phoma exigua* var. *foveata* when plants were grown in coarse sand, sandy loam, clay loam or loam soils. In sandy soils, the chemical exhibited a high rate of decomposition. On peat/sand compost or fen peat, however, uptake was not sufficient to prevent infection, and lesion size was inversely related to the amount of iprodione in the tissue. The authors concluded that because of the properties of sorption or decomposition observed in most soils, soil application of iprodione would not be practical for the control of potato diseases.

Sanders et. al. (55) demonstrated adsorption of the fungicides benomyl, pyroxychlor, iprodione and triadimefon to soil colloids. Using a variety of soil types, they found that initial and residual efficacy against *Sclerotinia homoeocarpa* were affected in all cases.
Benomyl proved least effective in high peat soils, while the latter three all did poorly in soils with a high clay content, presumably due to adsorption to the organic matter and clay colloids, respectively. Another researcher attributed low uptake of benomyl to high organic matter and low pH (61).

Carris (11) compared mobility and adsorption of metalaxyl in nine soils. Mobility of the fungicide was inversely related to the amount of organic matter and clay in the soil. Adsorption was relatively more efficient at higher fungicide concentrations.

Most of the reports discussed so far have been based partially, if not solely, on evidence obtained from bioassays. Bioassays constitute the most popular method for determining systemic movement of a fungicide. They are usually rapid, simple and highly sensitive. The simplest test of systemicity is to introduce a chemical into the potting medium or onto various plant parts, followed by inoculation of the plant with a suitable test organism (13, 17, 39). Variations on this method include inoculating excised leaves or leaf disks instead of the entire plant, which economizes materials and space (18, 47, 52).

Another variation involves macerating treated plant portions in a blender or tissue homogenizer (41, 43). The fungicide is extracted in a suitable solvent, usually acetone or benzene (10). Aliquots of the extract are then added to agar and the amount of fungicide is determined relative to the growth of an indicator fungus as measured by dry weight or radial growth. In most cases, therefore, this method
does not determine the absolute concentration present in tissue. One exception is a bioassay designed by Wynn and Cruite (80) to determine the amount of metalaxyl present in lettuce, tobacco and grape. Radial growth of *Pythium ultimum* on agar amended with metalaxyl alone was compared to growth on agar amended with extracts of plants treated with metalaxyl. The standard curve was confirmed by C14 studies. The bioassay method did not significantly differ from estimates using radioisotopes.

Bioassays do not differentiate between chemicals which are true systemics and those which are not translocated in a fungitoxic form, but instead induce resistance in the host through indirect means. In addition, there is no way to determine to what degree, if any, volatilization of the chemical contributes to disease control.

More specific information on the amount of chemical translocated and its distribution within the plant can be obtained from various methods of quantitative analysis and the use of radioisotope studies. Methods of residue analysis for some common systemics have been reviewed by Burchfield (10). Procedures for gas chromatography and thin-layer chromatography are available to determine the presence of most fungicides (12, 26, 38, 64, 69, 70, 77). These procedures are frequently tedious and may involve specialized equipment. The use of radioisotopes also involves special equipment and procedures, but it is a particularly useful method for identifying the quantity and distribution of a fungicide and its metabolites (11, 63, 82).
In 1981, forty-four chemicals listed as systemic were commercially available for the control of plant diseases (21). Among this group there are a number of fungicides for the control of Pythium diseases. On cool season grasses in established turf, several species of Pythium, including _P. graminicola_, and _P. ultimum_, but particularly _P. aphanidermatum_ are capable of causing a disease known as Pythium blight (53, 67). Control of disease on susceptible grass is usually based on the application of fungicides. The properties of five of these fungicides are the focus of research in this dissertation. Two of them, chloroneb and ethazole, are standard chemicals for control of Pythium blight. Three are relatively new fungicides which have recently been introduced on the market for control of Pythiaceous fungi: 1) fosetyl aluminum, 2) metalaxyl and 3) propamocarb. Metalaxyl and propamocarb are registered for Pythium blight on turfgrass as Subdue and Banol. Fosetyl aluminum is not yet registered for turfgrass, but research has shown it to be very effective for control of Pythium blight (58). There is evidence that all five fungicides are capable of systemic movement in certain crops.

Ethazole is registered on turfgrass under the trade name Koban. Researchers have demonstrated systemic activity of ethazole in cotton (1) and tomatoes (45). Gas chromatographic analysis of tomato tissues did not reveal discernible amounts anywhere but in stem tissue. Radioisotope (C14)-labelled ethazole applied to soybean seeds was translocated to the cotyledons of fourteen-day-old soybean seedlings (46). Despite this evidence, ethazole is often referred to in the
literature as a preventive fungicide (15, 51) that does not readily translocate and is only effective on the plant surface.

Chloroneb, (Tersan SP, Teremec SP) on the other hand, has been referred to as a systemic in several references (31, 51, 65, 66) based on work by Mukhopakhyay (44) and Kirk (36) who demonstrated its translocation in sugar beet and cotton, respectively. Littrell (40) found that soil drenches controlled P. aphanidermatum inoculated onto the foliage of tomato, pepper, bean and ryegrass, contradicting an earlier paper by Wells (78) who found no control of the fungus when chloroneb was applied as a soil drench or soil amendment. Foliar applications of the fungicide gave control according to both reports. Littrell (40) concluded that the differences in control, and thus the implications for systemic activity, might be attributable to a difference in time of exposure of the chemical to the plants before inoculation.

Propamocarb is a liquid fungicide completely soluble in aqueous solution. It is registered under the trade names Previcur N and Banol. Cohen demonstrated systemic activity of propamocarb in the control of downy mildew of cucumber (13). Control was better when the chemical was applied as a soil drench than as a foliar spray, with protection lasting longer than twenty-five days from a single application. Application three days prior to inoculation was more effective than one or two days.

Timmer found that propamocarb did not show significant systemic activity against Phytophthora parasitica when applied as a soil drench
to two-year-old grapefruit trees (72).

Metalaxyl has three labels. It is registered as Subdue on turf and ornamentals, as Ridomil for vegetable crops and as Apron, a seed dressing. The systemic nature of metalaxyl has been well documented (6, 7, 9, 11, 14, 18, 25, 34, 35, 50, 52, 56, 69, 72, 77, 80, 82).

Metalaxyl controlled Phytophthora root and foot rot of citrus when applied as a soil drench in two-year-old, but not five-year-old citrus (18). The difference was attributed to the inability of the chemical to penetrate heavy clay soils to reach the deeper root systems of the five-year-old trees. A bioassay of tissue from the tree trunk painted with metalaxyl suggested that the chemical moves efficiently in bark.

In work on potato plants for control of late blight, Rowe (52) found that metalaxyl was translocated acropetally when applied as a soil drench. Foliar sprays provided very little protection of the foliage indicating limited absorption by the leaves. There was no evidence of basipetal movement.

In another study with late blight on tomatoes, Cohen et al. (14) demonstrated acropetal movement of metalaxyl. They found that soil drenches gave excellent disease control for at least six weeks after application. Foliar sprays followed by inoculation with sporangial suspensions of Phytophthora infestans indicated some uptake by stems and petioles, but none by leaves.

Sanders et al. (56) reported similar results when metalaxyl was applied in wettable powder or granular formulation to control Pythium blight of ryegrass and creeping bentgrass. Efficacy after
three weeks was no different between irrigated and unirrigated plots. They concluded that absorption occurred in both roots and foliage, but that translocation was only acropetal.

Evidence of basipetal movement of metalaxyl was found by Zaki et al. (82) in avocado using radioactive isotopes. Application of radioisotopes to above-ground portions of the plants indicated that a small amount of the fungicide was absorbed by the leaves and translocated basipetally. Absorption was greater from the stem than from leaves. Foliar uptake was not enhanced either by the adjuvant Triton B-1956 (Rohm and Haas, Philadelphia, PA 19105) or an auxin, indole acetic acid. Using radioautography, soil-applied C14-labelled metalaxyl was found to be translocated acropetally in avocado, Persea indica and tomato.

Fosetyl aluminum is registered under the trade name Aliette for control of diseases caused by oomycetes. The fungicide exhibits in vitro toxicity against certain Pythiaceous fungi, especially Phytophthora species, but it is much less active in vitro against Pythium species (24). Sanders (58) found no in vitro activity against 25 Pythium isolates, representing 8 different species.

Experiments on citrus for the control of Phytophthora diseases suggest that fosetyl aluminum may be translocated basipetally (18, 37).

Despite the lack of direct fungitoxicity, fosetyl aluminum is capable of controlling diseases caused by Pythium species. On perennial ryegrass, the chemical was compared as foliar sprays or soil
drenches for the control of Pythium blight (58). As a soil drench, it failed to control disease. Foliar sprays conducted in the greenhouse suppressed the disease at three different concentrations, but only application at the highest concentration gave acceptable control. After two weeks, even plants sprayed at the highest concentration exhibited disease severity where well over fifty percent of the plants were diseased. Field data, however, indicated acceptable disease control up to three weeks after application. The authors (58) suggest that the chemical may be acting as an anti-gungal elicitor in the host. Absorption of the elicitor may stimulate the production of a phytoalexin toxic to Pythium, thus immunizing the plant against infection by the fungus. No phytoalexin has yet been identified, however, and the exact mechanism by which fosetyl aluminum controls disease is still unknown.
MATERIALS AND METHODS

Source of Pythium isolates. Five isolates of *Pythium aphanidermatum* were used in these studies. Isolate FS was obtained from A. F. Schmitthenner (OARDC, Wooster, OH). Isolates P-27, P-28, U-2 and U-3 were obtained from P. L. Sanders, (Dept. of Plant Pathology, Penn. St. Univ., University Park, PA).

Cultures were maintained on V-8 juice medium containing the following concentrations per liter: 1 g sucrose, 0.2 g yeast extract (Difco Laboratories, Detroit, MI), 100 mg neomycin sulfate (Calbiochem, San Diego, CA), 10 mg chloromycetin, 10 mg cholesterol, 27 mg pentachloronitrobenzene (Terraclor 75W, Olin Chemical Co., Little Rock, AR), 20 mg benomyl (Benlate 50W, E.I. du Pont de Nemours & Co., Wilmington, DE), 20 g Bacto agar (Difco Lab.) and clarified V-8 juice (Campbell Soup Co., Camden, NJ) extract prepared as follows: 40 ml of V-8 juice were steamed with 60 mg CaCO₃ for one hour in one liter of water, then centrifuged prior to being autoclaved. The resulting supernatant used in preparation of the storage medium. Cultures were stored at 12 C in 2 ml screw cap bottles containing V-8 juice media.
Plant cultivation and inoculation method. "Pennlawn" red fescue (Festuca rubra L. "Pennlawn") was seeded at a rate of 0.4 g per twelve ounce styrofoam cup (8.5 X 11 cm) filled with steam-sterilized soil mixture (soil:peat:perlite 1:1:1). Drainage holes were made in the bottom of each cup prior to planting. All plants were maintained at a height of 2 ½", and fertilized twice weekly with water-soluble fertilizer (200 μg/ml each N, P and K) with added trace elements.

Experiments were conducted with four to eight-week-old plants which were clipped immediately prior to inoculation. Inoculum was taken from 48-72 hr cultures of *P. aphanidermatum* grown on 1.5% corn meal agar (Difco Lab.) scattered with sterile millet seed. Cultures were incubated in a 30 C incubator (Percival Mfg., Boone, IA) under 12 hr light (20W, cool white, fluorescent with 40W supplemental incandescent lights, 4-5 Klux). The isolate FS was used in all experiments unless otherwise indicated. Plugs were taken with a No. 1 cork borer (5.0 mm diameter) such that each plug contained one millet seed. Plants were inoculated by placing an agar plug with the attached millet seed in the center of each pot using the agar to adhere the seed to the tips of the grass blades. The cups were then placed in plastic bags tied with a twist tie and incubated at 30 C under 12 hr of fluorescent light (20W, cool white, fluorescent with 40W supplemental incandescent lights, 4-5 Klux).

Tests for virulence of *P. aphanidermatum* isolates. The virulence of five isolates of *P. aphanidermatum*, FS, P-27, P-28, U-2 and U-3, was tested by comparing the rate of disease progression on
F. rubra using corn meal agar/millet seed inoculum. Plants were inoculated as described above. Disease was rated for 6 days at 24 hr intervals on a 0-10 rating scale, where 0=no disease, 1=1-10%, 2=11-20% 3=21-30%, 4=31-40%, 5=41-50%, 6=51-60%, 7=61-70%, 8=71-80%, 9=81-90% and 10=91-100%.

Minimum inhibitory concentration of fungicides in vitro. The minimum concentration required to inhibit linear growth of P. aphanidermatum was determined for the fungicides ethazole (Koban 30W, Mallinckrodt Inc., St. Louis, MO), chloroneb (Tersan SP 65W, E. I. du Pont de Nemours & Co., Wilmington, DE), fosetyl aluminum (Aliette 80W, Rhone-Poulenc Inc., Monmouth Junction, NJ), propamocarb (Banol 65, The Upjohn Co., Kalamazoo, MI) and metalaxyl (Subdue 2E, Ciba-Geigy Corp., Greensboro, NC). Fungicides were added at concentrations of 3, 6, 25, 50 and 100 μg active ingredient/ml to autoclaved corn meal agar cooled to approximately 50°C. The fungicide agar media were then poured into plastic petri dishes at 20 ml per plate. After solidifying, the center of each plate was seeded with an agar plug taken with a No. 1 cork borer from 48-72 hr corn meal agar cultures of P. aphanidermatum. The plates were sealed with parafilm (American Can Co., Greenwich, CT) and incubated at 30°C under 12 hr light as previously described. Radial growth was measured at 24 hr intervals for six days. Results were expressed as the mean of four separate measurements per plate with three plates per per treatment.
Preventive and curative fungicidal control. Evaluation of fungicides for preventive control of Pythium blight was determined by spraying the fungicides one hr prior to inoculation at the following recommended label rates of active ingredient per 93 m²: fosetyl aluminum, 90.7 g; propamocarb, 81.7 g; chloroneb, 73.7 g; ethazole, 59.5 g; and metalaxyl, 13.6 g. The fungicides were applied to the foliage in one ml water (The equivalent of 19.375 L of water per 93 m²) using an artist's air brush (Badger Air Brush, Co., Franklin Park, IL) at 25 psi. An hour after treatment, the grass plants were inoculated with corn meal agar/millet seed infested with P. aphanidermatum and incubated at 30 C. Disease severity was rated on a 0-10 scale as previously described.

In a second experiment for preventive control, fungicides were applied at the same rates outlined above, except that plants were sprayed 0, 3, 7, 14 and 21 days prior to inoculation. The treated plants were moved to the greenhouse and watered overhead and clipped as needed. All plants were inoculated on the same day, 21 days after the first plants were sprayed, and evaluated for disease development as previously described.

For curative control, the same method was used as described above, but in addition to the plants inoculated at the time of treatment, a second and third set of plants were sprayed 24 and 48 hr after inoculation and later evaluated for disease development.

Inoculated, untreated plants were included as controls in all experiments. Uninoculated, treated plants were included initially,
but when no evidence of phytotoxicity was observed, these treatments were omitted in subsequent experiments.

Determination of acropetal systemic movement in vivo. Five fungicides were injected into the soil to test acropetal systemic movement. Prior to treatment, the cups were watered and allowed to drain 2 hrs at room temperature. Fifty ml of each fungicide were injected approximately 1 cm into the soil in 10 ml aliquots at five sites in the pot using a 50 ml syringe. Fungicides were injected at one, two, four and eight times the recommended label rates for field use based on the active ingredient per 93 m² if foliarly applied. These rates in terms of active ingredient per cup (250 ml soil mixture/cup) were respectively: fosetyl aluminum, 5.1, 10.2, 20.4, and 40.8 mg; chloroneb, 4.2, 8.4, 16.7 and 33.4 mg; propamocarb, 4.6, 9.2, 18.4 and 36.8 mg; ethazole, 3.4, 6.8, 13.6 and 27.2 mg; metalaxyl, 0.77, 1.5, 3.1 and 6.2 mg. In subsequent experiments, metalaxyl was tested at rates of 0.3, 0.7, 1.5 and 2.2 mg active ingredient per cup.

Pots were placed in plastic trays after treatment for 24 hrs at 30 C prior to inoculation with P. aphanidermatum and incubated at 30 C as previously described.

Determination of basipetal systemic movement of fungicides in vivo. Three fungicides were evaluated to determine if they could be basipetally translocated. Cups of 15 cm high plants were inverted and dipped into 400 ml beakers containing fungicide solutions of the following concentrations: 7.2 and 3.6 g a.i./L, propamocarb; 1.2 g a.i./L metalaxyl; 6 g a.i./L fosetyl aluminum. When initial
rates proved phytotoxic, metalaxyl was also tested at concentrations of 1.2 g a.i./L and exposure times of 1 min, 5 min and 1 hr. Care was taken to insure that only the top 5 cm of leaf blades were immersed in solution. After 24 hr the plants were removed from the fungicide solutions, and, still inverted, left to dry at room temperature for one hour. The treated leaf portions were then clipped, and the remaining untreated portion was inoculated and incubated as previously described.

**Residual activity of fungicides applied to soil.** Three fungicides were tested for residual efficacy on *F. rubra* at various times after soil application. The rates applied, given as active ingredient per cup, were as follows: fosetyl aluminum, 5.1, 10.2 and 20.4 mg; metalaxyl, 0.3, 0.7 and 1.5 mg; propamocarb, 4.6, 9.2 and 18.4 mg. The fungicides were injected into the soil at 1, 3, 7, 14 and 21 days prior to inoculation of the plants. The treated plants were moved to the greenhouse and watered overhead as needed. All plants were inoculated on the same day, 21 days after the first plants were treated, and evaluated for disease development as previously described.

The experiments described in this study were repeated at least once with similar results. Results were subject to an analysis of variance, followed by an LSD when significant or t-test where appropriate.

**Vapor activity of fungicides.** Studies were initiated to determine whether fungicide vapor is responsible for disease suppression. In the first set of experiments, three fungicides were injected into the
soil mixture of potted $F$. rubra plants at the following concentrations of active ingredient per cup: ethazole 3.4, 6.8, 13.6 and 27.2 mg; metalaxyl, 0.3, 0.7 and 1.4 mg; and propamocarb, 4.6, 9.2, 18.4 and 36.8 mg. The pots were placed in plastic trays and maintained in a 30 C incubator for 24 hr prior to inoculation. The bottom of each treated pot was wrapped with parafilm to prevent fungicide from draining out of the pots and contaminating the untreated pots. Pots were placed two to a bag, one treated and one untreated. The pot of untreated plants was first inoculated with $F$. aphanidermatum before being placed in the bag, which was then sealed and placed in a 30 C incubator. Disease development was rated on the untreated pots as previously described.

In the second set of experiments, the same fungicides were added to cooled corn meal agar at a concentration of 100 $\mu$g/ml. For each fungicide, ten plates of fungicide-amended agar were placed in plastic bags with ten plates of unamended agar. The unamended agar plates were seeded with plugs of $F$. aphanidermatum. Radial growth was measured 24 hr intervals for six days. Controls consisted of ten plates of unamended agar seeded with $F$. aphanidermatum.

Effect of temperature on efficacy of fungicides applied to soil.

In order to evaluate the effect of temperature on uptake, two fungicides were applied to plants and subjected to two different temperature regimes prior to inoculation. Propamocarb was injected into the soil at rates of 4.6, 9.2, 18.4 and 36.8 mg and metalaxyl at rates of 0.3, 0.7 and 1.4 mg active ingredient per cup.
Plants were maintained in an incubator at 20°C and 30°C for 72 hr under a twelve hr cycle of light (20W cool white fluorescent) in ≥95% relative humidity. After 72 hr, plants were inoculated and incubated at 30°C in plastic bags. Disease progression was rated daily as previously described.

Gas chromatographic determination of metalaxyl. Metalaxyl was injected into pots of 6 week-old F. rubra approximately 1-2 cm below the soil line of each pot via hypodermic syringe at rates of 0.7, or 1.4 mg active ingredient per pot, applied in 50 ml water. After 24 hrs, the leaf tissue was clipped to approximately 1 cm above the crown, placed in plastic bags, and stored at -4°C until use.

The following extraction procedure was based on the method of Waliszewski and Szymazynski (77). Ten ml distilled water were added to ten grams of leaf tissue and macerated in a Virtis mixer (Gardiner, NY) for 30 sec. The homogenate was filtered through glass wool into a 250 ml separatory funnel containing 80 ml ethyl acetate and 20 ml acetone. The contents were agitated gently. After 2 hr, the bottom phase containing acetone and water was discarded, and the upper phase was drained into a large beaker to facilitate volatilization. One gram of sodium sulfate was added to the beaker. Depending upon the amount of water in the sample, more sodium sulfate was added as needed until flocculation occurred. The sample was evaporated to dryness. The residues were resuspended in 1-5 ml of ethyl acetate, decanted into centrifuge tubes and sealed with parafilm. The tubes were centrifuged at 2000 rpm for 5 min to remove any particulate
sodium sulfate. The supernatant was transferred to 5 ml screw cap vials.

Ten to fifty μl samples were injected into a 6' X 1/8" nickel column packed with 5% OV-101 on 100-120 mesh Anakrom using a Hewlett-Packard gas chromatograph Model 5750B (Hewlett-Packard Co., Pasadena, CA), with a flame-ionization detector and a Hewlett-Packard strip chart recorder, Model 7127A. The following parameters were used: carrier gas-nitrogen, flow rate 50 ml/min; hydrogen flow rate, 5 ml/min; air flow rate, 50 ml/min; column temperature, 210 C; detector temperature, 300 C; and injector port temperature, 250 C.

**Determination of soil moisture profile.** In order to determine the soil moisture content of the soil mix used in these studies, 400 ml of soil were placed in standard 4" pots and the amount of water loss was determined by the use of a "tension table", an apparatus used to extract water from soil when a known suction (negative pressure) is applied. Four pots of soil mixture were saturated by placing the pots in a waterproof container and slowly adding water until the level reached 2 cm from the rim of the pot. After 24 hr, the pots were weighed and placed on blotter paper on a tension table at 3.5 cm suction head (the average suction head at free drainage). The suction was increased 10 cm every 24 hr thereafter. The pots were weighed at 24 hr intervals to determine the amount of water loss.

When the suction had been lowered to 80 cm, the soil mixture from each pot was removed and placed in beakers in a drying oven at 105 C until a constant weight was reached. The dry weight of the
soil was used to determine the percent saturation and percent water content of the soil mix.

**Effect of soil moisture on efficacy of fungicides applied to soil.**

Using the data obtained from the soil moisture-suction curve, water contents at 30 cm suction and at free drainage were chosen in order to determine the effect of soil moisture on uptake of metalaxyl. *Festuca rubra* was grown in 4" standard pots containing 400 ml of soil. Soil containing three-month-old *F. rubra* plants were saturated as described above. After 24 hr, the set of plants to be held at free drainage was placed over a screen. Clear plastic was placed over the tops of the pots to eliminate water loss due to evaporation. A second set of plants was placed on a tension table at zero suction. The suction head was lowered 10 cm every 12 hr until 30 cm suction was obtained. Plants were held at 30 cm suction for 24-72 hr before treatment.

Plants at each suction were treated with metalaxyl at the following rates and in the following volumes of water: 0.7 and 1.4 mg a.i. per pot delivered in 12, 25 or 50 ml water. Treated plants were placed in plastic trays in a 30 C incubator with >95% relative humidity and 12 hr lighting. Twenty-four hr later, plants were clipped, inoculated, placed in plastic bags and incubated at 30 C for six days. Plants were rated for percent disease development as previously described.
RESULTS

Pathogenicity of Pythium isolates. Pythium aphanidermatum isolates FS, U-3, U-2, P-27 and P-28 were compared for virulence on F. rubra using millet seed inoculum. None of the isolates differed significantly in the degree of virulence at any time period up to 3 days, at which all plants were 100% diseased. Since comparable results were obtained with all P. aphanidermatum isolates, one isolate, FS, was chosen for subsequent experiments.

The pathogenicity of P. aphanidermatum isolate FS was compared using agar plugs or agar plugs imbedded with infested millet seed. The FS isolate resulted in a 100% disease rating on F. rubra after six days, regardless of which method of inoculation was used, although the rate of disease development was slightly higher with inoculum grown on millet seed plus agar than with inoculum grown on agar alone.

Minimum inhibitory concentration in vitro. The minimum fungicide concentrations required to inhibit linear growth of P. aphanidermatum isolate FS after 72 hr are given in Table 1. Fosetyl aluminum did not inhibit the growth of the fungus at any concentration tested. Ethazole reduced growth only slightly compared to controls, but there was no correlation between fungicide concentration and the rate of growth. In addition, ethazole altered the morphology of the culture, resulting
Table 1. Effect of five fungicides on in vitro linear growth of Pythium aphanidermatum.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Concentration (µg a.i./ml)</th>
<th>Radial growth (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Chloroneb 65W</td>
<td>38 a²</td>
<td>-</td>
</tr>
<tr>
<td>Ethazole 30W</td>
<td>38 a</td>
<td>30 b</td>
</tr>
<tr>
<td>Fosetyl aluminum 80W</td>
<td>38 a</td>
<td>38 a</td>
</tr>
<tr>
<td>Metalaxyl 2E</td>
<td>38 a</td>
<td>16 c</td>
</tr>
<tr>
<td>Propamocarb 6S</td>
<td>38 a</td>
<td>30 b</td>
</tr>
</tbody>
</table>

1Average radial growth (mm) after 72 hr on corn meal agar amended with fungicide. At 38 mm, growth had reached the edge of the petri dish.

2Means within columns followed by the same letter are not significantly different at the P=.05 level according to FLSD.
in fan-shaped patches of mycelium. Microscopic examination revealed that the mycelia in these fan-shaped areas were shortened and excessively branched.

Chloroneb, metalaxyl and propamocarb all demonstrated fungitoxicity in vitro. Chloroneb and metalaxyl were the most highly active, completely inhibiting growth at 12 µg a.i./ml.

Evaluation of preventive and curative fungicidal control. When fungicides were sprayed at label rates one hour prior to inoculation with isolate FS, all five fungicides gave significant disease control at the three day rating period when compared to the untreated control (Table 2). Slight disease activity was observed with ethazole and fosetyl aluminum treatments at the three day rating period, but by the six day rating period neither chemical provided acceptable control. Chloroneb, metalaxyl and propamocarb provided excellent control at both rating periods.

Fungicides were applied at various time intervals prior to inoculation (Table 3) to assess their preventive residual efficacy. Metalaxyl provided excellent control when applied up to seven days prior to inoculation. Propamocarb was very effective at the zero and three day intervals, but provided only moderate control after seven days. Disease control with chloroneb was comparable to that with propamocarb and metalaxyl when applied at the time of inoculation, but residual activity lasted less than three days. None of the chemicals controlled disease when applied 21 days prior to inoculation.

When fungicides were applied 24 hr after inoculation, ethazole and
Table 2. Comparison of fungicides applied to foliage of Festuca rubra one hour before inoculation with Pythium aphanidermatum.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Rate g a.i./93 m²</th>
<th>Disease severity₁</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Time after inoculation</td>
<td>3 days</td>
</tr>
<tr>
<td>chloroneb 65 W</td>
<td>74</td>
<td>0 a²</td>
<td>0 a</td>
</tr>
<tr>
<td>Ethazole 30W</td>
<td>60</td>
<td>1 a</td>
<td>10 c</td>
</tr>
<tr>
<td>fosetyl aluminum 80W</td>
<td>91</td>
<td>1 a</td>
<td>8 b</td>
</tr>
<tr>
<td>Metalaxyl 2E</td>
<td>14</td>
<td>0 a</td>
<td>0 a</td>
</tr>
<tr>
<td>Propamocarb 6S</td>
<td>82</td>
<td>0 a</td>
<td>0 a</td>
</tr>
<tr>
<td>Control</td>
<td>--</td>
<td>8 c</td>
<td>10 c</td>
</tr>
</tbody>
</table>

₁Disease rating scale 0-10 with 0= no disease, 1= 1-10% disease, and 10= 91-100% disease.

²Means within columns followed by the same letter are not significantly different at the P=.05 level according to FLSD.
Table 3. Residual activity of fungicides applied to foliage of Festuca rubra at various times prior to inoculation with Pythium aphanidermatum.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Rate g a.i./93 m²</th>
<th>Disease severity*&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Interval between treatment &amp; inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 days</td>
</tr>
<tr>
<td>Chloroneb 65W</td>
<td>74</td>
<td></td>
<td>0 a</td>
</tr>
<tr>
<td>Ethazole 30W</td>
<td>60</td>
<td></td>
<td>10 c</td>
</tr>
<tr>
<td>Fosetyl aluminum 80W</td>
<td>91</td>
<td></td>
<td>8 b</td>
</tr>
<tr>
<td>Metalaxyl 2E</td>
<td>14</td>
<td></td>
<td>0 a</td>
</tr>
<tr>
<td>Propamocarb 6S</td>
<td>82</td>
<td></td>
<td>0 a</td>
</tr>
<tr>
<td>Control</td>
<td>--</td>
<td></td>
<td>10 c</td>
</tr>
</tbody>
</table>

*<sup>1</sup>Disease rating scale 0-10 with 0=no disease, 1=1-10% disease, and 10=91-100% disease. Plants rated six days after inoculation.

Means within columns followed by the same letter are not significantly different at the P=.05 level according to FLSD.
fosetyl aluminum failed to halt the progress of the disease (Table 4). Metalaxyl provided good control, and chloroneb and propamocarb provided moderate control. When compared to untreated control plants, only chloroneb provided significant control when fungicides were applied 48 hr after inoculation. However, the degree of control with chloroneb was not significantly different from that obtained with any other fungicide tested.

**Systemic movement of fungicides.** Injection of fungicides into the soil mixture resulted in significant disease control with metalaxyl and propamocarb. Metalaxyl provided good to excellent control at all three rates applied (Table 5). Propamocarb provided good disease control only at the highest rate tested, 36.8 mg a.i./cup (Table 6). In subsequent experiments, the amount of disease development at each concentration varied, but in most cases less than 20% foliar blighting occurred at rates between 27.2 and 36.8 mg a.i./cup.

Three fungicides, chloroneb, fosetyl aluminum and ethazole, failed to suppress disease after six days at any rate tested when injected into the soil (Tables 7, 8 and 9). Ethazole suppressed disease when compared to control plants at the three day rating period, and only at the highest concentration.

When metalaxyl and propamocarb applied to the soil were tested for disease suppression with two different sources of inoculum, corn meal agar/millet seed inoculum did not result in significantly higher disease ratings than when corn meal agar alone was the source of inoculum. In addition, when metalaxyl applied to the soil was tested against five
Table 4. Efficacy of fungicides applied to foliage of *Festuca rubra* 24 and 48 hr after inoculation with *Pythium aphanidermatum*.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Rate g a.i./93 m²</th>
<th>Disease severity¹</th>
<th>Disease severity¹</th>
<th>Disease severity¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Interval between inoculation &amp; treatment</td>
<td>24 hr</td>
<td>48 hr</td>
</tr>
<tr>
<td>Chloroneb 65W</td>
<td>74</td>
<td>5.7 b²</td>
<td>8.3 a</td>
<td></td>
</tr>
<tr>
<td>Ethazole 30W</td>
<td>60</td>
<td>9.0 c</td>
<td>9.3 ab</td>
<td></td>
</tr>
<tr>
<td>Fosetyl aluminum 80W</td>
<td>91</td>
<td>9.7 c</td>
<td>9.3 ab</td>
<td></td>
</tr>
<tr>
<td>Metalaxyl 2E</td>
<td>14</td>
<td>1.7 a</td>
<td>8.7 ab</td>
<td></td>
</tr>
<tr>
<td>Propamocarb 6S</td>
<td>82</td>
<td>3.3 ab</td>
<td>9.3 ab</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>--</td>
<td>10.0 c</td>
<td>10.0 b</td>
<td></td>
</tr>
</tbody>
</table>

¹Disease rating scale 0-10 with 0=no disease, 1=1-10% disease, and 10=91-100% disease. Plants rated six days after inoculation.

²Means within columns followed by the same letter are not significantly different at the P=.05 level according to FLSD.
Table 5. Effect of metalaxyl applied to soil 24 hr prior to inoculation on suppression of Pythium blight of Festuca rubra.

<table>
<thead>
<tr>
<th>Fungicide conc. (mg a.i./cup)</th>
<th>Disease severity(^1)</th>
<th>Time after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>0</td>
<td>4.0 b(^2)</td>
<td>10.0 c</td>
</tr>
<tr>
<td>0.3</td>
<td>0.3 a</td>
<td>2.0 b</td>
</tr>
<tr>
<td>0.7</td>
<td>0.0 a</td>
<td>0.3 a</td>
</tr>
<tr>
<td>1.4</td>
<td>0.0 a</td>
<td>0.0 a</td>
</tr>
</tbody>
</table>

\(^1\)Disease rating scale 0-10 with 0=no disease, 1=1-10% disease, and 10=91-100% disease.

\(^2\)Means within columns followed by the same letter are not significantly different at the P=.05 level according to FLSD.
Table 6. Effect of propamocarb applied to soil 24 hr prior to inoculation on suppression of Pythium blight of *Festuca rubra*.

<table>
<thead>
<tr>
<th>Fungicide conc. mg a.i./cup</th>
<th>Disease severity&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time after inoculation</td>
</tr>
<tr>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>0</td>
<td>9.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4.6</td>
<td>8.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>9.2</td>
<td>8.3&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>18.4</td>
<td>4.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>36.8</td>
<td>1.3&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Disease rating scale 0-10 with 0=no disease, 1=1-10% disease, and 10=91-100% disease.

<sup>2</sup>Means within columns followed by the same letter are not significantly different at the P=.05 level according to FLSD.
Table 7. Effect of chloroneb applied to soil 24 hr prior to inoculation on suppression of Pythium blight of Festuca rubra.

<table>
<thead>
<tr>
<th>Fungicide conc. mg a.i./cup</th>
<th>Disease severity&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Time after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disease severity</td>
<td>3 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.0</td>
<td>8.7 a&lt;sup&gt;2&lt;/sup&gt;</td>
<td>10.0 a</td>
</tr>
<tr>
<td>4.2</td>
<td>8.7 a</td>
<td>10.0 a</td>
</tr>
<tr>
<td>8.4</td>
<td>9.7 a</td>
<td>10.0 a</td>
</tr>
<tr>
<td>16.7</td>
<td>9.3 a</td>
<td>10.0 a</td>
</tr>
<tr>
<td>33.4</td>
<td>9.7 a</td>
<td>10.0 a</td>
</tr>
</tbody>
</table>

<sup>1</sup>Disease rating scale 0-10 with 0=no disease, 1=1-10% disease, and 10=91-100% disease.

<sup>2</sup>Means within columns followed by the same letter are not significantly different at the P=.05 level according to FLSD.
Table 8. Effect of ethazole applied to soil 24 hr prior to inoculation on suppression of Pythium blight of Festuca rubra.

<table>
<thead>
<tr>
<th>Fungicide conc. mg a.i./cup</th>
<th>Disease severity(^1)</th>
<th>Time after inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>0</td>
<td>7.7 bc(^2)</td>
<td>10.0 a</td>
</tr>
<tr>
<td>3.4</td>
<td>8.0 c</td>
<td>10.0 a</td>
</tr>
<tr>
<td>6.8</td>
<td>8.0 c</td>
<td>10.0 a</td>
</tr>
<tr>
<td>13.6</td>
<td>6.0 b</td>
<td>10.0 a</td>
</tr>
<tr>
<td>27.2</td>
<td>2.3 a</td>
<td>10.0 a</td>
</tr>
</tbody>
</table>

\(^1\)Disease rating scale 1-10 with 0=no disease, 1=1-10% disease, and 10=91-100% disease.

\(^2\)Means within columns followed by the same letter are not significantly different at the P=.05 level according to FLSD.
Table 9. Effect of fosetyl aluminum applied to soil 24 hr prior to inoculation on suppression of Pythium blight of Festuca rubra.

<table>
<thead>
<tr>
<th>Fungicide conc. (mg a.i./cup)</th>
<th>Disease severity (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time after inoculation</td>
</tr>
<tr>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>0</td>
<td>8.7 a</td>
</tr>
<tr>
<td>5.1</td>
<td>9.3 a</td>
</tr>
<tr>
<td>10.2</td>
<td>8.7 a</td>
</tr>
<tr>
<td>20.4</td>
<td>8.0 a</td>
</tr>
<tr>
<td>40.8</td>
<td>8.7 a</td>
</tr>
</tbody>
</table>

\(^1\) Disease rating scale 0-10 with 0=no disease, 1=1-10% disease, and 10=91-100% disease.

\(^2\) Means within columns followed the same letter are not significantly different at the P=.05 level according to FLSD.
isolates of *P. aphanidermatum*, there were no significant differences in disease development among the isolates for each concentration tested (Table 10). When the foliage of *F. rubra* plants was immersed in solutions of fosetyl aluminum, metalaxyl and propamocarb at concentrations of 6 g a.i./L, 1.2 g a.i./L and 3.6 g a.i./L, and 7.2 g a.i./L respectively, no disease suppression was obtained on the untreated portions of the leaves, indicating no evidence of basipetal movement.

**Residual activity of fungicides applied to soil.** When pots of *F. rubra* were inoculated at various intervals following application of fungicides to the soil, metalaxyl and propamocarb controlled disease at intervals up to 21 days between application and inoculation (Tables 11 and 12). Fosetyl aluminum failed to suppress disease at any concentration or any pre-inoculation interval tested.

Metalaxyl provided good to excellent disease control at 0.7 and 1.4 mg active ingredient per cup. An analysis of variance indicated that for each rate, there were no significant differences in the level of suppression of disease at any pre-inoculation interval up to 21 days.

Good disease suppression was obtained with propamocarb only at the highest concentration of 18.4 mg active ingredient per cup. At this rate, propamocarb failed to control disease at the 1 day pre-inoculation interval. There were no significant differences in efficacy at the highest concentration of fungicides at pre-inoculation intervals between 3-21 days, according to a one-way analysis of
Table 10. Systemic activity of metalaxyl applied to soil 24 hr prior to inoculation with five isolates of *Pythium aphanidermatum*.

<table>
<thead>
<tr>
<th>Fungicide conc. mg a.i./cup</th>
<th>Isolate</th>
<th>Disease severity&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FS</td>
<td>10.0 a&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>0</td>
<td>P-27</td>
<td>10.0 a</td>
</tr>
<tr>
<td></td>
<td>P-28</td>
<td>10.0 a</td>
</tr>
<tr>
<td></td>
<td>U-2</td>
<td>10.0 a</td>
</tr>
<tr>
<td></td>
<td>U-3</td>
<td>10.0 a</td>
</tr>
<tr>
<td>0.3</td>
<td>FS</td>
<td>2.0 a</td>
</tr>
<tr>
<td></td>
<td>P-27</td>
<td>1.0 a</td>
</tr>
<tr>
<td></td>
<td>P-28</td>
<td>6.0 a</td>
</tr>
<tr>
<td></td>
<td>U-2</td>
<td>6.0 a</td>
</tr>
<tr>
<td></td>
<td>U-3</td>
<td>0.0 a</td>
</tr>
<tr>
<td>0.7</td>
<td>FS</td>
<td>0.3 a</td>
</tr>
<tr>
<td></td>
<td>P-27</td>
<td>0.3 a</td>
</tr>
<tr>
<td></td>
<td>P-28</td>
<td>1.3 a</td>
</tr>
<tr>
<td></td>
<td>U-2</td>
<td>3.0 a</td>
</tr>
<tr>
<td></td>
<td>U-3</td>
<td>0.3 a</td>
</tr>
<tr>
<td>1.4</td>
<td>FS</td>
<td>0.0 a</td>
</tr>
<tr>
<td></td>
<td>P-27</td>
<td>0.0 a</td>
</tr>
<tr>
<td></td>
<td>P-28</td>
<td>0.0 a</td>
</tr>
<tr>
<td></td>
<td>U-2</td>
<td>1.0 a</td>
</tr>
<tr>
<td></td>
<td>U-3</td>
<td>0.0 a</td>
</tr>
</tbody>
</table>

<sup>1</sup>Disease rating scale 0-10 with 0=no disease, 1=1-10% disease, and 10=91-100% disease. Plants rated six days after inoculation.

<sup>2</sup>For each rate, means followed by the same letter are not significantly different at the P=.05 level according to FLSD.
Table 11. Residual activity of fungicides applied at various times prior to inoculation of Festuca rubra with Pythium aphanidermatum.

<table>
<thead>
<tr>
<th>Fungicide conc. (mg a.i./cup)</th>
<th>Disease severity (^1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Interval between treatment &amp; inoculation</td>
</tr>
<tr>
<td></td>
<td>1 day</td>
</tr>
<tr>
<td>0</td>
<td>10.0 c(^2)</td>
</tr>
<tr>
<td>0.3</td>
<td>2.0 b</td>
</tr>
<tr>
<td>0.7</td>
<td>1.0 ab</td>
</tr>
<tr>
<td>1.4</td>
<td>0.0 a</td>
</tr>
</tbody>
</table>

\(^1\)Disease rating scale 0-10 with 0=no disease, 1=1-10% disease, and 10=91-100% disease. Plants rated six days after inoculation.

\(^2\)Means within columns followed by the same letter are not significantly different at the P=.05 level according to FLSD.
Table 12. Residual activity of propamocarb applied to soil at various times prior to inoculation of Festuca rubra with Pythium aphanidermatum.

<table>
<thead>
<tr>
<th>Fungicide conc. mg a.i./cup</th>
<th>Disease severity(^1)</th>
<th>Interval between treatment &amp; inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 day</td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>10.0 a(^2)</td>
</tr>
<tr>
<td>4.6</td>
<td></td>
<td>10.0 a</td>
</tr>
<tr>
<td>9.2</td>
<td></td>
<td>9.7 a</td>
</tr>
<tr>
<td>18.4</td>
<td></td>
<td>9.0 a</td>
</tr>
</tbody>
</table>

\(^1\)Disease rating scale 0-10 with 0=no disease, 1=1-10% disease, and 10=91-100% disease. Plants rated six days after inoculation.

\(^2\)Means within columns followed by the same letter are not significantly different at the P=.05 level according to FLSD.
Vapor activity of fungicides. When fungicides were tested for their volatile fungitoxic properties, only minimal disease suppression was observed (Table 13). In vivo studies indicated that three days after inoculation ethazole, metalaxyl and propamocarb all showed slight disease suppression compared with untreated pots, but control was usually less than twenty percent, and was significant only at the highest concentrations of ethazole and metalaxyl (27.2 mg and 1.4 mg, respectively). Propamocarb provided significant disease control at concentrations of 4.6 and 18.4 mg a.i. but not at 9.2 and 36.8 mg a.i. In subsequent experiments disease suppression did not correlate well with fungicide concentration. By six days after inoculation, all grass plants were 100% diseased, regardless of treatment.

In vitro experiments to determine vapor activity did not demonstrate any inhibition of linear growth of P. aphanidermatum on agar amended with ethazole, metalaxyl or propamocarb.

Effect of temperature on efficacy of fungicides applied to soil. Plants treated with metalaxyl and propamocarb were compared at two different temperatures (20 and 30 C) prior to inoculation. No difference was observed in the efficacy of metalaxyl applied to soil as a result of exposure to ambient temperatures of 20 and 30 C prior to inoculation (Table 15). Propamocarb at the highest concentration tested (36.8 mg a.i.) was significantly more effective in controlling disease at 30 C than at 20 C (Table 16). At lower rates there were no significant differences.
Table 13. Vapor activity of fungicides applied to soil 24 hr prior to inoculation of Festuca rubra with Pythium aphanidermatum.

<table>
<thead>
<tr>
<th>Fungicide</th>
<th>Concentration mg a.i./cup</th>
<th>Disease severity¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethazole</td>
<td>---</td>
<td>10.0 b²</td>
</tr>
<tr>
<td></td>
<td>3.4</td>
<td>9.3 ab</td>
</tr>
<tr>
<td></td>
<td>6.8</td>
<td>9.3 ab</td>
</tr>
<tr>
<td></td>
<td>13.6</td>
<td>9.3 ab</td>
</tr>
<tr>
<td></td>
<td>27.2</td>
<td>8.3 a</td>
</tr>
<tr>
<td>Metalaxyl</td>
<td>---</td>
<td>10.0 b</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>9.0 ab</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>9.7 ab</td>
</tr>
<tr>
<td></td>
<td>1.4</td>
<td>8.7 a</td>
</tr>
<tr>
<td>Propamocarb</td>
<td>---</td>
<td>10.0 b</td>
</tr>
<tr>
<td></td>
<td>4.6</td>
<td>8.3 a</td>
</tr>
<tr>
<td></td>
<td>9.2</td>
<td>9.0 ab</td>
</tr>
<tr>
<td></td>
<td>18.4</td>
<td>8.0 a</td>
</tr>
<tr>
<td></td>
<td>36.8</td>
<td>9.7 ab</td>
</tr>
</tbody>
</table>

¹Disease rating scale 0-10 with 0=no disease, 1=1-10% disease, and 10=91-100% disease. Plants rated three days after inoculation.

²Means within columns followed by the same letter are not significantly different at the P=.05 level according to FLSD.
Table 14. Effect of ambient temperature on efficacy of metalaxyl applied to soil 72 hr prior to inoculation of *Festuca rubra* with *Pythium aphanidermatum*.

<table>
<thead>
<tr>
<th>Fungicide conc. (mg a.i./cup)</th>
<th>Disease severity⋅</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Temperature prior to inoculation</td>
</tr>
<tr>
<td>0</td>
<td>10.0</td>
</tr>
<tr>
<td>0.3</td>
<td>3.3</td>
</tr>
<tr>
<td>0.7</td>
<td>0.0</td>
</tr>
<tr>
<td>1.4</td>
<td>0.0</td>
</tr>
</tbody>
</table>

*⋅Disease rating scale 0-10 with 0=no disease, 1=1-10% disease, and 10=91-100% disease. Plants rated six days after inoculation.*
<table>
<thead>
<tr>
<th>Fungicide conc. mg a.i./cup</th>
<th>Disease severity$^1$</th>
<th>Temperature prior to inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20 C</td>
</tr>
<tr>
<td>0</td>
<td>10.0$^2$</td>
<td>10.0</td>
</tr>
<tr>
<td>4.6</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>9.2</td>
<td>10.0</td>
<td>10.0</td>
</tr>
<tr>
<td>18.4</td>
<td>10.0</td>
<td>8.7</td>
</tr>
<tr>
<td>36.8</td>
<td>4.7</td>
<td>1.0$^*$</td>
</tr>
</tbody>
</table>

$^1$Disease rating scale 0-10 with 0=no disease, 1=1-10% disease, and 10=91-100% disease. Plants rated six days after inoculation.

$^2$For each rate, pairs of numbers followed by an asterisk are significantly different at the P=.05 level according to a Student's t test.
Table 16. Effect of soil moisture on efficacy of metalaxyl applied to soil 24 hr prior to inoculation of Festuca rubra with Pythium aphanidermatum.

<table>
<thead>
<tr>
<th>Fungicide conc. mg a.i./cup</th>
<th>Vol. of water applied to soil (ml)</th>
<th>Disease severity&lt;sup&gt;1&lt;/sup&gt; cm suction</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>50</td>
<td>3.0&lt;sup&gt;2&lt;/sup&gt; 3.0</td>
</tr>
<tr>
<td>0.7</td>
<td>50</td>
<td>3.0 1.5</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>2.8 2.0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>2.5 2.8</td>
</tr>
<tr>
<td>1.4</td>
<td>50</td>
<td>2.0 1.3</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>1.3 1.0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>1.8 1.3</td>
</tr>
</tbody>
</table>

<sup>1</sup>Disease rating scale 1-3, where 1=less than 50% area diseased, 2=more than 50% area diseased, and 3=100% area diseased.

<sup>2</sup>FLSD (0.05) = .52
Gas chromatographic analysis of leaf tissue. Gas chromatographic analysis of leaf tissue revealed the presence of metalaxyl in the tissue. A comparison of treated leaf tissue from plants treated with 1.4 mg a.i. metalaxyl with tissue fortified with 500 ug a.i./ml metalaxyl indicated that metalaxyl is present in leaf tissue in an apparently unaltered form (Figures 1-3). Because of the interference of peaks formed by other plant constituents, quantification of metalaxyl in the leaf tissue was not accomplished in this study.

Effect of soil moisture on efficacy of fungicides applied to soil.
The soil moisture-suction curve is given in Figure 4. When F. rubra plants were held on the tension table at a high soil moisture content and cool temperatures, they developed an infection which interfered with disease ratings for Pythium blight. As a result, the data for this experiment was rated on a 3 point scale, as given in Table 17.

The level of soil moisture and the concentration of metalaxyl both significantly affected the development of Pythium blight when P=0.05. Higher concentrations of fungicide and lower soil moisture levels resulted in lower disease ratings. The volume of water used to deliver the fungicide did not significantly affect results, but the interaction of soil moisture and the volume of delivery water was significant at the P=0.05 level.
Fig. 1. Gas chromatogram of metalaxyl standard at 500 µg/ml.
Fig. 2. Gas chromatogram of 10 g leaf tissue sample fortified with 500 μg/ml metalaxyl.
Fig. 3. Gas chromatogram of 15 g leaf tissue sample treated with 1.4 mg a.i. metalaxyl applied via soil injection.
Fig. 4. Soil moisture-suction curve of potting medium (soil:peat:vermiculite, 1:1:1).
DISCUSSION

Metalaxyl, propamocarb, and to a lesser degree, chloroneb, controlled Pythium blight on F. rubra under the conditions of these studies. Ethazole and fosetyl aluminum were not effective.

The results in vivo correlate with data from the in vitro fungitoxicity tests. Chloroneb and metalaxyl were equally effective at inhibiting linear growth of P. aphanidermatum in vitro. Propamocarb was less active. Fosetyl aluminum, on the other hand, was not fungitoxic. These results confirm earlier reports. Although fosetyl aluminum displays in vitro activity against Phytophthora spp. (24), it often lacks in vitro activity against Pythium spp. (24). Sanders et. al. (58) found no in vitro toxicity when the chemical was tested against eight species of Pythium, including P. aphanidermatum. Concentrations of the fungicide as high as 100 μg a.i./ml had no effect on the linear extension of P. aphanidermatum.

An unexpected result of the minimum inhibitory concentration studies was the failure of ethazole to provide adequate inhibition of mycelial growth. At a concentration as low as 6 μg a.i./ml, the fungicide reduced growth by 22%, but increasing the amount of fungicide did not result in any further inhibition. In addition, the colony morphology was altered, resulting in patches of fan-shaped mycelia
instead of the typical smooth pattern of radial growth. Previous studies have established the in vitro activity of ethazole to Pythiaceous fungi (29, 33, 79). Wheeler (79) found that concentrations of 10 µg a.i./ml prevented the mycelial growth of \textit{P. aphanidermatum}. In addition, 1-5 µg a.i./ml prevented zoospore formation in water. However, Rahimian et al. (49) could not suppress zoospore formation of \textit{P. aphanidermatum} with concentrations of ethazole as high as 100 µg a.i./ml. In addition, their work confirmed the observation reported in this study, i.e., that ethazole had no effect on mycelial growth of \textit{P. aphanidermatum} at concentrations of 100 µg a.i./ml.

An explanation for the failure of ethazole to completely inhibit linear growth of the \textit{P. aphanidermatum} isolate FS is provided by Halos (29). He found that \textit{P. sylvaticum}, \textit{P. vexans}, \textit{P. ultimum} and \textit{P. debaryanum} became tolerant to fungistatic activity of ethazole after a time proportional to the initial concentration. Mycelial growth was delayed up to 24 hr after the fungi were first exposed to the chemical, but thereafter growth proceeded at rates comparable to that of controls. Halos attributed this phenomenon to the ability of \textit{Pythium} spp. to bypass the mode of action of ethazole by means of a secondary pathway in the fungus.

Chloroneb was a highly effective fungicide for control of \textit{Pythium} blight when applied preventively. Other studies report differing results with chloroneb for control of \textit{Pythium} blight on turfgrass. Couch (15) found that three applications of chloroneb over a period of 21 days failed to provide any disease control. However, Littrell
(40) obtained satisfactory control of *P. aphanidermatum* on potted ryegrass using foliar sprays. At label rates, chloroneb demonstrated effective disease control even if applied 24 hr after inoculation. These results agree with similar findings by Wells (78) when comparable rates were used.

The principal limitation of chloroneb as a fungicide for control of Pythium blight on turfgrass appears to be its unusually short residual activity. In these studies, inoculations only three days after foliar application failed to provide disease control. Although the short residual activity might render chloroneb ineffective for control of Pythium blight under prolonged, severe disease pressure, the chemical may still be a tool for disease control under certain circumstances (8). The use of a forecast system to predict disease outbreak would allow application of the fungicide only under conditions favorable for disease. Using such a system, chloroneb, alternated or in combination with a long-term residual fungicide such as metalaxyl or propamocarb, may provide disease control while at the same time reducing the chance that resistance may develop to the more specific modes of action of the systemic fungicides. In addition, these studies suggest that chloroneb may be as effective as metalaxyl or propamocarb when applied after an infection period.

Applied on a preventive basis, ethazole and fosetyl aluminum suppressed the rate of disease development when compared with untreated controls, but the degree of disease control achieved was poor. The high degree of efficacy afforded by metalaxyl and propamocarb as
preventive sprays for the control of Pythium blight substantiates work by other researchers. Couch (15) found that one application of metalaxyl at 153 mg/m² and two applications of propamocarb at 612 mg/m² prevented symptoms of Pythium blight on bentgrass for 21 days in the field. In performance trials on perennial ryegrass conducted over several years, Sanders and co-workers (8, 27, 57, 59, 60) also observed excellent disease control with both fungicides under heavy inoculum pressure.

When applied as foliar sprays, the ability of both metalaxyl and propamocarb to control disease was significantly reduced after 14 days. These results may reflect the severity of disease pressure, and the high susceptibility of the host. Longer residual efficacy has been observed in field tests with both metalaxyl (8) and propamocarb (8, 27, 57, 59, 60). Unpublished work by P. L. Sanders, at Penn. St. Univ., and P. O. Larsen, at the Ohio St. Univ., indicate that propamocarb is an excellent preventive fungicide for the control of Pythium blight (Upjohn, internal report). Using agar plugs of \textit{P. aphanidermatum} growing on corn meal agar as the source of inoculum, Larsen found that rates of 41 and 82 g a.i./93 m² provided excellent disease control on fine fescue when inoculated 14 days after treatment. Sanders estimated the residual efficacy to be higher, with excellent control obtained 21 days after treatment, based on field trials conducted on perennial ryegrass. In another study, Sanders et. al. (56) demonstrated the effectiveness of metalaxyl against Pythium blight when applied either to foliage or soil and showed that residual efficacy had not yet begun to break down.
after three to four weeks.

Very little information exists on the ability of metalaxyl and propamocarb to act as "eradicants", i.e., control or eradicate established infections. Since Pythium blight develops so rapidly, experiments using pots of turfgrass may only accurately measure such "kickback" action up to the first 24 hr after inoculation. After that time, up to 70% of the grass may show visible symptoms. Chloroneb, metalaxyl and propamocarb all demonstrated the ability to stop the progress of the disease up to 24 hr after inoculation. Eradicant action up to 48 hr was possible, depending upon the progress of the disease at the time of application. The results of chloroneb and propamocarb generally agree with unpublished data obtained by Larsen (Upjohn, internal report) indicating both fungicides are capable of halting the progress of an infection. In Larsen's experiments, however, chloroneb was effective only the the three day rating period, whereas in these studies, the disease control achieved with chloroneb for preventive and "eradicant" activity was constant throughout the duration of the experiment. It was not determined whether the chemicals eliminated incipient infections, or merely acted as protectants on uninfected foliage, although the development of some disease after application of the chemicals suggests it may have been the latter.

The ability of a fungicide to act as an eradicant may or may not be associated with its translocation inside the leaf tissue. In the case of Pythium blight, discreet lesions are not formed. In the majority of cases where eradicant activity has been studied, the
fungicide has been able to prevent the sporulation of the fungus at some point after the infection process has begun, usually within a well-defined lesion. The systemic fungicides which effectively control Pythium blight may prevent the further colonization of the fungus without affecting established infections.

These studies failed to demonstrate effective control of Pythium blight when fosetyl aluminum was applied either by injecting it into the soil or as a foliar spray. Fosetyl aluminum may or may not inhibit a particular species in vitro, but many studies with the chemical show it to be a highly effective fungicide against Pythiaceous fungi (18, 37, 58). In a recent paper, however, Sanders et. al. (58) found results similar to those reported here. Foliar sprays of fosetyl aluminum on potted perennial ryegrass provided significant but commercially unacceptable disease control at 114 g/93 m². Doubling the concentration resulted in good disease suppression with a residual activity less than 14 days. In field trials, however, the same concentrations gave better control for longer periods of time.

The inability of fosetyl aluminum to provide adequate control in potted grass may indicate the need for the chemical to undergo chemical transformation prior to being active. Recent studies have found that phosphorous acid is a breakdown product of fosetyl aluminum in plants (73). Phosphorous acid exhibits greater inhibition in vitro than fosetyl aluminum when fungi are grown on solid media containing low amounts of phosphorous (24). *Pythium* spp. were inhibited by phosphorous acid to a lesser degree than *Phytophthora* spp. when both were grown on
corn meal agar (24).

The differences in disease control achieved when fosetyl aluminum was applied to pot-grown grass and field trials are not easily explained. The grass used in these tests is seedling grass which is more susceptible to infection by Pythium blight than mature turfgrass. In addition, the soil mixtures used in these studies are high in organic matter (33%). Sanders (58) experiments on potted turfgrass were conducted in soil mixtures high in vermiculite. Both vermiculite and organic matter have high cation exchange capacities, which may result in high adsorption of the fungicide to the soil mixture, thus reducing the amount of chemical available for uptake by plant roots. For this to significantly affect the performance of fosetyl aluminum in these studies, however, the fungicide would have to be taken up by plant roots and act systemically. Other researchers have found that fosetyl aluminum may be taken up by plants (18, 37). The experiments in this dissertation, however, did not indicate systemic activity as evidenced by disease suppression when applied to the soil prior to inoculation. The only disease suppression occurred when fosetyl aluminum was applied foliarly prior to inoculation. The treated plants were immediately placed in plastic bags to incubate. Therefore, the fungicide was not in contact with the roots during the experiment. Either the fungicide was acting directly to inhibit P. aphanidermatum, or it was being translocated via foliar absorption. The fungicide could then be biodegraded to phosphorous acid or could elicit an anti-fungal response in the F. rubra plants.
Although Davis (18) has suggested basipetal movement of fosetyl aluminum based on in vivo control of a root pathogen as a result of application of the fungicide to bark, this is not direct evidence of phloem transport. Since fosetyl aluminum may act indirectly by eliciting a phytoalexin response in the host, it is possible that fosetyl aluminum itself is not transported to the roots, but rather, that a metabolite produced by the plant in response to the fungicide is transported by the plant in response to the fungicide is transported in the xylem or phloem to the site of infection.

Chloroneb applied to the soil in concentrations as high as 33.4 mg a.i./cup did not appear to have systemic activity in _F. rubra_. Littrell (40) controlled Pythium blight on ryegrass with soil drenches of approximately 4.1 mg a.i./pot using a 48 hr exposure time prior to inoculation. An earlier paper by Wells (78) failed to observe any evidence of systemic movement in ryegrass using a 24 hr exposure period. The results reported here for fine fescue support Wells' data. Systemic uptake could not be demonstrated using a 24 hr exposure time prior to inoculation. Although a longer exposure period was not tested, soil applications as high as ten times the amount of active ingredient used in Littrells' and Wells' studies, failed to indicate any evidence of uptake.

The evidence of systemic movement of metalaxyl reported in this study supports a considerable body of evidence that the chemical is highly systemic in a wide range of crops against species of both _Pythium_ and _Phytophthora_ (6, 7, 9, 11, 14, 18, 25, 34, 35, 50, 52, 56, 69, 72,
At concentrations as low as 0.7 mg a.i./cup, metalaxyl was as effective when applied to soil as when applied to the foliage. In an earlier report, Sanders et. al. (50) found that metalaxyl was translocated in perennial ryegrass grown in pot culture. The concentrations used in Sanders' research were similar to those reported here, indicating that the performance of metalaxyl was not altered, regardless of the species of turfgrass tested. The high degree of systemic movement achieved in these studies and elsewhere (11, 14, 52, 56) does suggest that a greater efficacy might actually occur from soil application of the chemical than from foliar sprays. If longer residual is achieved from soil application, then a second consideration must be whether soil applications may reduce or increase the frequency of resistance to the chemical (52). Although resistance of Phytophthora spp., especially Phytophthora infestans (19), has been known to occur for some time, the resistance of Pythium spp. under field conditions is a recent development. Sanders has observed the development of resistant strains of P. aphanidermatum after exposure to metalaxyl on turf research plots for several years (Sanders, unpublished).

Ethazole failed to provide disease control of Pythium blight on red fescue when applied via soil injection. Evidence shows, however, that the compound is systemic in crops other than turfgrass (45, 46). The slight reduction in disease severity at rates as high as 27.2 mg a.i./cup suggest that ethazole may be systemically translocated in small quantities when applied to the soil.

The evidence for systemic activity is not conclusive, however.
Other studies have indicated that ethazole is not highly mobile in the soil (30). It has also been shown to be a highly effective fumigant (35). It is possible that the disease activity attributed to systemic movement at the higher rates of application may in fact be due to vapor activity. Ioannou and Grogan (35) studied the fumigant activity of ethazole against Phytophthora crown and root rot of tomato. Growth inhibition through fumigant action occurred at concentrations of 100 μg a.i./ml. Metalaxyl also demonstrated vapor activity against Phytophthora, but was not as effective as ethazole. It is significant to note that ethazole was extremely effective at inhibiting growth of Phytophthora in vitro.

In the test of vapor activity, none of the chemicals was highly effective in controlling disease via fumigant action alone. At high rates, metalaxyl, propamocarb and ethazole all reduced disease significantly when compared with plants exposed to water-drenched pots, but the degree of control achieved was no more than a 20% reduction over plants exposed to untreated pots. Also, the response did not necessarily increase with increasing concentrations of fungicides. These results suggest that the degree of control demonstrated by metalaxyl and propamocarb could not be achieved solely via fumigant activity. With ethazole, however, the suppression of disease development at the highest rates used in soil application experiments was not enough to exclude the possibility that vapor activity may have accounted for the disease control observed. The degree of control after soil injection varied. Whereas the results presented represent the
most average, rates of 27.2 mg a.i./cup sometimes reduced disease significantly even at the six day rating. These results suggest that low levels of systemic movement may occur, although perhaps not in sufficient quantities to contribute to disease control under field conditions.

Propamocarb effectively reduced Pythium blight when applied to roots at rates between 18.4 and 36.8 mg a.i./cup. Although the degree of disease suppression always increased with increasing concentrations of fungicide, the actual results at a particular rate varied greatly during repetitions of the experiment. Grass plants were used between 4-8 weeks of age, and usually 6-8 week old plants were tested. By eight weeks of age, however, the grass was becoming pot-bound, and plants which varied even slightly in age and condition appeared to affect the degree of uptake of propamocarb. At best, propamocarb applied via soil injection gave good control at 18.4 mg, and excellent disease control at 36.8 mg. At worst, the highest tested concentration did not control disease. Complete disease control was rarely obtained via soil application. At the highest rates, disease symptoms developed within the first 24-48 hr after inoculation, and the progress of the disease was often halted after that time. These results suggest that 24 hr if not sufficient time for adequate uptake of propamocarb.

Results comparing inoculation at 24 and 72 hr after treatment indicate that after 72 hr disease control is more effective. Data supporting this observation comes from Cohen (13), who found that a three day pre-inoculation interval gave better control than one or two days when propamocarb was applied as a soil drench for control of downy mildew.
on cucumber. The concentrations required for disease control on cucumber were similar to those required for control of Pythium blight in these studies using a similar soil mixture. Thus, it appears that at longer pre-inoculation intervals, propamocarb may be effective at lower concentrations when applied to soil.

By comparison, metalaxyl appears to be very rapidly taken up by plants. Optimal disease control was achieved within 24-48 hr after treatment. In addition, very little variability occurred between replications of experiments, indicating that uptake is not as affected by the condition of the plant roots as with propamocarb. Application at rates of 0.7 mg a.i./cup were usually sufficient to provide less than 10% disease development over the test period and often completely inhibited symptom development. By contrast with propamocarb, if significant symptoms developed within the first 24 hr after inoculation, disease often progressed beyond acceptable levels in subsequent days.

Even at half the standard rate, however, good disease control was often obtained. The amount of metalaxyl required to control Pythium blight is considerably less than the amount of propamocarb required. Similar results were found by Reilly (50) when metalaxyl and propamocarb were applied via transplant water to control black shank of tobacco.

Edgington (20) suggested that the differences in control which Reilly observed between metalaxyl and propamocarb are due to differences in toxicity of the two chemicals and not systemicity. The results in these studies suggest otherwise. Other researchers have found metalaxyl is translocated in quantities sufficient to provide significant
disease control of late blight of tomato in one hour (14). Such
effectiveness implies that the mode of uptake for metalaxyl may be
different from that of most other systemic fungicides. Translocation
of metalaxyl did not appear to be affected by the overall health of the
plant roots, as evidenced by the high degree of control achieved from
plants with roots which were visibly unhealthy at the time of
application. These observations suggest that metalaxyl may be
actively taken up by plants. Propamocarb, by contrast, may be
passively taken up by plants with the degree of uptake dependent upon
the total area of root surface.

There was no evidence to support the possibility of basipetal
movement of fosetyl aluminum, metalaxyl or propamocarb. Immersion of
the foliage in high concentrations of these fungicides did not result
in any disease suppression on the untreated portions of the plants.
In the previous literature describing translocation of metalaxyl,
a limited amount of basipetal movement has been shown using both
bioassays and physical evidence. Zaki (82) used autoradiography to
show that a slight amount of metalaxyl was translocated to the roots
of avocado after foliar application. A higher amount of absorption
appeared to occur in the stems, compared with leaves. Carris (11)
applying metalaxyl to strawberry plants, found that translocation
from the parent plant via stolons to the roots of sucker plants
occurred if transpiration was unencumbered in the recipient plant.
Overall, however, the amount of metalaxyl translocated via phloem
tissue would not appear to be sufficient to suggest that disease control
could be achieved in this manner.

Researchers have demonstrated that factors which affect transpiration affect uptake of systemic fungicides. Carris (11) found that wrapping strawberry leaves with material which reduced transpiration, reduced translocation of metalaxyl to that leaf. When water was withheld from the donor plant or leaves removed from either plant, the pattern of translocation was altered.

Temperature is a factor which also affects transpiration, and therefore could affect the uptake of a fungicide. Cohen (14) tested uptake of metalaxyl at two different temperature regimes in tomato and found that increases in temperature from 15 to 20 to 25 C increased the amount of metalaxyl translocated as evidenced by disease control of Phytophthora infestans.

The data presented in this study show that varying the temperature from 20 to 30 C increased the degree of disease control observed with propamocarb but not with metalaxyl. At the highest concentration, propamocarb significantly reduced the level of disease development when subject to a 72 hr pre-inoculation period of 30 C compared with 20 C. Contrary to the results observed by Cohen (14), however, temperature had no effect on the degree of disease control achieved with metalaxyl. In fact, lack of variability between disease ratings for the two temperatures was noteworthy. The level of disease control observed post-inoculation was well within the average for other experiments, but the pre-inoculation period was three times as long. The long pre-inoculation interval may have obscured differences in
uptake in the first 24 hrs after treatment.

Propamocarb was initially chosen to study the effect of soil moisture and volume of delivery water on disease control. Several factors, however, rendered the fungicide unsuitable for these studies. Firstly, the concentrations of chemical used were four to eight times greater than the highest label rate recommended for control of Pythium blight on turfgrass, making it impractical for soil application under actual field use. Secondly, other researchers had found that propamocarb is highly adsorbed to organic matter in the soil and extremely resistant to movement by soil water (John Bowers, personal communication). And thirdly, the regime required to bring pots of turfgrass to a constant soil moisture using the tension table required soil saturation followed by several days at moisture levels near field capacity. Fine fescue is low-oxygen intolerant and suffered root damage as a result. Initial tests with propamocarb failed to provide any disease control at any concentration, presumably because root damage severely limited uptake.

There is little literature on the effects of soil moisture on the uptake of fungicides. Evidence suggests that moisture may have an effect. The degree of effect may depend on the volume of water with which fungicides are applied to soil. Cohen found that increasing the amount of water with which fungicides were applied increased the amount of fungicide translocated with both metalaxyl (14) and propamocarb (13).

The experiments on the effect of soil moisture on the uptake of metalaxyl were complicated by the fact that during the incubation period, the grass developed a secondary, low-level infection which
interfered with ratings for Pythium blight. Enough disease control was achieved, however, to show that the efficacy of metalaxyl for control of Pythium blight was affected by the moisture level of the soil mixture. When the fungicide was applied at maximum soil water retention with free drainage ("field capacity"), there was a trend toward less effective disease control than when the same concentrations were applied to pots at a lower soil moisture level. These results would suggest that at or near field capacity, some fungicide leaches from the pot at the time of application. Conversely, one might also hypothesize that at a lower soil moisture, lack of distribution of the fungicide within the soil becomes a problem. At higher rates, enough metalaxyl appears to be available to the plant to achieve good disease control despite these factors.

The problems encountered in this experiment might be solved in one of two ways. Either another variety of turfgrass should be used which can better withstand the pre-inoculation regime, or the use of the tension table should be bypassed, and the soil moisture levels determined on a weight basis extrapolated from the soil moisture content profile.

A standard recommendation for systemic fungicides is that they should be watered-in after foliar sprays. The assumption is that better uptake and translocation will occur from this treatment. For such a procedure to be effective, several factors must be considered. If the fungicides are applied on a preventive basis, then a significant amount of fungicide presumably is present at all times. If, however,
the fungicides are applied on a curative spray program, based on a forecast system, then application of water after the fungicide is sprayed onto foliage requires that either enough fungicide is left to act as a protectant for uninfected foliage, or that uptake of the chemical occurs in high concentrations in a short period of time to provide control before the disease can become established. With Pythium blight, this is a matter of hours. Thus, metalaxyl is the only fungicide which would appear to be effective applied in this manner. Experiments in these studies and elsewhere (52, 56) indicate that metalaxyl is as effective as a systemic when applied to roots as when it is sprayed onto foliage. It is highly adsorbed to soil organic matter and is not readily leached (11, 62). The organic matter then serves as a reservoir. In two out of three experiments for residual activity, metalaxyl injected into the soil provided longer residual activity against Pythium blight than when the same rates were sprayed onto foliage.

Longer residual is often cited as one of the advantages of systemic fungicides over non-systemics (21, 23, 75). While it is true that systemic fungicides often exhibit longer residual, there is very little research to determine whether a foliarly applied fungicide is actually redistributed within the plant in concentrations sufficient to provide disease control.

To answer such questions, quantitative determination of fungicide concentrations within leaf tissue is required. Although some quantitative bioassays exist (38, 80), previous researchers have
usually relied on radiographic analysis (11, 82) or complicated extraction procedures for chromatography (69, 70). The method of Waliszewski and Szymczynski (77) was easily adapted to use with turfgrass. The gas chromatograms demonstrated physical evidence for the presence of metalaxyl in leaf tissue. Interfering peaks by indigenous plant constituents did not allow quantification of the active ingredient in the test samples. Further work with variables such as oven temperature and gas flow rates, however, may allow the separation of metalaxyl from the interfering peaks. In addition, the use of an electron capture detector to replace the flame ionization detector would render the technique a thousand times more sensitive. This method shows promise for yielding a quick, simple, inexpensive technique for the determination of metalaxyl in plant tissues.

The data presented in this study indicate that metalaxyl and propamocarb are very effective fungicides for the control of Pythium blight on F. rubra. The results demonstrate that they are efficacious for both preventive and curative control. Moreover, control obtained from soil applications suggest that longer residual activity may be achieved under actual field use as a result of systemic uptake of the fungicides. Chloroneb was found to be an effective preventive fungicide, but the lack of residual efficacy may limit its use. Ethazole was not effective in these studies, presumably due to the high inoculum pressure used to evaluate disease control. The results obtained with fosetyl aluminum do not support direct evidence of acropetal or basipetal systemic
uptake. Further research is necessary to elucidate the mode of action of this fungicide before conclusions on its systemic nature can be reached.
LITERATURE CITED


APPENDIX A. Tension table apparatus.

- Pots of grass
- Metal frame
- Blotter paper & glass plate
- Metal screen
- Plastic tube
- H₂O
- 30 cm