FUNGICIDAL CONTROL AND RELATED STUDIES ON BLACK ROOT ROT OF APPLE (*MALUS PUMILA* MILL.) CAUSED BY *XYLARIA MALI* FROMME

by

David A. Smith

Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Plant Pathology

APPROVED:

K. D. Hickey, Chairman

R. J. Stipes

H. B. Couch

May, 1973

Blacksburg, Virginia

ACKNOWLEDGMENTS

The author expresses sincere thanks to Dr. K. D. Hickey and Dr. R. J. Stipes for their guidance and assistance in the developement of the research program reported in this thesis and for their helpful criticisms of this manuscript. The comments and suggestions of the faculty and staff of the Department of Plant Pathology and Physiology were also helpful in this study.

The author wishes to thank for her technical assistance in the laboratory. The help received from the Winchester field station personnel in the greenhouse experiments is also appreciated.

The author is indebted to his parents who provided encouragement and financial assistance during his college career. Thanks are due to of The Pennsylvania State University whose course in plant pathology determined the author's choice of graduate study.

This manuscript is dedicated to the author's wife, , whose patience, understanding, encouragement and unselfish sacrifice in typing this thesis will always be appreciated.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
TABLE OF CONTENTS	iii
LIST OF TABLES	iv
LIST OF FIGURES	vi
INTRODUCTION	1
LITERATURE REVIEW	4
MATERIALS AND METHODS	22
RESULTS	49
DISCUSSION	87
LITERATURE CITED	93
VITA	97

LIST OF TABLES

Table		Page
1.	Mycelial dry weight of *Xylaria mali* obtained in liquid medium containing various concentrations of benomyl, thiabendazole, captan and triarimol fungicides	51
2.	Growth response of *Xylaria mali* to agar incorporations of benomyl and thiabendazole	53
3.	Growth response of *Xylaria mali* to agar incorporations of captan and triarimol	55
4.	Growth responses of *Xylaria mali* to thiabendazole impregnated bioassay discs	56
5.	Growth responses of *Xylaria mali* to benomyl impregnated bioassay discs	58
6.	Growth responses of *Xylaria mali* to triarimol impregnated bioassay discs	59
7.	Growth responses of *Xylaria mali* to captan impregnated bioassay discs	60
8.	The effects of the various fungicide treatments on the root system of container grown apple (*Malus pumila*) seedlings inoculated with *Xylaria mali* in greenhouse studies	62
9.	Inhibition of *Penicillium expansum* by leaf extracts from apple seedlings treated with benomyl at 300 and 1,000 ppm drenches	72
10.	Inhibition of *Penicillium expansum* by leaf extracts from apple seedlings treated with benomyl at 300 and 1,000 ppm incorporations	73

Table		Page
11.	Inhibition of *Penicillium expansum* by leaf extracts from apple seedlings treated with thiabendazole at 300 and 1,000 ppm drenches	74
12.	Inhibition of *Penicillium expansum* by leaf extracts from apple seedlings treated with thiabendazole at 300 and 1,000 ppm incorporations	76
13.	Inhibition of *Saccharomyces pastorianus* by leaf extracts from apple seedlings treated with captan at 1,000 and 4,000 ppm drenches	77
14.	Amount of benomyl recovered from apple leaf tissue 47 weeks after benomyl drench or incorporated treatments	84
15.	Amount of thiabendazole recovered from apple leaf tissue 47 weeks after thiabendazole drench or incorporated treatments	86

LIST OF FIGURES

Figure		Page
1.	Healthy root system of apple (Malus pumila) seedlings on which no Xylaria mali lesions were found. The dense root system was considered to be excellent and was rated '0'	32
2.	Healthy root system of apple (Malus pumila) seedlings on which no Xylaria mali lesions were found. The density of the root system was considered only fair and was rated '1'	34
3.	Healthy root system of apple (Malus pumila) seedlings on which no Xylaria mali lesions were found. The density of the root system was considered only fair and was rated '2'	36
4.	Diseased root system of apple (Malus pumila) seedlings rated '3'. Xylaria mali lesions occurred in a limited area on one or two of the larger roots	38
5.	Diseased root system of apple (Malus pumila) seedlings rated '4'. Xylaria mali lesions occurred on most of the major roots with vascular discoloration extending a short distance up the infected roots toward the crown	40
6.	Diseased root system of apple (Malus pumila) seedlings rated '5'. Extensive Xylaria mali lesions occurred on the roots with vascular discoloration extending through the crown area into the lower stem	42
7.	Composite of the six catagories of the root rating standards of apple (Malus pumila) seedlings inoculated with Xylaria mali infested water sprouts	44

Figure Page

8. Phytotoxicity of triarimol (EL-273) 300 ppm drench treatment to container grown apple (Malus pumila) seedlings in greenhouse studies 66

9. Phytotoxicity of triarimol (EL-273) 1,000 ppm drench treatment to container grown apple (Malus pumila) seedlings in greenhouse studies 68

10. Closeup of triarimol (EL-273) 300 ppm drench treatment showing the proliferation of knurled, club-shaped adventitious roots 70

11. Standard fungicide inhibition curve for benomyl. Penicillium expansum was the bioassay organism 80

12. Standard fungicide inhibition curve for thiabendazole. Penicillium expansum was the bioassay organism 82

INTRODUCTION

The black root rot disease of cultivated apple, Malus pumila Mill. (19) is caused by the fungus, Xylaria mali Fromme. This root-destroying soil-borne pathogen was first identified and described by F. D. Fromme in 1928 (14), and apple is the only suscept of economic importance readily susceptible to it. The only wild hosts observed to be naturally infected are wild dewberry, Rubus spp. and sassafras, Sassafras albidum (Nutt.) Nees (14, 19). Infection in sassafras is limited to the cortical tissue, but the fungus is found throughout the other root tissues of other hosts.

Black root rot is principally a disease of older fruit-bearing apple trees, and seldom causes extensive losses in younger, rapidly growing specimens (19). Maturity and fruit bearing may be considered as factors associated with infection along with factors that weaken or damage the tree such as mouse and drought injury. The survival of replants made in locations from which diseased trees have been removed is too low to justify replanting.

Roots of declining trees must be carefully observed, since there are no symptoms on the aerial parts of the apple tree which are alone diagnostic for black root rot (14). Characteristically, the growth of the tree is noticeably decreased for a period of a year or commonly more, up to a decade before death. The foliage becomes thinner, and leaves are smaller and off-color. Single branches may die or exhibit pronounced symptoms of disease in advance of others, and the trunk may

have a decided lean. Excessive blooming and fruiting usually accompanies the decline.

The presence of stromata of X. mali attached to the main root and trunk is an unfailing evidence of black root rot in addition to black incrustations on the roots and brittle, punky wood throughout the invaded roots (14). Because of the extreme brittleness of the wood, even large trees in late stages of disease development may be easily uprooted as a stub.

A large number of own-rooted varieties, scion-rootstock combinations and various Malus species have been tested for possible resistance to black root rot both by artificial inoculations and replanting in known infested sites. No indications of resistance to infection were regarded as established (19).

Localized soil treatments were employed by Groves (18, 19) in an attempt to destroy the fungus within a limited area so as to increase replant survival. The treatment of small areas appeared feasible (19), since the spread of the fungus through the soil is very slow, if it occurs as all independently of the root system of a susceptible host. Unfortunately, no treatments to date have been demonstrably successful, although the record of some treatments has been good over a period of years, notably carbon disulfide and chloropicrin injections and Uramon (carbamide) incorporations (18, 19).

The purpose of this research was to evaluate chemical treatments

that could possibly lead to increased replant survival, either by eradication of the fungus in the infested site or by prophylaxis and therapy with soil incorporation or drench treatments. The chemicals used were the systemic fungicide, benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazole carbamate) (4, 24, 25, 45, 17, 5, 46, 31, 36, 28, 33) and thiabendazole (2-(4-thiazolyl)-benzimidazole) (45, 17, 26), triarimol (a-(2, 4-dichlorophenyl)-a phenyl-5-pyrimidine methanol) (11), and captan (N-trichloromethylthiotetrahydrophthalimide). Uptake and translocation of these fungicides were monitored by bioassay techniques (34, 9, 10, 29, 30, 35, 38). Disease control was evaluated by examining artificially-inoculated, treated greenhouse seedlings. Methyl bromide treatments were also applied to known infested sites in a commercial orchard before replanting. Harvest of these trees to examine the root system will be made in three to five years unless symptoms occur prior to that time.

LITERATURE REVIEW

The first published record of the black root rot disease of apple appeared in 1913 by Reed and Crabill (40) in "Plant Diseases in Virginia in the Years 1911 and 1912". They suggested that the disease may be caused by one or more mushrooms. A further note (41) in their observation which described the symptoms of the disease was published in 1915 and claimed that it was the least controllable of all apple diseases at that time and one of the most destructive to Virginia growers.

Crabill (8) reported in 1916 that he consistently isolated Trichoderma koenigni Oudemans from the woody tissue of infected roots. T. koenigni was therefore labeled as the causal organism of the black root rot disease although proof of pathogenicity was not accomplished. Crabill also mentioned that some roots yielded an unidentified non-sporulating fungus.

Fulton and Cromwell (16) in 1916 expanded the area of known occurrence of this disease to Pennsylvania and North Carolina. Their work reported that the fungus first produced a white mycelium which later became a black incrustation on diseased roots. Characteristic rotting could be produced by inoculating wounds on apple roots with pure cultures of the isolate. No spore production was observed under either artificial or natural conditions and the

organism remained unidentified.

Fromme and Thomas (12) in 1917 (Science) reported the destructive rotting of the roots of apple trees prevalent in the chief orchard growing sections of Virginia. The infectiousness of this condition was shown in the death of adjoining trees in groups of considerable numbers irrespective of soil conditions or topography. Isolation from these diseased roots yielded cultures of an imperfect fungus which Fromme and Thomas believed to be the conidial stage of a species of Xylaria. Perithecial stromata of X. polymorpha (Pers.) Grev. had also been found on roots of apple trees in various stages of typical root rot attack and on stumps of several deciduous trees in a small patch of woodland immediately adjoining the diseased orchard in question. Fromme and Thomas therefore felt reasonably certain that a species of Xylaria was responsible for the root rot disease of apple in Virginia. They also stated that possibly more than one species of Xylaria was involved since certain constant cultural distinctions existed between some of the isolations. Their observations indicated that the disease may be spread by cultivation, or in the removal of tree borers, or by contact between healthy and diseased roots, and by surface washing of spores.

In 1917 Wolf and Cromwell (47) published data of their investigations on the black root rot disease of apple collected over the previous three years at the North Carolina Experiment Station. Although no careful survey to determine the exact distribution of this

disease had been made, they believed this root rot was generally present in the orchards in the western part of the state, based on the widely separated places of collection and the ease in finding trees characteristically affected in any of the sections devoted to apple growing.

Observations in the field indicated that soil type, drainage, exposure, elevation, age, and variety of trees had no bearing upon the presence of the disease, since root rot was found under the widest variations of these factors. Diseased trees occurred on previously cultivated lands and on land planted soon after the timber had been removed. Trees in well-maintained orchards appeared to be as subject to this disease as those in neglected orchards.

Contrary to Fromme and Thomas, not more than four trees in a group had been found to be diseased and most often such diseased trees occurred singly. Wolf and Cromwell suggested that the dissemination of the pathogen was probably accomplished by cultivation, rodents, and surface washing of soil.

A detailed description of the development of this disease in apple roots was given by Wolf and Cromwell. The affected roots became covered with a thin compact growth of mycelium which was snowy white at first but after a few days the superificial portion developed into a black incrustation or stroma. Minute, black, thread-like rhizomophs radiated from the margin of the stroma and extended for several inches along the surface of the root. These rhizomorphs

anastomosed and formed a network in such intimate relationship with the cortex that it was impossible to separate even small portions. These strands became obscured in the stroma except in its most recent growth. The disintegration of the cortex beneath the stroma proceeded rapidly and followed closely upon its advance. The bark became fissured, and when it had dried, could be crumbled readily. Affected roots soon became girdled, and the distal portions died. New roots sometimes developed above the diseased parts, enabling diseased trees to live for a term of years.

The effects of root rot upon the woody tissues were much less profound than upon the cortex. Histological sections made by Wolf and Cromwell showed that the fungal hyphae appeared to traverse all of the xylem tissues equally well and in all directions. Perforation of the cell wall appeared to be accomplished at any point and no evidence was noted that the pores served as the places of passage. No considerable delignification occurred as indicated by treating normal and diseased wood with phloraglucin and hydrochloric acid. Starch, however, was shown to disappear rapidly from invaded tissues as indicated by its absence when sections were treated with iodine.

Confirmation of the digestion of starch by this fungus was secured by growth on starch agar. A halo extending beyond the margin of the colony resulted on this medium, thus presenting a visible demonstration of the excretion of amylase. Other media including casein, fibrin and aspargine produced halos which indicated certain

proteolytic enzymes (erepsin, protease and amidase) were also secreted by X. mali.

Isolations from diseased roots from several sources made at various times by Wolf and Cromwell constantly yielded an organism with a very characteristic mycelial growth. Conidial fructifications appeared in certain of them and the conidia were confirmed to be those of the causal organism. Typical Xylaria stromatic arms formed in these cultures. Conidia of X. mali, similar to those of other species of Xylaria, were formed either on these arms or on elevations arising from the incrustation on the surface of the culture medium. Perithecia never appeared in any of the cultures on a variety of media placed under several environmental regimes by Wolf and Cromwell.

Perithecial stromata, however, had been found upon the roots of trees which had succumbed to root rot. All attempts to germinate ascospores failed, making it impossible to determine whether this perithecial form was genetically connected with the conidial stage which appeared in culture. The perithecial material was morphologically similar to Xylaria hypoxylon, (L.) Grev., a species which embraces a wide variety of forms.

Pathogenicity of the pure cultures was demonstrated by Wolf and Cromwell in inoculations of both wounded and uninjured roots with inoculum of mycelia grown on steamed rice. Infections were uniformly successful regardless of whether or not the tissues had been injured. Resulting lesions varied from only localized infections to those

that extended a foot or more beyond the point of inoculation in a period of six weeks.

In a later publication in 1917, Fromme and Thomas (13) noted finding numerous stromata of X. hypoxylon (L.) Grev. at the base of a dead apple tree. X. hypoxylon had also been isolated in pure culture from specimens obtained from nine different locations. Several pure cultures of X. polymorpha and a Xylaria species were also obtained. X. hypoxylon and other species of Xylaria proved to be equally vigorous wound parasites in inoculation studies while X. polymorpha appeared to be only slightly or occasionally parasitic.

Fromme (14) in 1928 found in abundance the stromata of the causal fungus of the black root rot disease of apple. The relationship of these stromata to the disease was firmly established. The causal organism was not X. hypoxylon as had been previously suggested by Fromme and Thomas in 1917 but X. digitata (L. ex Fr.) Grev. as the species was described and understood by Ellis in his mycology text, North American Pyrenomycetes as cited by Fromme.

Fromme's further examination of this species in collections at the Lloyd Herbarium, the United States Department of Agriculture and in the personal collection of Dr. C. L. Shear indicated that the American species of Xylaria described by Ellis and Fromme was not the same species as X. digitata found in the European collections. The major features demonstrating differences between the American Xylaria species and X. digitata were the greatly-branched stromata,

the compressed, blunt tips of the stromatal arms, the dark gray-pigmented area in the stroma in which the perithecia were deeply embedded and ascospore size.

Since the name X. digitata was properly applied to the species of Europe, Fromme renamed the American species X. mali and completely described this species. The causal organism of Xylaria root rot or black root rot of apple therefore became known as X. mali Fromme and was positively identified in Arkansas, Illinois, Indiana, Kentucky, Tennessee, West Virginia, Virginia and North and South Caroline. Fromme also expected the occurrence of X. mali in the Cumberland Valley of Pennsylvania and Maryland but associated X. polymorpha with the Xylaria root rot disease in New York and Pennsylvania which was reported by Thomas in 1926 as cited by Fromme.

The only unfailing symptoms diagnostic for this disease were the presence of stromata, the characteristic black incrustation on the roots, and the brittle, punky character of the wood throughout the invaded root. No other known disease of apple roots affected the wood in such a manner. Even large trees in the late stages of disease could be pushed over with little effort. The affected roots broke off close to the main axis which was uprooted as a stub that had perhaps been supported by only one or two living roots.

The incidence of apple tree death from black root rot and other causes was determined in special surveys in 1926 for a limited number of orchards in Augusta and Frederick counties in Virginia.

R. H. Hurt made the survey in Augusta county and F. J. Schneiderhan made the survey in Frederick county, both in collaboration with Fromme (14). Thirty eight orchards totalling over 20,000 original trees were surveyed for trees that had died and been removed, of replanted trees, of dead and standing trees and of diseased trees, with the most obvious cause of death recorded. The orchards which averaged twenty one years of age were classified into two categories; the non-root rot orchard in which root rot was absent or of only minor importance, and root rot orchards in which root rot was of major importance. Eighteen orchards were classified in the non-root rot group and twenty orchards in the root rot group. The average percentage of dead or diseased trees in the non-root rot orchards was 9.04 percent while that in the root rot orchards was 18.28 percent. The nine percent average difference between non-root rot and root rot orchards was considered to be the percentage of trees killed by the disease. In individual instances, the loss in root rot orchards was much greater; in these orchards the importance of the disease was most apparent. In two of the twenty root rot orchards the tree loss exceeded forty percent; it exceeded thirty percent in one and twenty percent in five. The fact that few trees set as replants, following trees dead of root rot, survive until bearing age was also attested to the destructive nature of this disease.

Studies of the susceptibility of various rootstock-scion

combinations and of commercial seedlings demonstrated that any resistance to root rot existed as a property of individuals rather than of varieties or combinations. Scion roots of Northern Spy had shown considerable resistance to infection in inoculation tests, but when set as replants, appreciable percentages died of root rot. York Imperial appeared to be the most susceptible under comparable conditions.

Other hosts for the pathogen, which when inoculated gave vigorous development of infection, were Norway maple (Acer platanoides L.) and Mahaleb cherry (Prunus mahaleb. L). Less vigorous infection resulted in elm (Ulmus americana L.), honey locust (Gleditsia tricanthos L.) and pear (Pyrus communis L. and P. ussuriensis maximowicz).

Andes (1) in 1935 stated that only X. mali Fromme had been found in connection with black root rot diseased roots in Tennessee. Preliminary tests conducted by him suggested that the disease could be arrested by sterilizing the immediate area of diseased trees. A treatment with a combination of steam and formaldehyde appeared to be the most economical method tried for this purpose.

Schneiderhan (42) in 1937 reported that a wide range of Malus species and apple rootstocks tested had not demonstrated superior resistance to the root rot disease. Some resistance appeared in several clones but was insufficient to prevent infection. The long term viability of the organism was determined to be at least 16 years

in the larger roots left undisturbed in the soil after the removal of the diseased tree.

Fromme and Schneiderhan (15) in 1938 reported further resistance studies with various seedling varieties which were planted in known diseased sites. Areas 10 X 10 feet around each diseased site were spaded and raked to remove the old roots. Several trees set in the prepared areas were examined after three years and showed infection rates from 46 to 97 percent. Infection in only one side or one area of the prepared site was common which demonstrated that only one side or section of the original tree roots had been infected. Again, no evidence of resistance was found among the 44 clones tested.

Their work also verified the reliability of the technique of inoculating wounded roots with cultures of X. mali. Ninety five percent infection within 171 days was obtained by this method on two-year-old seedlings.

Cooley (7) in 1942 expanded the known area of occurrence of black root rot to Georgia. He found the disease to be concentrated in the fruit growing regions of West Virginia and Virginia and the states to the south and also in the Ozark region. His explanation for this range was the high optimum temperature (25°C) for growth of this soil-borne pathogen. Whether this temperature favored the pathogen or adversely affected the host resistance was not clarified.

Variations in pathogenicity of the 27 isolates he tested were

observed. Cooley also felt the resistance of one and two year old seedlings was greater than when three or more years of age. No clones tested were highly resistant although some were less susceptible than others.

His observations further concluded that Xylaria dies soon after the death of the roots if disturbed. Trichoderma koenigni Oudemans was also isolated from roots previously killed by X. mali. T. koenigni caused a collapse of the mycelium of X. mali upon physical contact. Cooley reported no known occurrence of X. mali on either wild or cultivated plants anywhere outside of the United States through 1942.

Groves (18) in 1944 began soil treatments to improve the survival rate of replants in sites of previously diseased trees. He stated that throughout the Cumberland-Shenandoah fruit growing region the survival of replants made in locations from which diseased trees have been removed was too low to justify replanting. After seven years, carbon disulfide treatments appeared to be the most promising with other promising preliminary results from urea and chloropicrin treatments.

In 1951 Groves (19) offered a compendium of information secured to date on black root rot of apple. He confirmed the short-lived nature of the fungus if the diseased roots were disturbed. When such roots were dug and reburied or wrapped and stored recovery seldom occurred. Similar results were experienced when artificially-

inoculated material was buried. Many more isolations of T. koenigni Oudemans and other organisms were obtained in such instances which suggested that the environmental and biological circumstances which permitted X. mali to persist were disrupted when the roots were dug from the soil and that these circumstances could not be re-established by re-burial.

X. mali was, he concluded, a soil-inhabiting and not a soil-invading fungus. It was clearly able to exist saprophytically for prolonged periods. Although of insignificant importance under natural circumstances, it could build up rather rapidly under favorable circumstances provided by the planting of a susceptible host. Tree loss was seldom void of such predisposing factors as mouse or drought injury, though it was firmly established that infection and killing could occur without the aid of such predisposing factors.

During the duration of Groves' investigations, over five hundred X. mali isolates were obtained and were carried in culture for several years on Leonian's malt extract, or general purpose agar, modified through a reduction to half strength of the organic nutritive elements. While it was possible to classify many of the isolates into groups, no correlation between type and virulence was apparent. All types were pathogenic. The pathenogenicity of isolates, carried five to six years in culture, was not lost and virulence did not diminish. The cultures were all pathogenic and equally as virulent as isolates obtained only a few weeks previously.

The search for resistance to artificial inoculation was expanded. Approximately 900 trees, principally own-rooted variety material with numerous rootstock and scion combinations were tested. No indications of resistance to infection were regarded as established.

A number of soil treatments were tested to improve replant survival. The materials were either placed into the hole when planting or worked into or injected into the soil in the area centered around the site. Materials used were 6:6:100 Bordeaux mixture (drenched), formaldehyde (worked in and later uncovered to aerate), copper phosphate, sodium thiocyanate, soil sulfur, wettable sulfur, 26 copper fungicide, borax, Cyanamid, paradichlorobenzene crystals, and Uramon (a synthetic urea) as incorporated treatments, and carbon disulfide, chloropicrin and D-D mixture as injected treatments.

The information obtained neither established the effectiveness of soil treatments upon apple replant survival nor even the validity of the approach. A few methods appeared promising particularly the carbon disulfide and Uramon treatments.

The systemic nature of benomyl (1-(butycarbomyl)-2-benzimidazole carbamate) in elm (Ulmus americana L.) when the fungicide was drenched or injected into the soil has been reported in various publications by several investigators over recent years (4, 24, 25, 45). Stipes (45) also reported the systemic activity of thiabendazole, (2-(4-thiazolyl)benzimidazole) in elm seedlings when treated with a fungicide drench.

Gilpatrick (17) demonstrated the systemic activity of benomyl and thiabendazole on powdery mildew (Podosphaera leucotricha (Ell. and Ev.) Salm.) of apple and powdery mildew (Podosphaera oxyacanthae (D. C.) d By.) of cherry when applied as soil drenches. Benomyl was much more effective than thiabendazole.

Cimanowski et. al. (5) found benomyl, when applied as a soil drench, to be an effective systemic fungicide for control of powdery mildew on apple leaves. Its effectiveness was equal to that of Karathane (2-(1-methylheptyl)-4, 6-dinitrophenyl crotonate) applied as a spray. Benomyl was also an effective systemic fungicide for controlling cherry leaf spot (Coccomyces hiemalis Higgins) when applied as a drench. Leaf surface area was also increased 168.9 percent over that of the control.

Stover (46) reported the translocation of benomyl from the soil trhough the roots up to the leaf blades in banana (Musa sapientum (L.) Kuntze). Plants two feet tall which had been grown for five days in pots containing benomyl-treated soils were inoculated with ascospores of the banana leaf spot pathogen (Mycosphaerella musicola Leach = Cercospora musae Zimm.). The results showed a fifty percent decrease in germ tube length and a fifty-two percent decrease in germination on foliage from plants grown in benomyl amended soils. Also the germ tubes were curved, swollen, and stunted on these plants indicating benomyl was readily transported from the soil through the roots up to the leaves.

Jaynes and Anagnostakis (31) reported a significant limitation of growth of cankers caused by the chestnut blight fungus (Endothia parasitica (Murr.) P. J. and H. W. Anderson) on the American chestnut, Castanca dentata (March.) Borkh., when benomyl was applied as a soil drench. Growth of the fungus in the trees treated with 350 and 750 pounds per acre of 50% W. P. benomyl was 55% and 46%, respectively, of that in untreated trees.

Palmer and Semeniuk (36) reported benomyl applied as a drench to cuttings of Rosa dilecta Rehd. 'HT Pink Radiance' retarded symptom developement of rose blackspot (Diplocarpon rosae Wolf = Marssonia rosae (Lib) Lind), for a period of about forty days before losing effectiveness. The number of acervuli was markedly reduced, but their conidia were still infectious.

Horst and Hoitink (28) reported 100 percent control of Cylindrocladium blights on azalea (Rhododendron species) caused by C. scoparium (F-68) Boedyn and Reitsma and C. floridanum Sobers and Seymour with soil drench treatments of benomyl. This fungicide controlled the disease even when the drench was applied as late as five days after soil inoculations.

Hoitink and Schmitthenner (26) found that a 5% benomyl or 10% thiabendazole-amendmendment of indolebutric acid (IBA) used as a dip treatment on rhododendron (Rhododendron catawbiense Michx.) cuttings, significantly increased rooting over the commonly-used thiram-IBA product. Benomyl and thiabendazole controlled losses caused by

Pestalotia species, Cylindrocladium species, Botryosphaeria ribis Gross & Dvg., Fusarium roseum (Cke.) Snyder and Hansen, and Rhizoctonia species and also shortened the propagation period.

Manning et al. (32) reported benomyl soil drenches to be effective in reducing poinsettia (Euphorbia heterophylla L.) root rot caused by Thielaviopsis basicola (Berk & Br.) Ferraris in newly-potted rooted cuttings and established plants with root rot symptoms. The drenches also resulted in reductions in the number of colonies of T. basicola isolated from soil and roots.

Several bioassay procedures have been developed to determine the uptake and translocation of the systemic benzimidazole fungicides. The most commonly used technique was the leaf disc method which consisted of cutting discs from the leaf tissue and placing them on a suitable medium before or after overspraying the plate with a spore solution of a test fungus sensitive to the fungicide present (34, 9, 10, 29, 30, 3, 35). Peterson and Edgington (38) modified the techniques by freezing the leaf tissue prior to plating in order to rupture cell walls and release more fungitoxicant. The most commonly used test fungus was Penicillium expansum Link ex Thom (9, 29, 30, 3).

Hine, Johnson, and Wenger (23) utilized P. expansum Link ex Thom as the test fungus for both benomyl and thiabendazole in a bioassay to determine the persistence of these fungicides in soil. The persistence of benomyl was determined to be at least twenty-two weeks

in greenhouse studies. Thiabendazole and benomyl could be detected in field applications for twelve weeks. After eighteen weeks only benomyl at its highest concentration (50 ppm) was detectable by bioassay. Erwin (11) also detected a fungitoxic substance in soil collected in the field at least six months after treatment with benomyl.

Netzer and Dishon (35) reported that during the period of vigorous growth of muskmelon (Cucumis melo L.) benomyl, when applied to the soil, reached the apical leaves in relatively small quantities. Toward the end of the season a steep rise in its content in the apical leaves was noted.

Peterson and Edgington (39) reported the translocation of benomyl from the soil through the roots up to the leaves. Studies showed that the fungicide was transported primarily to the foliage. A tendency for benomyl to accumulate at the leaf tips and margins was reported by Biehn and Dimond (2, 3) in tomato (Lycopersicon esculetum Mill.) and Peterson and Edgington (38, 39) in bean (Phaseolus vulgaris L.). Biehn and Dimond reported that the fungitoxicant was detected in only very small amounts in the central leaf blade area (3). Peterson and Edgington (38) described the translocation of benomyl as passive uptake by the roots, movement via cell walls to the xylem, movement through the xylem to the leaves and then movement to regions of evaporation via the cell walls of the leaf resulting in accumulation at the tip and margin.

Several researchers, when bioassaying plants grown in a medium

containing benomyl, detected another fungitoxic compound (37, 6, 44, 38, 39), methyl-2-benzimidazolecarbamate (MCB) which can be produced from benomyl by the hydrolysis of the butylcarbamoyl group. MCB itself was systemic and fungitoxic and was relatively stable in plant tissues. Clemons and Sisler (6) stated that since benomyl breaks down rapidly in aqueous solution to MCB the antifungal activity at sites removed from the point of application should possibly be attributed to MCB or a MCB derivative and not to benomyl.

The translocation pattern of thiabendazole was demonstrated by Erwin (11) in an experiment utilizing ^{14}C labeled thiabendazole. When ^{14}C thiabendazole was applied to roots, the concentration in the plant decreased gradually with increasing height. The greatest concentration of ^{14}C labeled thiabendazole occurred in the xylem of the roots and in the lower stems, with only slight evidence of ^{14}C labeled thiabendazole in upper leaves and then only in the veins.

Triarimol (EL-273 or a-(2, 4-dichlorophenyl)-a phenyl-5-pyrimidine methanol), a highly effective fungicide, was released for testing in 1969 by the Eli Lilly Co., Elanco Products Division. The compound is reported to have local systemic activity in several host plants as cited by Erwin (11). Its effectiveness as a foliar spray against apple scab fungus (Venturia inaequalis (Cke.) Wint.) and powdery mildew of apple caused by Podosphaera leucotricha (E. & E.) Salm. at very low rates has been reported in numerous independent tests in The Fungicide and Nematicide Tests, Results of 1969 (20), 1970 (21) and 1971 (22) respectively.

MATERIALS AND METHODS

1.0 In vitro fungitoxicity studies.

Three different in vitro studies were used to evaluate fungitoxicity of benomyl, thiabendazole, captan and triarimol to X. mali. The first two procedures consisted of the "poisoned food" technique of Horsfall (27) in both liquid culture and agar incorporation. The third technique was the modified bioassay disc procedure described by Sharvelle (43) to determine fungitoxicity to non-sporulating fungi.

1.1 Incorporation of fungicide into liquid medium.

The fungitoxicity of benomyl, thiabendazole, captan, and triarimol fungicides to X. mali was determined in a liquid culture medium. The medium contained 10.0 gm soluble starch, 1.0 gm $MgSO_4$, 1.0 gm KH_2PO_4, and 3.0 gm yeast extract and was prepared by first steaming in the autoclave to dissolve all amendments. Two hundred milligrams of chloroamphenicol, dissolved in ethanol, was added to the medium still hot from the autoclave to inhibit any bacterial contaminants. When the medium cooled to room temperature, 49 ml aliquots were pipetted into 250 ml erlenmeyer flasks (checks received 50 ml aliquots). The flasks were then sterilized at 120°C for 15 minutes.

Fungicide solutions in concentrations containing the proper

amount of fungicide in 1.0 ml of acetone were then added bringing the total volume in each flask to 50 ml and producing the following fungicide concentrations which were replicated five times; 0 ppm (check with acetone), 0.5 ppm, 1.0 ppm, 2.0 ppm, 3.0 ppm, 5.0 ppm, 10 ppm, 20 ppm, 50 ppm and 100 ppm. The 50 and 100 ppm concentrations were deleted in the benomyl and thiabendazole studies because these fungicides precipitated out at these concentrations when added to the liquid medium.

An agar plug taken with a 5 mm cork borer from the mycelial mat of a pure culture of X. mali was placed in each flask. All plugs were taken near the margins of newly transferred and rapidly growing colonies. The amendments of the agar medium on which the cultures grew were identical to those in the liquid culture except for the addition of 20 gm agar per liter. The seeded flasks were then allowed to stand at room temperature for 14 days before mycelial dry weight was determined.

Filter paper discs were dried in an oven at 110°C for 24 hours. After drying the discs were placed in a desiccator and cooled to room temperature. Each disc was tare weighed to 1/1,000th of a gram on a Mettler balance. A drying agent ($CaCl_2$) was kept in the pan area of the balance to reduce the rate of absorption of water vapor into the disc from the air.

The cultures were harvested after 14 days by the following procedure. Each flask was emptied onto a tared filter paper disc in

a Buchner funnel. The liquid was drawn through the filter by aspirator vacuum. The vacuum was alternately released and the mycelial mat was washed several times with distilled water. The agar plug could then be lifted from the mycelial mat.

The filter paper discs with the mycelium were then oven dried at 110°C for 36 hours. The discs were again placed in a desiccator to cool and were then weighed. The dry weights were used to indicate the growth response of the hyphae to the presence of the fungicide.

1.2 Incorporation of fungicide into agar medium.

The fungitoxicity of the four fungicides, benomyl, thiabendazole, triarimol, and captan to X. mali was determined by their incorporation at various concentrations into an agar culture medium. The medium used was a modified GYEA (2.0 gm agar, 0.5 gm dextrose, 0.2 gm yeast extract) with 20 mg chloroamphenicol (dissolved in ethanol) added per 100 ml in a 250 ml erylenmeyer flask. After autoclaving for 15 minutes at 120°C the flasks were placed in a water bath and allowed to stabilize at 53°C. The desired amounts of fungicide (dissolved in acetone) were added individually to each flask prior to pouring plates. The concentrations varied with each fungicide based on their demonstrated toxicity in the liquid culture experiment. The concentrations used for benomyl and thiabendazole were 0, 0.5, 1.0, 2.0, 20, 40, 70, and 100 ppm. For captan the concentrations used were 0, 30, 40, 50, 60, 80, 100 and 150 ppm. Triarimol was used at 0,

40, 50, 60, 70, 100, 150 and 200 ppm. After the fungicide was thoroughly mixed with the medium, five glass petri dishes 9 cm in diameter were poured for each fungicidal concentration. A check solution with acetone was also compared in growth to the standard check.

All plates were seeded with an agar plug of X. mali mycelium inverted on the center of each plate. The diameter of the growth of the mycelium was measured in centimeters daily initially and later over several day intervals for the higher concentrations of fungicides until the fungal hyphae reached the margins of the plate.

1.3 Impregnation of fungicide onto bioassay discs.

Sharvelle's (43) modified paper-disc bioassay was used to determine the minimum inhibition concentration (m. i. c.) for each fungicide on X. mali. This fungicide bioassay is especially designed for use with fungi that do not sporulate readily in culture. It is especially suitable for comparing the effectiveness of fungicides without subjecting them to hydrolysis when placed in water or tied up (adsorbed) in agar medium.

A modified GYEA medium (20 gm agar, 5 gm dextrose, and 2 gm yeast extract) with 200 mg per liter of chloroamphenicol (dissolved in ethanol before adding) was used as the culture medium. After autoclaving for 15 minutes at 120°C the medium was allowed to cool to 50°C before pouring plates.

Bioassay discs (13 mm in diameter) were impregnated with a

solution containing the desired amount in micrograms of fungicide dissolved in acetone. The concentration of the fungicide solutions were designed so that 200 µl contained the desired amount of fungicide for each concentration. The 200 µl of fungicide solutions were pippetted onto the individual paper discs external to the culture plates.

A mycelial suspension of X. mali was made by grinding three cultures in a Waring blender with sterile distilled water. One drop of this suspension from a 2 ml calibrated pippette was carefully placed on the center of each treated disc before being placed in the culture plate. The fungicide impregnated and mycelial seeded discs were then asceptically transferred to the agar medium. Seeded discs containing no fungicide were used in this experiment and served as checks.

The cultures were incubated at 25°C for five days before growth responses were recorded. The experiment was continued through 15 days to determine if the fungus would ultimately grow on the fungicide-impregnated discs which displayed fungistasis.

2.0 In vivo fungitoxicity studies in greenhouse.

The effectiveness of the four fungicides was evaluated on container-grown apple seedlings inoculated with X. mali in greenhouse studies. Two methods of application of benomyl and thiabendazole were used to detect any differences resulting from application

techniques. Upon the harvest of the first trees from this experiment a rating procedure was developed to evaluate the root systems of the test trees.

2.1 Greenhouse-fungicide treatments.

A number of fungicide treatments were evaluated for control of black root rot on inoculated apple trees in the greenhouse. Treatments were evaluated by (1) incorporating the fungicide into the potting medium and (2) fungicide drenches applied after planting. The fungicides evaluated and the method of application were as follows: incorporated, benomyl and thiabendazole 300 and 1,000 ppm; drench captan 1,000 and 4,000 ppm, triarimol, thiabendazole and benomyl, all at 300 and 1,000 ppm.

Trees were grown in forty-six ounce (404 X 700) juice cans which were perforated at the base and filled with a soil mixture composed of 1:1:1 ratio of peat, sand, and top soil. The soil mixture was sterilized before use with methyl bromide (MC-2) at the rate of three pounds per cubic yard. The soil mixture from six cans was oven dried and the average weight determined. All fungicide rates expressed as ppm were based on this average amount of oven-dry soil mixture per can.

The specific amount of fungicide required for each concentration was weighed individually for each can. In the soil-incorporated test the fungicides were thoroughly mixed into the soil with a hand

trowel. Fungicide amounts applied as drenches were suspended in sufficient water in a beaker to produce a slurry which was poured over the soil surface. Each beaker was thoroughly rinsed several times with water which was poured into the can containing the test seedling. Each can was then carefully watered sufficiently to drench in the fungicide without percolating the water and fungicide from the base of the can.

Dormant apple seedlings, one-fourth inch in stem diameter, were used in these experiments. Prior to planting tree roots were wounded by scraping lightly with the dull edge of a knife on the crown area and in one or two places on each of the largest roots. A total of eight to twelve wounds were made on the roots of each tree. Trees in the inoculated checks and all fungicide treatments were inoculated with X. mali.

Inoculation was accomplished while planting by loosely placing 15 pieces of inoculum per can within the area of greatest root density. The inoculum pieces consisted of three-fourths to one inch lengths of apple water sprouts infested with X. mali. The water sprout inoculum had been prepared by cutting shoots into the desired length and sterilizing in 250 ml erylenmeyer flasks in the autoclave for two hours at 120°C. Mycelium of a pure culture of X. mali which had been isolated from infected apple roots were placed on the sterile medium and allowed to incubate at 25°C with high humidity for three months. The inoculum became a solid fungal mat and was separated into pieces with sterilized forceps for use in the experiments.

2.2 Root rating procedure.

Trees in the greenhouse experiment were harvested after eight and eleven months respectively (approximately one half of the total per harvest). The trees were removed from the cans and all soil shaken loose from the roots. The roots were then washed in running tap water to remove the remaining soil particles. The surface moisture on the root system was allowed to dry before being examined for X. mali lesions.

A large number of trees were harvested to give a broad spectrum of root samples. Root samples were classified into six categories which served as a standard for all further evaluations.

Healthy trees were considered to be those in which no X. mali lesions could be located. These root systems were assigned '0', '1' and '2' numerals based on the size and density of the root systems. Trees marked with a '0' had a dense root system and were considered to be excellent (Fig. 1). Those marked with a '1' had a sufficiently developed root system to be considered satisfactory (Fig. 2), while those marked '2' had a less than ideal root system and were considered only fair (Fig. 3).

Ratings of '3', '4' and '5' were applied to root systems on which typical black root rot lesions could be observed. All lesions were examined thoroughly and knife cuts were made in and above each lesion to determine the extensiveness of the vascular discoloration. The rating numerals increased directly in proportion to the severity of the infection. A '3' rating was applied to a root system on which

X. mali lesions occurred in a limited area on one or two of the larger roots (Fig. 4). When X. mali occurred on most of the major roots and when discoloration extended a short distance up these infected roots toward the crown the rating was a '4' (Fig. 5). A '5' rating was applied to all root systems with extensive X. mali lesions on the roots and with vascular discoloration extending through the crown area into the lower sten (Fig. 6). Figure number seven is a composite of the six catagories of root rating standards.

3.0 Uptake and translocation of fungicides.

Initially the uptake and translocation of the four fungicides was determined by the presence or absence of inhibition zones of a suitable test fungus around bioassay discs treated with leaf tissue extracts. Later the area of the inhibition zone of P. expansum to a known quantity of fungicide (benomyl or thiabendazole) was determined and a standard curve illustrating the area of inhibition per microgram of fungicide was developed. In the last leaf tissue bioassay of this experiment the appropriate standard curve was used to calculate the quantity of fungicide (benomyl or thiabendazole) contained in the leaf samples.

3.1 Leaf tissue bioassays for fungicides.

A bioassay technique to determine the presence or absence of the various fungicides in leaf tissues was initiated six weeks after planting dormant apple seedlings in the greenhouse and was continued

Figure 1. Healthy root system of apple (Malus pumila) seedlings on which no Xylaria mali lesions were found. The dense root system was considered to be excellent and was rated '0'.

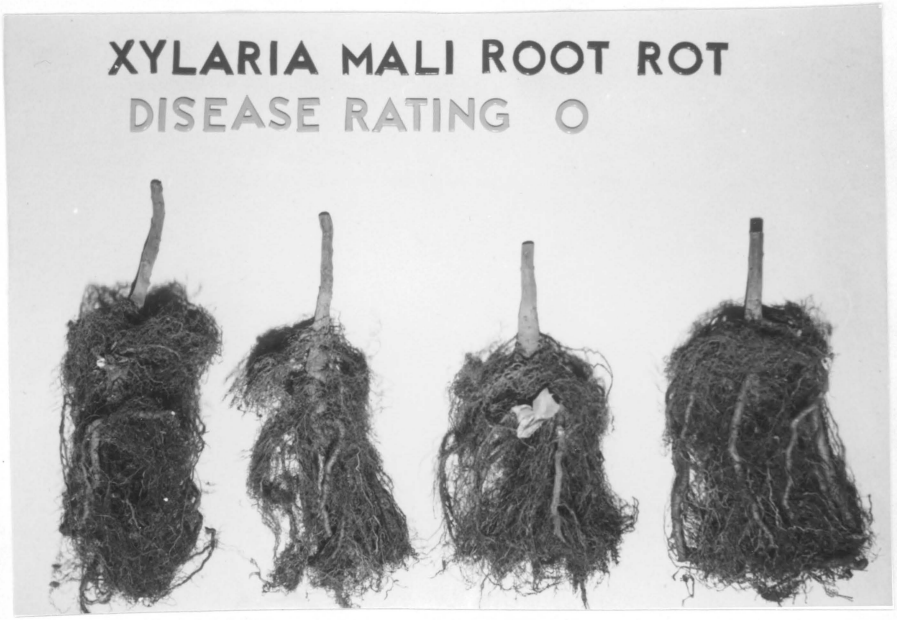

XYLARIA MALI ROOT ROT
DISEASE RATING 0

Figure 2. Healthy root system of apple (Malus pumila) seedlings on which no Xylaria mali lesions were found. The density of the root system was considered satisfactory and was rated '1'.

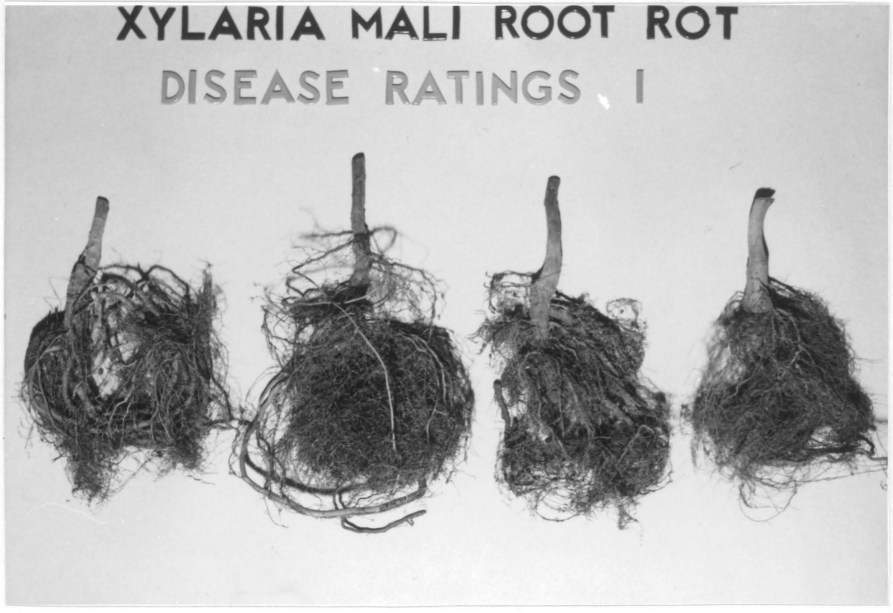

XYLARIA MALI ROOT ROT
DISEASE RATINGS 1

Figure 3. Healthy root system of apple (Malus pumila) seedlings on which no Xylaria mali lesions were found. The density of the root system was considered only fair and was rated '2'.

XYLARIA MALI ROOT ROT
DISEASE RATINGS 2

Figure 4. Diseased root system of apple (Malus pumila) seedlings rated '3'. Xylaria mali lesions occurred in a limited area on one or two of the larger roots.

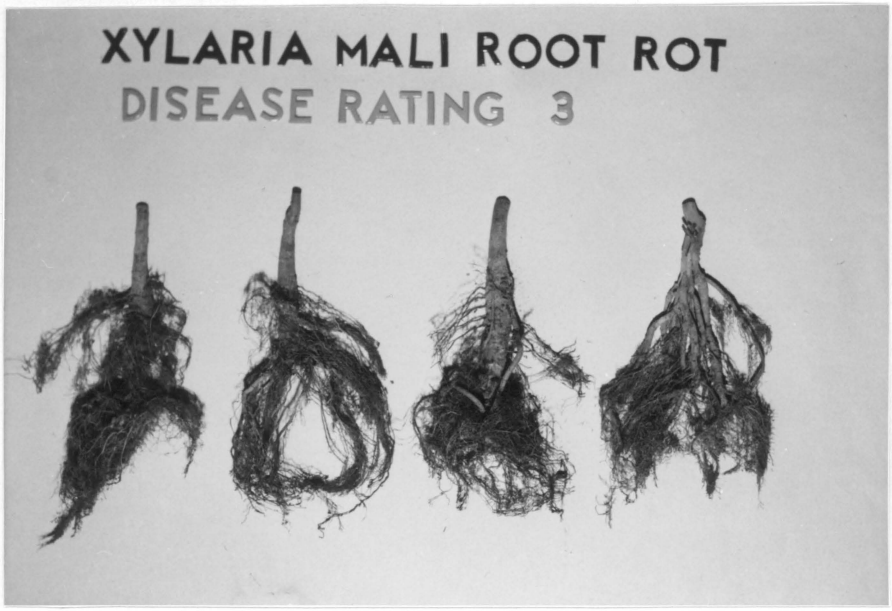

XYLARIA MALI ROOT ROT
DISEASE RATING 3

Figure 5. Diseased root system of apple (Malus pumila) seedlings rated '4'. Xylaria mali lesions occurred on most of the major roots with vascular discoloration extending a short distance up the infected roots toward the crown.

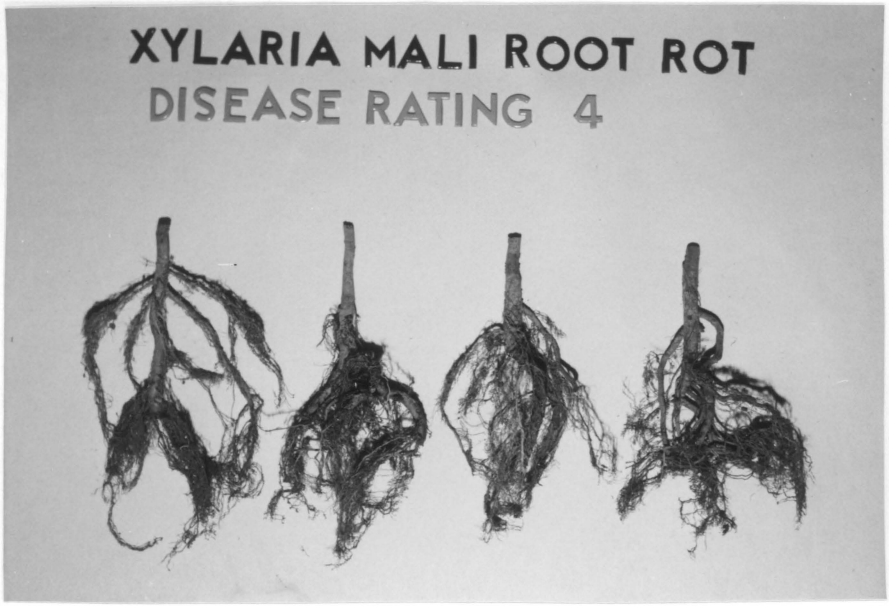

XYLARIA MALI ROOT ROT
DISEASE RATING 4

Figure 6. Diseased root system of apple (Malus pumila) seedlings rated '5'. Extensive Xylaria mali lesions occurred on the roots with vascular discoloration extending through the crown area into the lower stem.

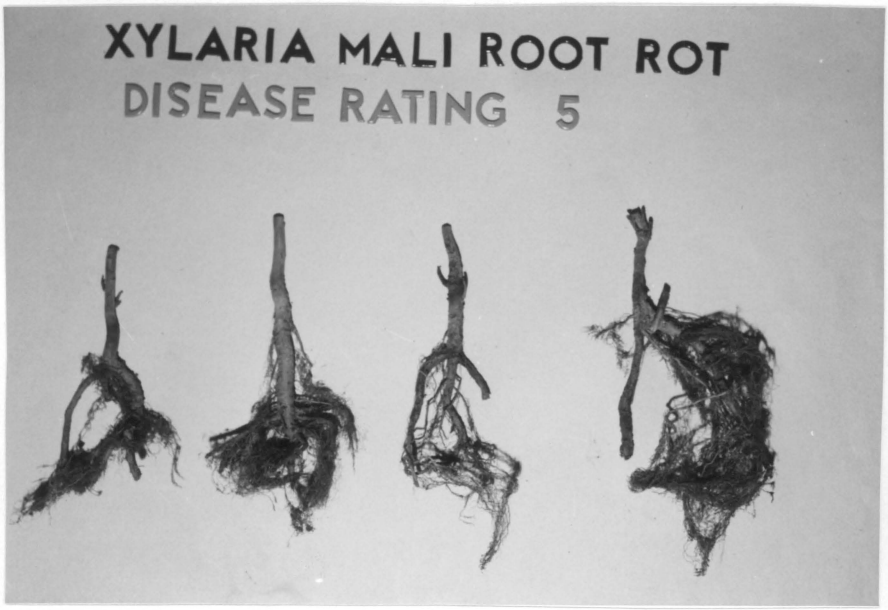
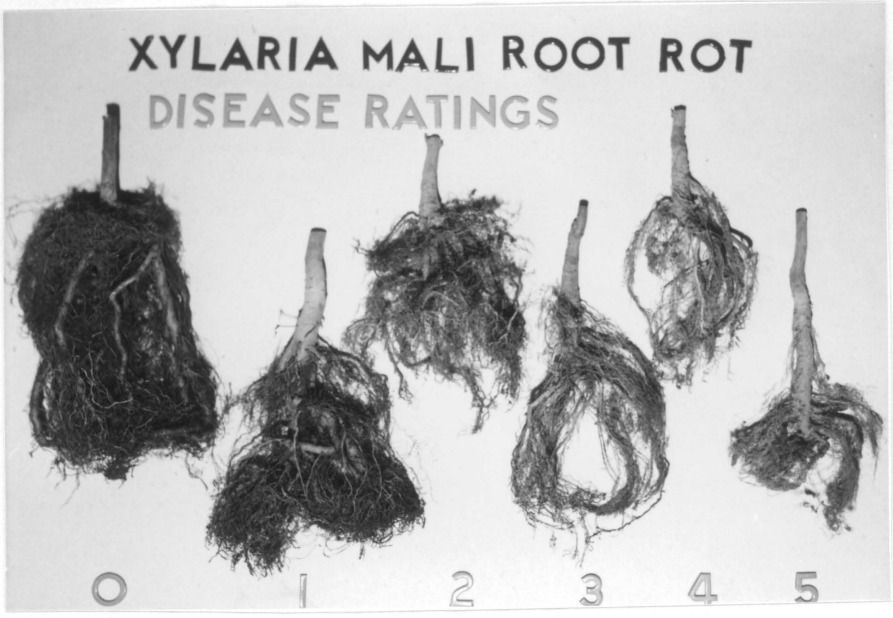

XYLARIA MALI ROOT ROT
DISEASE RATING 5

Figure 7. Composite of the six catagories of the root rating standards of apple (Malus pumila) seedlings inoculated with Xylaria mali infested water sprouts.


XYLARIA MALI ROOT ROT
DISEASE RATINGS
0
1
2
3
4
5

for one year. The leaves bioassayed were taken from trees used in the greenhouse fungicide evaluation studies. The extraction and plating technique was similar for all fungicide treatments with only the sensitive test fungus being changed.

Several leaves were selected from ten trees in each treatment beginning no closer than the fifth leaf from the terminal. All trees had been stripped of auxillary shoots throughout the experiment allowing only the terminal shoot to elongate. One gram fresh-weight of leaf tissue (petioles removed) was ground in a mortar and pestle. Washed sand was added to thoroughly macerate the tissue. The macerated tissue was extracted with a 1:1 mixture of acetone and absolute ethanol. Extracts were filtered and evaporated to dryness at room temperature before being stored in a refrigerator at $4^{o}C$ until all samples were processed.

A modified GYEA medium (20 gm agar, 5.0 gm dextrose, 2.0 gm yeast extract per liter) was used to culture the various test fungi. Two hundred mg of chloroamphenicol dissolved in 5 ml of absolute ethanol was added to the medium immediately upon its removal from the autoclave to inhibit bacterial contaminants. Ten ml of this medium was poured into plastic petri dishes (180 X 15 mm) to produce a uniform thickness of agar. The plastic petri dishes were selected because of their more perfectly flat bottoms than glass petri dishes, especially along the margins.

The extract residue was redissolved in one ml of 1:1 acetone/

ethanol. Micro-capillary pippettes were used to transfer 200 ul of this solution onto a 13 mm bioassay disc. The disc was then placed in the center of the petri dish containing the 10 ml of the modified GYEA medium. The plates were placed in an incubator at 25°C for 36 hours to allow diffusion of the extract into the medium before overspraying with a spore solution of the sensitive test fungus. Penicillium expansum was used as the test fungus for both benomyl and thiabendazole. Cladosporium cucumerinum as supplied by I. W. Brown of Eli Lilly Co. was the test fungus for triarimol and the yeast, Saccharomyces pastorianus, was used as the test organism for captan.

The oversprayed plates were then returned to the incubator at 25°C for 72 hours. At this time the cultures were examined for a zone of fungus inhibition surrounding the bioassay disc. The average diameter of the zone if present was measured and recorded. Check cultures with the 1:1 acetone/ethanol solvents and also extracts from check trees were bioassayed to verify that the inhibited area was caused by the fungicide which had been translocated to the leaf tissue sampled.

3.2 Developement of standard curves for benomyl and thiabendazole.

After translocation of the fungicides benomyl and thiabendazole in apple seedlings was established and prior to the 47 week bioassay in this research, a procedure to determine the area of inhibition of Penicillium expansum in proportion to an exact quantity of

fungicide was developed. Flat-bottom plastic petri dishes, containing 10 ml of modified GYEA medium (20 gm agar, 5 gm dextrose, and 2 gm yeast extract) with 200 mg per liter of chloroamphenicol (dissolved in ethanol before adding), were also used in this evaluation. Two hundred microliters of redistilled acetone containing the desired amount of fungicide in micrograms were pippetted onto a 13 mm paper bioassay disc external to the culture plates. A single disc was then placed on the agar medium in the center of the petri dish. The petri dishes were incubated at 25°C for 36 hours to allow the diffusion of the fungicide into the agar. These plates were oversprayed with a spore suspension of P. expansum in sterile distilled water. The cultures were then incubated at 25°C for 72 hours. The average diameter of the inhibition zone was measured in centimeters and the total area calculated for each amount of fungicide. The average area for the five replicates was plotted on a graph to produce a standard curve.

3.3 Calculations of fungicide (benomyl or thiabendazole) content of leaf tissue samples.

The amount or range of fungicide per gram of leaf tissue (fresh weight) after 47 weeks was calculated by reading the value from the standard curve and multiplying by a factor of five since only 200 µl of the one ml of solvent was pippetted onto the bioassay disc. Other multiplication factors occasionally were two or ten

depending upon the amount of dilution of the leaf sample extract required to produce a measurable inhibition zone on the plate.

RESULTS

1.0 In vitro fungitoxicity studies.

In each of the three methods used to determine the fungitoxicity of the four fungicides to *X. mali* a different fungicide or related fungicides were rated as being the most effective. Benomyl and thiabendazole were equally fungitoxic to *X. mali* when suspended in the liquid medium in which the fungus was grown. Triarimol proved to be the most fungitoxic to *X. mali* when incorporated into the agar culture medium. Captan was the most effective fungicide impregnated on the bioassay discs.

1.1 Incorporation of fungicide into liquid medium.

Benomyl and thiabendazole were highly fungitoxic to *X. mali* when suspended in liquid medium in which the fungus was grown. At rates of 0.5 ppm both completely inhibited mycelial growth throughout the 14 days the test was conducted. The fungus in the agar-mycelium plugs used to seed the liquid cultures was, however, still viable after being washed several times with distilled water and plated on PDA medium. Mycelial growth occurred within 12 days after plating from all plugs placed in concentrations of 0.5, 1, 2, 3, 5, 10 and 20 ppm. The results, thus, showed that both benomyl and thiabendazole were fungistatic and not fungicidal at concentrations up to 20 ppm. Concentrations higher than 20 ppm were not tested because both fungicides

precipitated at concentrations of 50 and 100 ppm.

Captan and triarimol, each at 50 ppm concentrations, effectively inhibited mycelial growth through the 14-day test period. No apparent inhibition of growth was observed from concentrations up to 20 ppm. Since there were no concentrations between 20 and 50 ppm tested, growth curves showing fungicide concentration effect could not be obtained.

Mean mycelial dry weight obtained during the 14-day period in five replicate cultures for each captan concentration ranged from 171 to 191 mg compared to 179 mg in the acetone check (Table 1). Somewhat less growth but a similar pattern was obtained with the same concentration of triarimol. Although some variation in growth among replicates occurred, the amount was not considered excessive and was attributed to variation in the amount of mycelium present on each of the agar-mycelium plugs used in seeding the cultures.

It was noted that the amount of mycelial growth in the check treatments decreased with storage time from the initial isolation date of the culture to the time of its use. This gradual reduction in vigor occurred even though repeated transfers of the culture to fresh media were made.

1.2 Incorporation of fungicide into agar medium.

The check and check with acetone cultures required six days for mycelial growth to reach the margins of the plates. No difference in growth could be observed between the checks and checks with acetone.

Table 1. Mycelial dry weight of Xylaria mali obtained in liquid medium containing various concentrations of benomyl, thiabendazole, captan and triarimol fungicides.

Concentration	Milligrams of mycelial dry weight			
parts per million	benomyl	thiabendazole	captan	thiarimol
0.0	276	285	201	110
0.0 + acetone	270	299	179	106
0.5	0	0	191	119
1.0	0	0	185	122
2.0	0	0	171	120
3.0	0	0	175	121
5.0	0	0	180	126
10.0	0	0	177	134
20.0	0	0	170	141
50.0	-	-	0	0

The acetone, to a large extent, flash evaporated when added to the flasks at 53°C.

The number of days required for mycelial growth to reach the margins of the plates in the benomyl treatments varied between six and 16 days depending upon fungicide concentrations (Table 2). The concentrations 0.5 ppm, 1.0 ppm and 2.0 ppm all required six days. Eight days were required for 20 ppm, ten days for 40 ppm, 13 days for 70 ppm and 16 days for 100 ppm. A definite difference in the density of the mycelium was noted in benomyl 40 ppm concentrations and higher. The greatest mycelial density occurred within a 1.5-2.0 cm radius immediately surrounding the agar plug of inoculum.

Growth of X. mali on the thiabendazole treatments was similar to benomyl and required six to eighteen days for the mycelium to reach the margins of the plates for the various fungicide concentrations (Table 2). The concentrations 0.5 ppm, 1.0 ppm and 2.0 ppm all required six days for the mycelium to grow to the margins of the plate. Eight days were required for 20 ppm and nine days for 40 ppm. Four of five plates at 70 ppm grew to the margins of the plates in 11 days while one required 13 days. At the 100 ppm concentration, one plate required 13 days to reach the margins of the plate, one plate required 16 days and two required 18 days. The remaining plate was not measured again until 38 days at which time it had grown to the margins of the plate. The different zones of mycelial density as described with the higher

Table 2. Growth response of Xylaria mali to agar incorporations of benomyl and thiabendazole.

	Days required for hyphae to reach margins of plate	
Conc. ppm	benomyl	thiabendazole
0.0	6	6
0.0 + acetone	6	6
0.5	6	6
1.0	6	6
2.0	6	6
20	8	8
40	10	9
70	13	11[a]
100	16	18[b]

[a] Three of five replicates grew to margins by 11 days, one required 13 days, final one required 16 days.

[b] One replicate required 13 days, one required 16 days, two required 18 days, final one not determined.

concentrations of benomyl was observed in the 20 ppm and higher concentrations of thiabendazole.

Captan was the least effective fungicide when incorporated into agar and growth to the margins of the plate required six to ten days depending upon fungicide concentration (Table 3). The concentration of 30 ppm required six days for the mycelium to grow to the margins of the plate. The concentrations of 40 ppm, 50 ppm and 60 ppm all required seven days. Eight days were required for 80 ppm, nine days for 100 ppm and ten days for 150 ppm.

Triarimol proved to be the most effective fungicide incorporated into agar. At 40 ppm concentration, 38 days were required for the mycelium to grow to the margins of all five plates (Table 3). Four of five plates at 50 ppm and three of five at 60 ppm had reached the margins of the plates at 38 days. The average diameters of the mycelial growth for the remaining fungicide concentrations at 38 days were 6.7 cm for 70 ppm, 6.4 cm for 100 ppm, 4.9 cm for 150 and 4.7 cm for 200 ppm. The different zones of mycelial density as described with benomyl were observed on all concentrations of triarimol.

1.3 Impregnation of fungicide into bioassay discs.

In these tests thiabendazole proved to be ineffective since the fungus grew to the margins of the plates within ten to 12 days (Table 4). The mycelial growth rate was slower at the higher concentrations (50-100 µg) and was reduced only 20 percent by the

Table 3. Growth response of Xylaria mali to agar incorporations of captan and triarimol.

	Days required for hyphae to reach margins of plate	
Conc. ppm	captan	triarimol
0.0	6	6
0.0 + acetone	6	6
30	6	-
40	7	38
50	7	38[a]
60	7	38[b]
70	-	-[c]
80	8	-
100	9	-[d]
150	10	-[e]
200	-	-[f]

[a] Four of five replicates grew to margins of plate.

[b] Three of five replicates grew to margins of plate.

[c] Average dia. of mycelial growth 6.7 cm.

[d] Average dia. of mycelial growth 6.4 cm.

[e] Average dia. of mycelial growth 4.9 cm.

[f] Average dia. of mycelial growth 4.7 cm.

Table 4. Growth responses of Xylaria mali to thiabendazole impregnated bioassay discs.

Amount of thiabendazole in ug on bioassay discs	Days incubated				
	6	8	10	12	15
0.0	+[a]	+[a]	x[b]	x[b]	x[b]
0.0 + acetone	+	+	x	x	x
1	+	+	x	x	x
2	+	+	x	x	x
5	+	+	x	x	x
10	+	+	2(x)[c]	x	x
20	3(+)[c]	+	3(x)[c]	1(x)[c]	x
50	+	+	+	x	x
100	+	+	+	x	x

[a] + indicates growth of Xylaria mali from bioassay discs onto agar for all replicates.

[b] x indicates complete growth of Xylaria mali to the margins of the plates for all replicates.

[c] Number preceding parentheses indicates the number of replicates demonstrating the growth response designated within the parentheses.

100 µg rate when compared to the checks.

Benomyl was almost equally ineffective as thiabendazole. All X. mali cultures grew to the margins of the plates in ten to 15 days (Table 5). As with thiabendazole the growth rate was slower at concentrations of 50-100 µg with only 50 percent reduction by the 100 µg rate. Hyphal growth into the agar around the bioassay disc was delayed for two days by this concentration.

Triarimol greatly reduced the rate of mycelial growth of X. mali in these tests. Hyphal growth into the agar was delayed through 15 days at rates of 100, 150 and 200 µg (Table 6). A small tuft of hyphae growing vertically from the bioassay disc did, however, appear after 12 days. No measurable growth occurred between the twelfth and fifteenth day which was the termination time of the experiment. The initiation of hyphal growth into the agar was delayed for two and four days at rates of 40 µg and 50 µg, respectively.

Captan proved to be the most effective fungicide when evaluated by this method. No hyphal growth into the agar occurred at any fungicide rate used in this experiment during the 15 day duration (Table 7). A tuft of vertical hyphae did appear by the fifteenth day on three bioassay discs containing 10 µg and on one containing 20 µg of captan.

Table 5. Growth responses of Xylaria mali to benomyl impregnated bioassay discs.

Amount of benomyl in ug on bioassay discs	Days incubated				
	6	8	10	12	15
0.0	+[a]	+[a]	x[b]	x[b]	x[b]
0.0 + acetone	+	+	x	x	x
1	+	+	x	x	x
2	+	+	x	x	x
5	+	+	x	x	x
10	+	+	2(x)[c]	x	x
20	4(+)[c]	+	x	x	x
50	4(+)[c]	+	+	4(x)[c]	x
100	1(+)[c]	4(+)[c]	+	+	x

[a] + indicates growth of Xylaria mali from bioassay discs onto agar for all replicates.

[b] x indicates complete growth of Xylaria mali to the margins of the plates for all replicates.

[c] Number preceding parentheses indicates the number of replicates demonstrating the growth response designated within the parentheses.

Table 6. Growth responses of Xylaria mali to triarimol impregnated bioassay discs.

Amount of triarimol in ug on bioassay discs	Days incubated				
	6	8	10	12	15
0.0	+[a]	+[a]	x[b]	x[b]	x[b]
0.0 + acetone	+	+	x	x	x
20	3(+)[c]	+	+	+	x
30	1(+)[c]	4(+)[c]	4(+)[c]	4(+)[c]	4(+)[c]
40	1(+)	3(+)	+	+	+
50	-[e]	2(+)	4(+)	+	+
100	-[e]	-[e]	-[e]	t[d]	t[d]
150	-	-	-	t	t
200	-	-	-	4(t)	t

[a] + indicates growth of Xylaria mali from bioassay discs into agar for all replicates.

[b] x indicates complete growth of Xylaria mali to the margins of the plates for all replicates.

[c] Number preceding parentheses indicates the number of replicates demonstrating the growth response designated within parentheses.

[d] t indicates only vertical tufts of Xylaria mali mycelium on bioassay discs not growing into agar for all replicates.

[e] - indicates no growth of Xylaria mali for all replicates.

Table 7. Growth responses of Xylaria mali to captan impregnated bioassay discs.

Amount of captan in ug on bioassay discs	Days incubated				
	6	8	10	12	15
0.0	+[a]	+[a]	x[b]	x[b]	x[b]
0.0 + acetone	+	+	x	x	x
10	-[e]	-[e]	-[e]	1(t)[cd]	3(t)[cd]
20	-	-	-	-[e]	1(t)[cd]
30	-	-	-	-	-[e]
40	-	-	-	-	-
50	-	-	-	-	-
100	-	-	-	-	-
150	-	-	-	-	-
200	-	-	-	-	-

[a] + indicates growth of Xylaria mali from bioassay discs into agar for all replicates.

[b] x indicates complete growth of Xylaria mali to the margins of the plates for all replicates.

[c] Number preceding parentheses indicates the number of replicates demonstrating the growth response designated within parentheses.

[d] t indicates only vertical tufts of Xylaria mali mycelium on bioassay discs not growing into agar for all replicates.

[e] - indicates no growth of Xylaria mali for all replicates.

2.0 In vivo fungitoxicity studies in greenhouse.

Black root rot disease of apple in X. mali infested soil was substantially reduced by several of the fungicide treatments (Table 8). Drenches were more effective than incorporated tretments at similar rates. Captan drenched at the rate of 4,000 ppm was rated significantly better at the 5 percent level than other treatments. Rated second in effectiveness was the captan drench at 1,000 ppm. Benomyl drench at 300 ppm was rated third, but there was no significant difference at the 5 percent level between the latter two treatments.

The normally high loss of replants in X. mali infested soil was demonstrated by 81 percent infection of inoculated checks. Fifty percent of the infected trees were moderately or severely infected. The lower infection rate for the best treatments, captan 4,000 ppm drench 47.5%, captan 1,000 ppm drench 51.4% and benomyl 300 ppm drench 58.1% is amplified when the severity of the infection is considered. Only 12.5% of the captan 4,000 ppm drench treatment, 21.6% of the captan 1,000 ppm drench treatment and 34.9% of the benomyl 300 ppm drench treatment were moderately or severely infected.

Treatments that were only slightly better than the inoculated check include thiabendazole 300 ppm drench (rated fourth), benomyl 300 ppm incorporated (rated fifth), and benomyl 1,000 ppm drench (rated sixth) and thiabendazole 300 ppm incorporated (rated seventh).

Table 8. The effects of the various fungicide treatments on the root system of container grown apple (Malus pumila) seedlings inoculated with Xylaria mali in greenhouse studies.

Treatments	Percentage of trees in each catagory of the root system ratings						
	Healthy trees			Diseased trees			
ppm	'0'	'1'	'2'	'3'	'4'	'5'	$\bar{X}$ rating[1/]
Check	56.7	33.3	10.0	0	0	0	0.53 a
Inoculated ck.	0	9.5	9.5	30.9	21.4	28.6	3.50 gh
Drench							
thiabendazole							
1,000	0	0	5.7	28.6	22.9	42.9	4.09 i
300	9.3	20.9	9.3	16.3	18.6	25.6	2.91 d
benomyl							
1,000	7.5	10.0	7.5	25.0	25.0	25.0	3.25 ef
300	16.2	16.2	9.3	23.3	20.9	14.0	2.58 c
captan							
4,000	17.5	17.5	17.5	35.0	10.0	2.5	3.10 b
1,000	10.8	16.2	21.6	30.0	13.5	8.1	2.43 c

[1/] Means not followed by a small letter indicates a significant difference between treatments at the 0.05 level (DMT).

Table 8. Cont'd.

Treatments	Percentage of trees in each catagory of the root system ratings						
	Healthy trees			Diseased trees			
ppm	'0'	'1'	'2'	'3'	'4'	'5'	$\bar{X}$ rating[1/]
triarimol							
1,000	0	0	5.9	20.6	11.7	61.8	4.29 i
300	3.4	3.4	6.9	27.6	41.4	17.2	3.52 gh
Incorporated							
thiabendazole							
1,000	0	0	5.7	28.5	25.7	40.0	4.00 i
300	0	7.9	13.2	34.2	21.1	23.7	3.39 fg
benomyl							
1,000	0	11.4	2.9	28.5	25.7	31.4	3.63 h
300	2.5	7.5	20.0	27.5	25.0	17.5	3.18 e

[1/] Means not followed by a small letter indicates a significant difference between treatments at the 0.05 level (DMT).

Root growth was very poor on trees receiving triarimol drenches at rates of 300 and 1,000 ppm. Similar results were obtained with thiabendazole 1,000 ppm drench, thiabendazole 1,000 ppm incorporated and benomyl 1,000 ppm incorporated treatments. The percent of the roots infected on these trees was equal to or higher than that of the inoculated check (Table 8). Phytotoxicity to the roots treated with triarimol drenches at both 300 ppm (Fig. 8) and 1,000 ppm (Fig. 9) was apparent by the proliferation of knurled, club-shaped adventitious roots (Fig. 10).

All fungicides with the exception of captan allowed better root growth at the lower concentration. Correlated with better root systems was a decrease in infection in all treatments.

3.0 Uptake and translocation of fungicides.

The uptake and translocation of benomyl, thiabendazole and captan was demonstrated by clearing zones of fungal inhibition around bioassay discs. Inhibition zones for benomyl and thiabendazole treatments were produced from leaf tissue extracts made 47 weeks after fungicide treatments. Captan demonstrated no clear inhibition zone after 15 weeks.

Two standard bioassay curves, one for benomyl and one for thiabendazole, showing the area of inhibition of P. expansum per microgram of fungicide, were developed. Thiabendazole required four to seven times the amount of benomyl to produce the same area of inhibition.

Figure 8. Phytotoxicity of triarimol (EL-273) 300 ppm drench treatment to container grown apple (Malus pumila) seedlings in greenhouse studies.

EL-273 300 PPM DR.

Figure 9. Phytotoxicity of triarimol (EL-273) 1,000 ppm drench treatment to container grown apple (Malus pumila) seedlings in greenhouse studies.

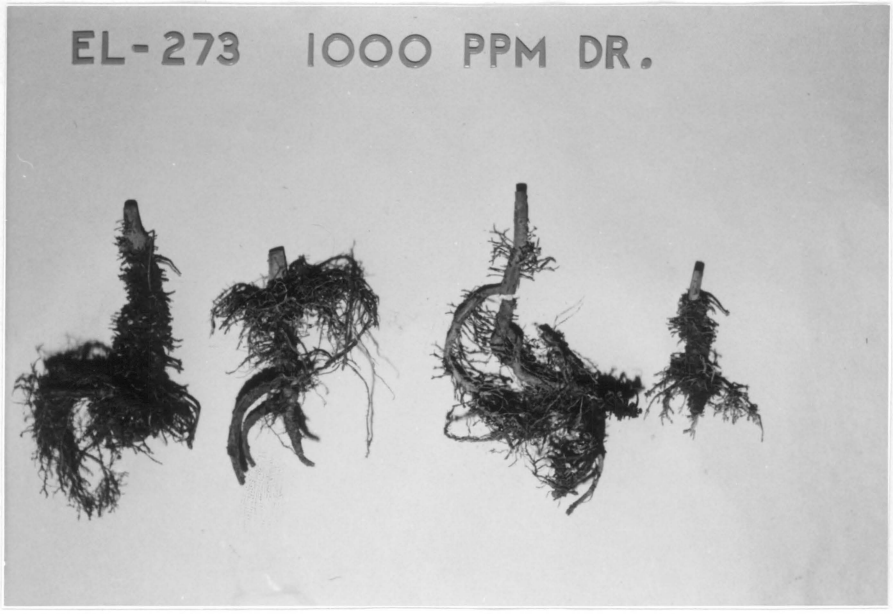
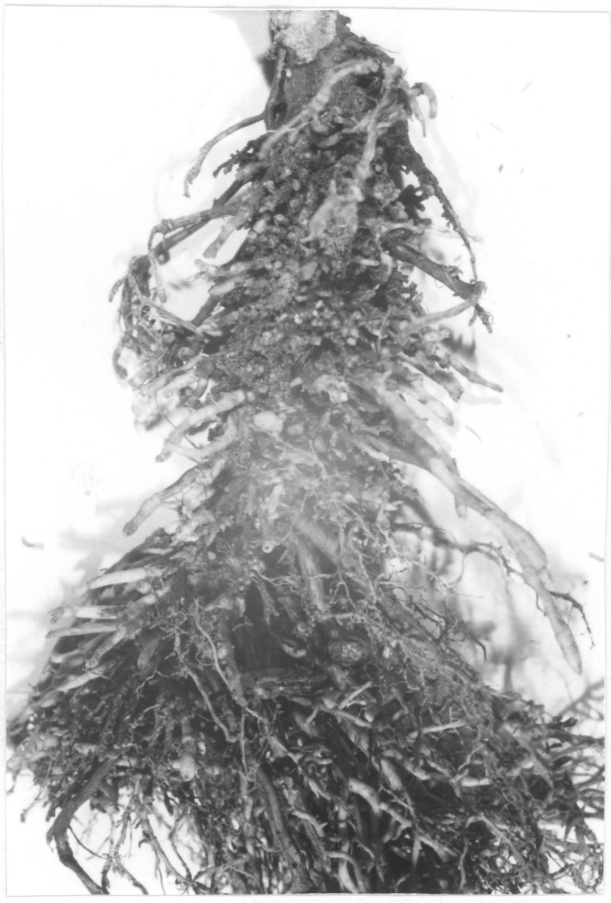

EL-273 1000 PPM DR.

Figure 10. Closeup of triarimol (EL-273) 300 ppm drench treatment showing the proliferation of knurled, club-shaped adventitious roots.



The amount of benomyl or thiabendazole was calculated for each gram (fresh weight) of leaf tissue sampled at 47 weeks. Leaf samples of the benomyl 1,000 ppm incorporated treatment contained the greatest amount of benomyl. The greatest amount of thiabendazole was found in the 1,000 ppm incorporated treatment.

3.1 Leaf tissue bioassay for fungicides.

The uptake and translocation of benomyl, thiabendazole and captan in apple seedlings were verified in these experiments. The data obtained were based on observations of clear zones of fungus inhibition in bioassay dishes.

Leaf tissue extracts taken from apple seedlings receiving benomyl drench treatments at 300 and 1,000 ppm produced clear inhibition zones of P. expansum when sampled at six, 15, 22 and 47 weeks after treatment (Table 9). Translocation to the leaves appeared to be dalayed initially since there were some discs with no inhibition zones at the six and 15 week samplings. The first bioassays of the benomyl 300 and 1,000 ppm incorporated treatments were made 15 weeks after planting. Large inhibition zones were produced by leaf extracts taken at 15, 22 and 47 weeks after planting (Table 10).

Thiabendazole 300 and 1,000 ppm drench treatments also generally produced inhibition zones of P. expansum when sampled at six, 15, 22 and 47 weeks (Table 11). As with benomyl there appeared to be some delay during the first six to 15 weeks in uptake and translocation to the leaves. The incorporated treatments of

Table 9. Inhibition of Penicillium expansum by leaf extracts from apple seedlings treated with benomyl at 300 and 1,000 ppm drenches.

	Diameter of inhibition zones (cm) weeks from initial treatment							
	6		15		22		47	
Tree	Conc.ppm		Conc. ppm		Conc. ppm		Conc. ppm	
number	300	1,000	300	1,000	300	1,000	300	1,000
1	4	8.5	7	8.5	8.0	8.5	10(7.5)[a]	10(7.6)[a]
2	6.5	8.5	8.5	8.5	8.5	8.5	10(6.9)	10(7.6)
3	3.5	8.5	7.7	8.5	8.5	8.5	10(6.6)	10(7.1)
4	6	8.5	6.2	8.5	8.5	8.5	10(7.1)	10(6.8)
5	5	8.5	8.5	8.5	8.5	8.5	10(8.5)	10(7.4)
6	4.5	8.5	7	8.5	8.5	8.5	10(7.3)	10(7.6)
7	3.5	7.0	7.5	8.5	8.5	8.5	10(7.1)	10(7.5)
8	3.5	7.0	6.7	8.5	8.5	8.5	10(6.0)	10(7.8)
9	7	8.5	6.5	8.5	8.5	8.5	10(6.8)	10(7.0)
10	8.5	8.5	8.5	8.5	8.5	8.5	10(6.8)	10(7.6)

[a] Number outside parentheses indicates the dilution factor of that sample.

Table 10. Inhibition of Penicillium expansum by leaf extracts from apple seedlings treated with benomyl at 300 and 1,000 ppm incorporations.

	Diameter of inhibition zone (cm) weeks from initial treatment					
	15		22		47	
Tree	Conc. ppm		Conc. ppm		Conc. ppm	
number	300	1,000	300	1,000	300	1,000
1	8.5	8.5	8.5	8.5	10(7.4)[a]	10(8.5)[a]
2	8.5	8.5	8.5	8.5	10(7.3)	10(8.5)
3	8.5	8.5	8.5	8.5	10(7.4)	10(8.5)
4	8.5	8.5	8.5	8.5	10(7.4)	10(8.5)
5	8.5	8.5	8.5	8.5	10(7.3)	10(7.9)
6	8.5	8.5	8.5	8.5	10(8.3)	10(7.9)
7	8.5	8.5	8.5	8.5	10(7.5)	10(7.2)
8	8.5	8.5	8.5	8.5	10(7.6)	10(7.9)
9	8.5	8.5	8.5	8.5	10(7.5)	10(7.9)
10	8.5	8.5	8.5	8.5	10(8.5)	10(7.9)

[a] Number outside the parentheses indicates the dilution factor of that sample.

Table 11. Inhibition of *Penicillium expansum* by leaf extracts from apple seedlings treated with thiabendazole at 300 and 1,000 ppm drenches.

	Diameter of inhibition zone (cm)							
	weeks from initial treatment							
	6		15		22		47	
Tree	Conc. ppm		Conc. ppm		Conc. ppm		Conc. ppm	
number	300	1,000	300	1,000	300	1,000	300	1,000
1	3.5	0.0	3.2	3.0	5.0	6.2	4.1	2(7.6)[a]
2	5.0	0.0	2.5	4.0	6.2	5.2	4.7	5.3
3	2.0	2.5	0.0	0.0	4.2	4.0	6.5	2(7.0)[a]
4	0.0	4.5	3.5	5.5	4.5	5.0	2(7.3)[a]	6.9
5	0.0	0.0	3.7	3.2	7.0	5.0	4.1	6.3
6	0.0	0.0	4.0	3.7	5.2	6.5	6.7	5.0
7	0.0	0.0	3.0	4.5	4.5	4.2	6.6	6.4
8	0.0	2.5	5.2	4.0	5.7	6.5	5.8	5.1
9	4.0	4.0	3.0	0.0	7.0	6.0	5.7	4.9
10	2.0	3.5	3.7	5.0	3.7	6.5	2(7.0)[a]	5.8

[a] Number outside parentheses indicates the dilution factor of that sample.

thiabendazole were not bioassayed until 15 and 22 weeks after planting. The 300 ppm treatment produced inhibition zones from all samples taken at 15, 22 and 47 weeks (Table 12). The 1,000 ppm treatment was not bioassayed until 22 weeks because the trees were stunted and had insufficient growth to provide leaves for sampling. Inhibition zones were produced by all samples collected at 22 and 47 weeks after planting.

Captan 4,000 ppm drench treatments produced inhibition zones to S. pastorianus on all ten samples after six weeks (Table 13). Clear inhibition zones were evident in only one of ten samples after 15 weeks, although a less densely colonized wet-appearing zone appeared around the bioassay disc. The fungus at the margin of this zone increased in density and grew toward the bioassay disc in concentric rings. This phenomenon also occurred in the 22 week bioassay, although the density of colonization in the wet-appearing zone was nearly similar to that near the edges of the plate. Captan 1,000 ppm drench treatment produced wet-appearing zones similar to captan 4,000 ppm drench. Only three of ten samples at six weeks and one of ten after 15 weeks produced clear inhibition zones around the bioassay disc. Wet-appearing zones were produced up to 22 weeks after treatment. Bioassays of both captan treatments were discontinued after the 22 week sampling.

Triarimol 300 and 1,000 ppm drench treatments were bioassayed after six and 15 weeks. No inhibition zones of the test organism

Table 12. Inhibition of Penicillium expansum by leaf extracts from apple seedlings treated with thiabendazole at 300 and 1,000 ppm incorporations.

	Diameter of inhibition zone (cm)					
	weeks from initial treatment					
	15		22		47	
Tree	Conc. ppm		Conc. ppm		Conc. ppm	
number	300	1,000	300	1,000	300	1,000
1	4.5	---	7.2	5.2	6.6	2(6.9)[a]
2	6.2	---	5.0	6.0	6.3	2(7.4)
3	4.5	---	6.5	4.5	5.4	2(7.4)
4	5.2	---	5.0	7.5	3.2	2(7.7)
5	5.2	---	5.5	6.2	2(7.3)[a]	2(7.6)
6	4.2	---	6.5	5.2	2(8.0)	6.7
7	4.7	---	5.5	6.5	7.1	2(7.3)
8	6.2	---	6.5	5.5	7.3	2(7.5)
9	4.7	---	5.7	6.0	2(6.7)	6.6
10	4.7	---	5.2	5.5	5.7	2(7.9)

[a] Number outside parentheses indicates the dilution factor of that sample.

Table 13. Inhibition of Saccharomyces pastorianus by leaf extracts from apple seedlings treated with captan at 1,000 and 4,000 ppm drenches.

	Diameter of inhibition zone (cm) weeks from initial treatment					
	6		15		22	
Tree	Conc. ppm		Conc. ppm		Conc. ppm	
number	1,000	4,000	1,000	4,000	1,000	4,000
1	0.0	3.5	0.0	0.0	0.0	0.0
2	2.5	3.5	0.0	0.0	0.0	0.0
3	0.0	3.5	0.0	0.0	0.0	0.0
4	0.0	3.5	0.0	2.5	0.0	0.0
5	0.0	4.5	0.0	0.0	0.0	0.0
6	0.0	3.5	2.0	0.0	0.0	0.0
7	2.0	3.0	0.0	0.0	0.0	0.0
8	2.0	3.5	0.0	0.0	0.0	0.0
9	0.0	4.0	0.0	0.0	0.0	0.0
10	0.0	3.0	0.0	0.0	0.0	0.0

Cladosporium cucumerinum were produced by any of the leaf sample extracts. Since no uptake and translocation was demonstrated the bioassays were discontinued after 15 weeks.

3.2 Developement of standard curves for benomyl and thiabendazole.

Standard curves for area of inhibition per microgram of fungicide for benomyl and thiabendazole were established. The results obtained showed that the standard curves are not linear since the area of inhibition per unit of fungicide decreases as fungicide quantity increases (Fig. 11 and Fig. 12).

Benomyl produced a much larger area of inhibition per microgram of fungicide than thiabendazole. Four to seven times as much thiabendazole was required to produce the same area of inhibition. The ratio decreases inversely with the amount of fungicide. At the lower end of the curve approximately 2.8 µg of thiabendazole was needed to produce the same area of inhibition as 0.4 µg of benomyl or a ratio of 1:7. At the upper end of the curve a ratio of 1:4 occurred with approximately 4.8 µg of thiabendazole being required to equal the area of inhibition of 1.2 µg of benomyl. Accuracy of the standard curves decreased as the inhibition zone increased. For this reason an upper limit for inhibition zone diameter from leaf extract samples was established at 6.5 cm. Any leaf extract sample producing an inhibition zone greater than 6.5 cm was diluted with 1:1 ethanol/acetone to reduce the inhibition zone for increased accuracy.

Figure 11. Standard fungicide inhibition curve for benomyl. *Penicillium expansum* was the bioassay organism.

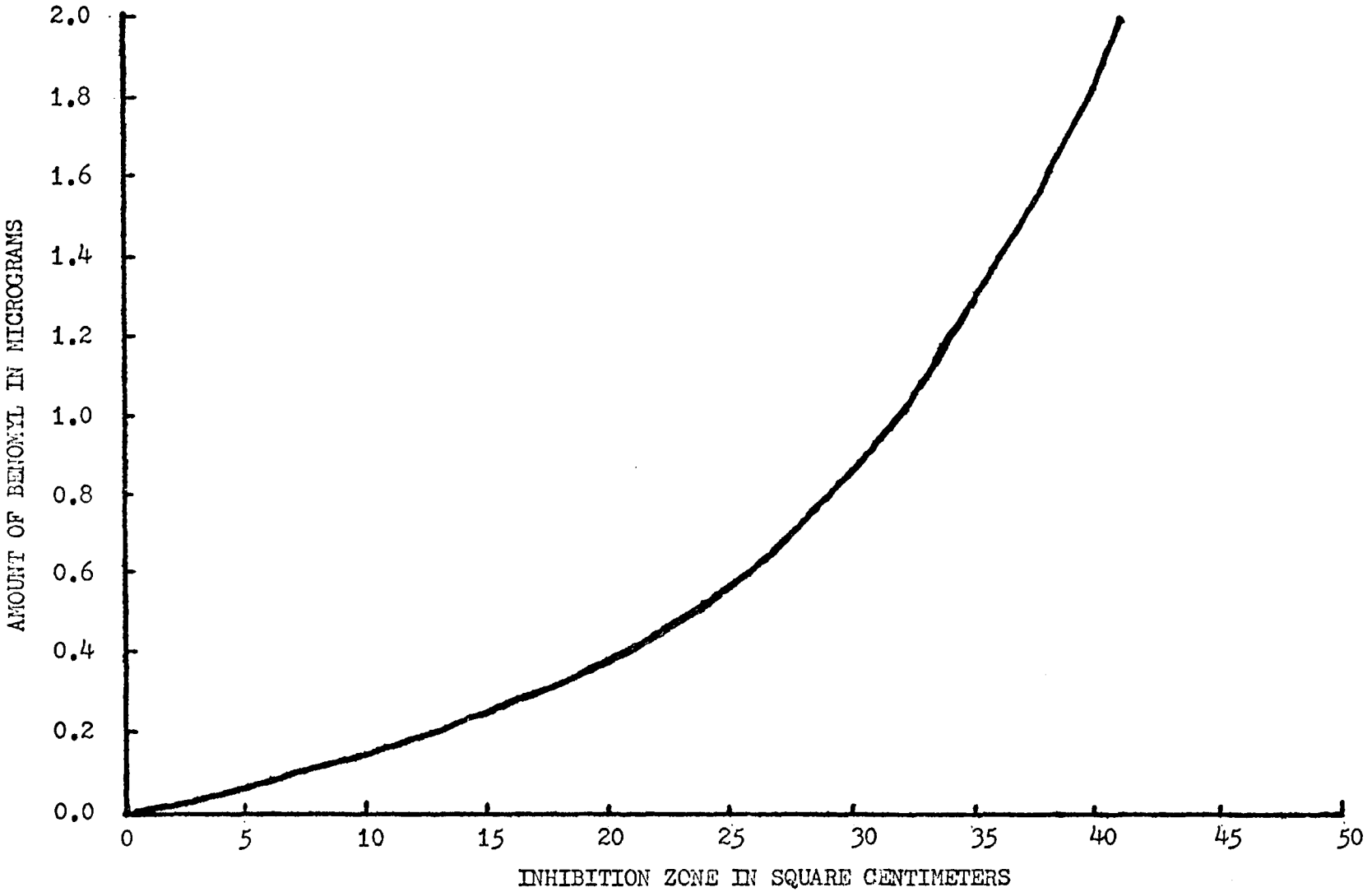
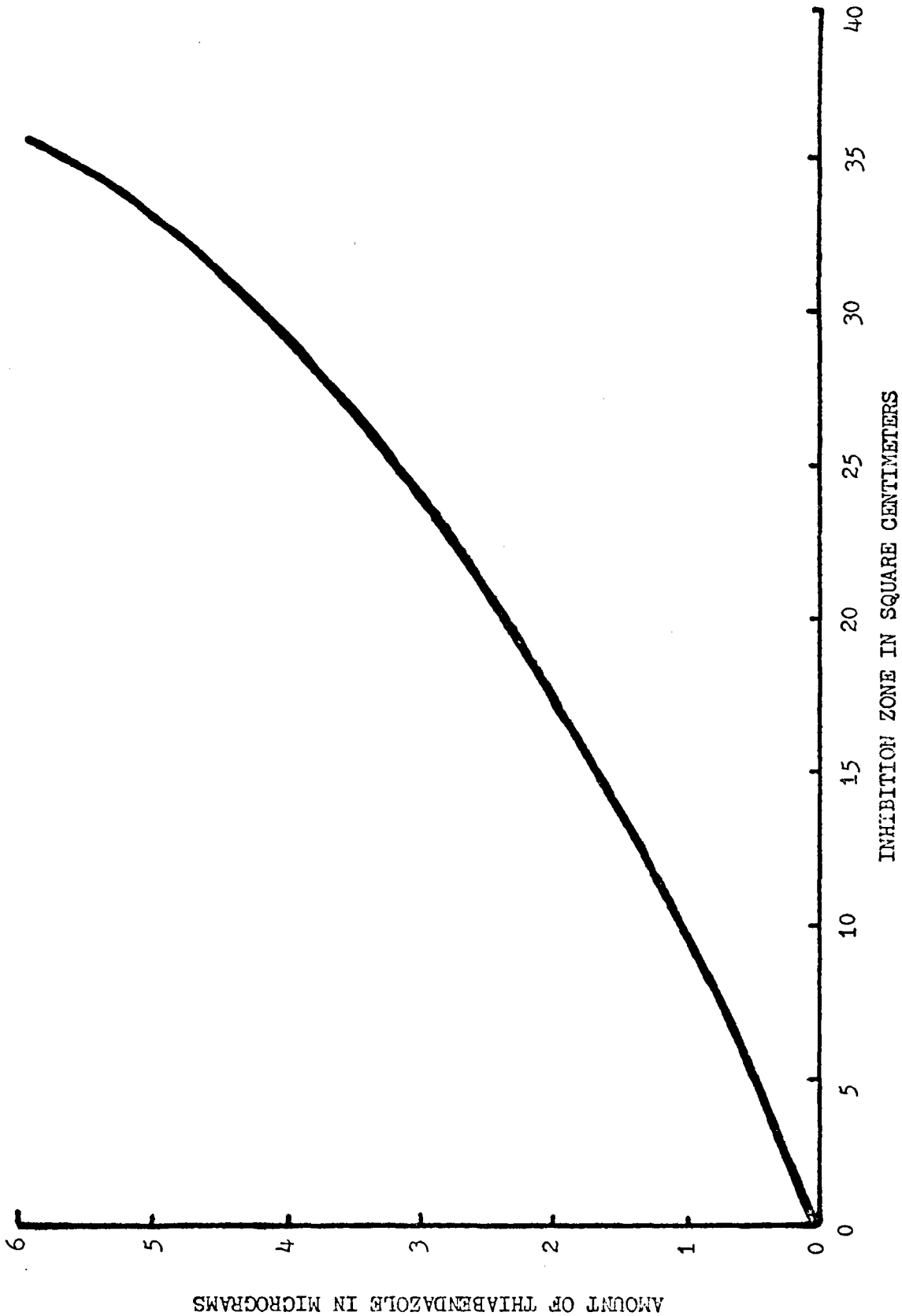

AMOUNT OF BENOMYL IN MICROGRAMS
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
5
10
15
20
25
30
35
40
45
50
INHIBITION ZONE IN SQUARE CENTIMETERS

Figure 12. Standard fungicide inhibition curve for thiabendazole. *Penicillium expansum* was the bioassay organism.


AMOUNT OF THIABENDAZOLE IN MICROGRAMS
0
1
2
3
4
5
6
INHIBITION ZONE IN SQUARE CENTIMETERS
0
5
10
15
20
25
30
35
40

3.3 Calculations of fungicide (benomyl or thiabendazole) content of leaf samples.

In establishing the standard curve it was found that at the higher concentrations tested very little separation between areas of inhibition per unit change of fungicide was possible. As a result, leaf tissue extracts producing a large area of inhibition could be calculated only to within a range of fungicide concentration present.

The amount of benomyl in one gram of leaf tissue varied between 70 and 150 µg in the 1,000 ppm drench treatments after 47 weeks (Table 14). The mean was in the 100-150 µg range. Benomyl amounts varied between 40 and 250 µg per gram of leaf tissue in the 300 ppm drench treatment. Amounts of 100 or more was found in only three samples out of ten with the remainder ranging between 40 and 85 µg. The amounts most commonly found ranged between 70 and 85 µg.

Benomyl amounts found in leaf tissue from the 300 ppm incorporated treatments were generally higher than either the 300 or 1,000 ppm drench treatments after 47 weeks (Table 14). The amount most commonly found was 100-150 µg. The amounts ranged between 100 and 250 µg with only one out of ten samples as low as 100 µg. Two of the ten samples had amounts of 200 µg or higher.

The benomyl 1,000 ppm incorporated treatment produced the

Table 14. Amount of benomyl recovered from apple leaf tissue 47 weeks after benomyl drench or incorporated treatments.

Tree number	Amount of benomyl ug per gram of leaf tissue			
	Drench		Incorporated	
	300 ppm	1,000 ppm	300 ppm	1,000 ppm
1	100-150	100-150	100-150	250
2	75	100-150	100	250
3	60	85	100-150	250
4	85	70	100-150	250
5	250	100-150	100	150-200
6	100	100-150	200	150-200
7	85	100-150	100-150	100
8	40	150	100-150	150-200
9	70	85	100-150	150-200
10	70	85	100-150	150-200
Mean	96	120	146	198
Median	80	125	125	175

greatest amount of fungicide per gram of leaf tissue in this experiment. Five out of ten samples ranged between 150-200 µg, four were greater than or equal to 250 µg and one was 100 µg.

The amount of thiabendazole found in leaf tissue in the 1,000 ppm drench treatment ranged between 11 and 100-150 µg (Table 15). Two samples contained 60 µg or more of fungicide and seven samples ranged between 11 and 24 µg. One sample ranged between 30-50 µg.

The amount of thiabendazole found in leaf tissue in the 300 ppm drench treatment ranged between seven and 100 µg (Table 15). Eight of the ten samples ranged within a seven to 30 µg range and two samples were 60 µg or higher. Very little difference in fungicide uptake could be determined between the 300 and 1,000 ppm drench treatments.

The thiabendazole 300 ppm incorporated treatment varied from four µg to 150-200 µg per gram of leaf tissue (Table 15). Four samples contained 50 µg or more of fungicide and five samples contained 30 µg or less. One sample ranged between 30-50 µg. The amount was generally greater than in the 300 and 1,000 ppm thiabendazole drench treatments.

Thiabendazole 1,000 ppm incorporated treatment produced the highest amounts of fungicide per gram of leaf tissue among all the thiabendazole treatments. The amount of fungicide varied from 27.5 to 150-200 µg per gram of leaf tissue. Only two samples contained 30 µg or less, one sample was in the 60-100 µg range and six samples contained 100 µg per gram of leaf tissue.

Table 15. Amount of thiabendazole recovered from apple leaf tissue 47 weeks after thiabendazole drench or incorporated treatments.

	Ug thiabendazole per gram of leaf tissue			
Tree	Drench		Incorporated	
number	300 ppm	1,000 ppm	300 ppm	1,000 ppm
1	7	100-150	27.5	60-100
2	9.5	13	22	100-150
3	25	60-100	14	100-150
4	100	30-50	4	100-150
5	7	22	100	100-150
6	30	11	150-200	30
7	27.5	24	30-50	100
8	17	12	50	100-150
9	16.5	11	60	27.5
10	60-100	17	16.5	150-200
Mean	32	36	60	104
Median	21	20	34	125

DISCUSSION

The results of the various *in vitro* fungitoxicity studies demonstrated differences in the fungicides and in the tests themselves. In each of the three studies a different fungicide was demonstrated to be the best for that particular test.

In the incorporation of fungicide into liquid medium both benomyl and thiabendazole were equally effective in the inhibition of growth of *X. mali* at 0.5 ppm. Captan and triarimol effectively inhibited growth at 50 ppm. Since all fungicides were dissolved in acetone before being added to the liquid medium, and no precipitation occurred at the concentration used, solubility should not have been a factor in the results. The toxicity of the fungicide molecules themselves and the influence of their hydrolysis products should have been factors in the results of this experiment.

In the studies where fungicides were incorporated into agar, triarimol was the most effective fungicide tested. It delayed the growth of *X. mali* to the margins of the plate for 38 days at 40 ppm. Captan delayed the growth of hyphae to the margins of the plate for 10 days at 100 ppm and thiabendazole for 18 days at 100 ppm. Fungal toxicity, solubility, thermo stability at 53°C, hydrolysis products and adsorption to the agar could all be factors affecting the results of this experiment.

An explanation for the different areas of mycelial density as noted in benomyl (40 ppm and higher), thiabendazole (20 ppm and

higher) and triarimol (at all concentrations) could be the utilization of the nutrients in the agar plug of inoculum to the exclusion of those in the "poisoned" agar. As the nutrients in the agar plug neared depletion the hyphal strands would become more sparse and the growth rate would be reduced. Also as more nutrients were obtained from the "poisoned" culture medium and more hyphal surface area was exposed to fungicide in the agar similar results could be produced by the "poisoned" fungus.

Captan proved to be the most effective fungitoxicant in the fungicide impregnated bioassay disc studies. It effectively prevented the growth of X. mali into the agar during the 15 day duration of this experiment at 10 µg per disc. Vertical tufts of hyphae appeared in three of five cultures at 10 µg per disc and one of five at 20 µg per disc after 15 days. Triarimol also prevented the growth of X. mali into the agar at 100, 150 and 200 µg per disc, although vertical tufts of hyphae appeared after 12 days. Both benomyl and thiabendazole were nearly equally ineffective with all concentrations tested having grown to the margins of the plates in 15 days.

The fungicide treated bioassay disc essentially measured contact activity. The several strands of hyphae were placed in direct contact with the fungicide tested. The results were not influenced by adsorption to agar or by hydrolysis in a large quantity of water. This test is especially useful in testing

non-sporulating fungi.

The in vivo fungitoxicity studies demonstrated the effectiveness of captan drenches in reducing severe infection of apple seedlings by X. mali. Fifty percent of container-grown seedlings in the greenhouse which were inoculated with X. mali were moderately to severely infected. In the captan 4,000 ppm drench only 12.5% were moderately to severely infected and was significantly the best treatment. Captan 1,000 ppm drench with 21.6% moderate to severe infection was rated second and benomyl 300 ppm drench rated third although no statistical difference was shown.

In projecting these results to the field, moderate to severely infected trees would ultimately die before bearing fruit or at least be unproductive for a short period of time before death. The results of both captan treatments may offer growers a practical application method for the maintenance of dense orchard plantings with replants in X. mali-infested sites.

As noted in the results all fungicides with the exception of captan allowed better root growth at the lower fungicide concentration. Correlated with better root systems was a decrease in infection. Since phytotoxicity was obviously apparent with triarimol, and since the root systems and the top growth were stunted by both benomyl and thiabendazole at the 1,000 ppm rates in both incorporated and drench treatments, phytotoxicity decreasing tree vigor could account for this increased infection. This correlation was previously noted

by Groves (19) who observed that any reduction in tree vigor as caused by mouse injury, drought, excessively heavy fruit production, etc. led to increased susceptibility of apple to X. mali.

Drench treatments were initially bioassayed after six weeks and not all extracts produced inhibition zones of P. expansum when plated. This can be explained by a limited fungicide to root surface contact early in the experiment due to lack of feeder root developement. All incorporated treatments produced large inhibition zones initially but leaf tissue was not bioassayed until 15 weeks for these treatments (22 weeks for thiabendazole). Therefore no comparison of the initiation of fungicide uptake and translocation can be made between the two application techniques.

During the duration of the greenhouse experiment, leaf tissue bioassays verified the uptake and translocation of benomyl and thiabendazole through 47 weeks. Uptake and translocation of captan was also shown early in the experiment but could not be demonstrated after 15 weeks. No inhibition zones of Cladosporium cucumerinum by leaf extracts of triarimol treatments were produced and the non-systemic nature of the material was concluded.

While the developement of a standard curve for the fungicide was a simple procedure, accuracy was completely dependent upon an individual's ability to be consistent in his technique. Sources of inaccuracy include the weighing of fungicides for stock solutions, the pouring of 10 ml of agar medium per plate, the evaporation of

solvent from stock solutions, and failure to stir spore suspension before overspraying plates.

Two standard curves were developed during this study, one for benomyl and one for thiabendazole. Thiabendazole required four to seven times the amount of benomyl to produce the same area of inhibition of P. expansum. This could result from the diffusion characteristics of the two compounds or from differences in adsorption to agar, since both fungicides are considered to be equally toxic to the test fungus.

The results obtained in these studies has placed in question the results of some previously published experiments rating the systemicity of these two compounds. While later experiments have confirmed the more systemic nature of benomyl versus thiabendazole earlier conclusions based entirely on inhibition zone diameters were in fact, a measure of more than the amount of chemical present.

By calculations of the amount of benomyl or thiabendazole contained in leaf tissue after 47 weeks I concluded that benomyl either persists longer than thiabendazole or is more readily translocated and characteristically becomes more highly concentrated in leaf tissue. Incorporated treatments produced higher concentrations of fungicide, both benomyl and thiabendazole, in leaf tissue than the corresponding drench treatments. Several reasons for the greater amounts of fungicide in leaf tissue from incorporated treatments are as follows: (1) the drench treatments applied as a slurry to

the surface of the container were less uniformly distributed through the potting mix than the incorporated treatments which would reduce the fungicide to root surface contact, (2) the potting mixture served as a filter which trapped the fungicide particles from the slurry near the surface of the mix, (3) the potting mix could have served as an ion exchange medium, adsorbing the fungicide in the slurry on organic and clay colloids near the surface of the mix, and (4) the wetted fungicide particles in the slurry could have been percolated through the potting mix by watering. The latter reason is, however, unlikely since by design excessive percolation of the fungicide in the drench treatments from the container was held to a minimum by careful light watering. The first three reasons, all ultimately producing a less uniform distribution of the drench fungicide, would most correctly explain the lower amounts of fungicide found in leaf tissue from drench treatments.

Literature Cited

1. Andes, J. O. 1935. Black root rot of apple. Phytopathology 25:976. (Abstr.)

2. Biehn, W. L. and A. E. Dimond. 1969. Reduction of tomato Fusarium wilt symptoms by 1-(butylcarbamoyl)-2-benzimidazole carbamic acid, methyl ester. Phytopathology 59:397. (Abstr.)

3. Biehn, W. L. and A. E. Dimond. 1970. Reduction of tomato Fusarium wilt symptoms by benomyl and correlation with a bioassay of fungitoxicant in benomyl-treated plants. Plant Disease Reptr. 54:12-14.

4. Biehn, W. L. and A. E. Dimond. 1970. Protective action of benomyl against Dutch elm disease. Phytopathology 60:571. (Abstr.)

5. Cimanowski, J., A. Masternals and D. F. Millikan. 1970. Effectiveness of benomyl for controlling apple powdery mildew and cherry leaf spot in Poland. Plant Disease Reptr. 54:81-83.

6. Clemons, G. P. and H. D. Sisler. 1969. Formation of a fungitoxic derivative from Benlate. Phytopathology 59:705-706.

7. Cooley, J. S. 1942. Factors affecting distribution and severity of black root rot of apples. Jour. Agr. Res. 65:299-311.

8. Crabill, C. H. 1916. Note on apple-rot in Virginia. Phytopathology 6:159-161.

9. Erwin, D. C., H. Mee and J. J. Sims. 1968. The systemic effect of 1-(butylcarbamoyl)-2-benzimidazole carbamic acid, methyl ester. on Verticillium wilt of cotton. Phytopathology 58:528-529.

10. Erwin, D. C., J. J. Sims and J. Partridge. 1968. Evidence for the systemic fungitoxic activity of 2-(4-thiazolyl)benzimidazole in the control of Verticillium wilt of cotton. Phytopathology 58:860-865.

11. Erwin, D. C. 1970. Progress in the development of systemic fungitoxic chemicals for control of plant diseases. FAO Plant Protection Bull. 18:73-82.

12. Fromme, F. D. and H. E. Thomas. 1917. The root rot disease of the apple in Virginia. Science 45:93.

13. Fromme, F. D. and H. E. Thomas. 1917. Black root rot of the apple. Jour. of Agr. Res. 10:163-174.

14. Fromme, F. D. 1928. The black root rot disease of apple. Va. Agr. Exp. Sta. Tech. Bull. 34:51p.

15. Fromme, F. D. and F. J. Schneiderhan. 1938. Studies of black root rot of apple. Phytopathology 28:483-490.

16. Fulton, H. R. and R. O. Cromwell. 1916. Black root rot of apple. Phytopathology 6:110. (Abstr.)

17. Gilpatrick, J. D. 1969. Systemic activity of benzimidazoles as soil drenches against powdery mildew of apple and cherry. Plant Disease Reptr. 53:721-725.

18. Groves, A. B. 1944. Soil treatments and apple replant survival in Xylaria mali infested locations. Phytopathology 34:1001. (Abstr.)

19. Groves, A. B. 1951. Black root rot of apple. Va. Agr. Exp. Sta. Tech. Bull. 118. 51p.

20. Hickey, K. D. (ed.). 1969. Fungicide and nematicide tests, results of 1969. Vol. 25, 148p. American Phytopathological Society, St. Paul, Minn.

21. Hickey, K. D. (ed.). 1970. Fungicide and nematicide tests, results of 1970. Vol. 26, 196p. American Phytopathological Society, St. Paul, Minn.

22. Hickey, K. D. (ed.). 1971. Fungicide and nematicide tests, results of 1971. Vol. 27, 206p. American Phytopathological Society, St. Paul, Minn.

23. Hine, R. B., D. L. Johnson and C. J. Wenger. 1969. The persistency of two benzimidazole fungicides in soil and their fungistatic activity against Phymatotrichum omnivorum. Phytopathology 59:798-801.

24. Hock, W. K., L. R. Schreiber and B. R. Roberts. 1970. Suppression of Dutch elm disease in American elm seedlings by benomyl. Phytopathology 60:391-392.

25. Hock, W. K., L. R. Schreiber and B. R. Roberts. 1970. Factors influencing uptake, concentration, and persistence of benomyl in American elm seedlings. Phytopathology 60: 1619-1622.

26. Hoitink, H. A. J. and A. F. Schmitthenner. 1970. Disease control in Rhododendron cuttings with benomyl or thiabendazole in hormone mixtures. Plant Disease Reptr. 54:427-430.

27. Horsfall, J. G. 1956. Principles of fungicidal action. Chronica Botanica Co., Waltham, Mass. 260p.

28. Horst, R. K. and H. A. J. Hoitink. 1968. Occurrence of Cylindrocladium blights on nursery crops and control with fungicide 1991 on azales. Plant Disease Reptr. 52:615-617.

29. Jacobsen, B. J. and P. H. Williams. 1969. Cabbage clubroot control using Benlate. Phytopathology 59:1033. (Abstr.)

30. Jacobsen, B. J. and P. H. Williams. 1970. Control of cabbage clubroot using benomyl fungicide. Plant Disease Reptr. 54: 456-460.

31. Jaynes, R. A. and S. L. Anagnostakis. 1971. Inhibition of Endothia parasitica by benomyl in field-grown American chestnut trees. Plant Disease Reptr. 55:199-200.

32. Lilly, V. G. and H. L. Barnett. 1951. Physiology of the fungi. McGraw-Hill Book Co. Inc., New York. 464p.

33. Manning, W. J., F. J. Campbell, P. M. Papia and P. A. Hughes. 1970. Effectiveness of benomyl soil drenches for control of Thielaviopsis root rot of poinsettias. Plant Disease Reptr. 54:328-330.

34. Netzer, D. and I. Dishon. 1970. Field control of powdery mildew on muskmelons by root application of benomyl. Plant Disease Reptr. 54:232-234.

35. Netzer, D. and I. Dishon. 1970. Effect of mode of application of benomyl on control of Sclerotinia sclerotiorum on muskmelons. Plant Disease Reptr. 54:909-912.

36. Palmer, J. G. and P. Semenuik. 1969. Effects of three soil-applied fungicides on development of rose blackspot. Phytopathology 59:1559. (Abstr.)

37. Peterson, C. A. and L. V. Edgington. 1969. Quantitative estimation of the fungicide benomyl using a bioautograph technique. Jour. Agr. Food Chem. 17:898-899.

38. Peterson, C. A. and L. V. Edgington. 1970. Transport of the systemic fungicide, benomyl, in bean plants. Phytopathology 60:475-478.

39. Peterson, C. A. and L. V. Edgington. 1971. Transport of benomyl into various plant organs. Phytopathology 61:91-92.

40. Reed, H. S. and C. H. Crabill. 1913. Plant diseases in Virginia in the years 1911 and 1912. Va. Agr. Exp. Sta. Rept. for 1911. Vol. 12, 35-50.

41. Reed, H. S. and C. H. Crabill. 1915. Notes on plant disease in Virginia observed in 1913 and 1914. Va. Agr. Exp. Sta. Tech. Bull. 2:44-47.

42. Schneiderhan, F. J. 1937. Rootstocks in relation to the black root rot (Xylaria mali) disease of apples. Va. Fruit 25:69-74.

43. Sharvelle, E. G. 1961. The nature and uses of modern fungicides. Burgess Pub. Co., Minneapolis, Minn. 380p.

44. Sims, J. J., H. Mee and D. C. Erwin. 1969. Methyl 2-benzimidazole-carbamate, a fungitoxic compound isolated from cotton plants treated with methyl 1-(butylcarbamoyl)-2benzimidazole-carbamate (benomyl). Phytopathology 59:1775-1776.

45. Stipes, R. J. 1969. Chemotherapeutic patterns of methyl 1-(butylcarbamoyl)-2benzimidazolecarbamate and thiabendazole in the control of Dutch elm disease. Phytopathology 59:1560. (Abstr.)

46. Stover, R. H. 1969. The effect of benomyl on Mycosphaerella musicola. Plant Disease Reptr. 53:830-833.

47. Wolf, F. A. and R. O. Cromwell. 1917. Xylaria rootrot of apple. Jour. Agr. Res. 9:269-276.

The vita has been removed from the scanned document

FUNGICIDAL CONTROL AND RELATED STUDIES ON BLACK ROOT ROT OF APPLE (*MALUS PUMILA* MILL.) CAUSED BY *XYLARIA MALI* FROMME

by

David A. Smith

(ABSTRACT)

Attempts were made to control black root rot of apple with the fungicides, benomyl, captan, thiabendazole and triarimol, and to determine their fungitoxic effects on the causal pathogen *Xylaria mali*. The pathogen responded differently in various bioassay methods, but all fungicides were inhibitory to its vegetative growth.

Disease control was evaluated by applying the fungicides as amendments or as drenches to the soil in which the roots were artificially inoculated. The best therapy was obtained with a captan drench (4,000 ppm), followed by captan (1,000 ppm) and benomyl (300 ppm) drenches. Root growth was suppressed at all higher concentrations except with captan; root development was directly correlated with chemotherapy.

Fungitoxicants were recovered from leaves of seedlings treated with all fungicides except triarimol. Higher bioassay activity was observed in plants treated by the soil amendment method. Leaves from benomyl-treated plants exhibited the greatest bioassay activity.