FUNGICIDAL INVESTIGATIONS WITH *PYTHIUM ULTIMUM* AND STUDIES WITH SEED-BORNE PATHOGENS OF CULTIVARS OF *PELARGONIUM HORTORUM* BAILEY

By

HUA-FU CHAO

Bachelor of Science

Tunghai University

Taichung, Taiwan

1973

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
December, 1979

OKLAHOMA STATE UNIVERSITY LIBRARY

FUNGICIDAL INVESTIGATIONS WITH *PYTHIUM ULTIMUM*

AND STUDIES WITH SEED-BORNE PATHOGENS

OF CULTIVARS OF *PELARGONIUM*

HORTORUM BAILEY

Thesis Approved:

R. N. Payne
Thesis Adviser

D. E. Wadsworth

Grant Fox

John L. Johnson

Norman N Durham
Dean of Graduate College

1042912

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Dr. Richard N. Payne, my major adviser, and Dr. Dallas F. Wadsworth of the Plant Pathology Department, for their guidance, assistance, and encouragement throughout this study and my entire master's program.

Additional gratitude is extended to the other committee members, Dr. Grant Vest and Dr. Johnny Johnson of the Horticulture Department. Appreciation is also extended to Dr. Robert Morrison of the Mathematics and Statistics Department for his valuable assistance in statistically analyzing the research data.

Special gratitude is extended to Garry Sites, greenhouse superintendent, and Rocky Walker, technician in the Plant Pathology Department, for their many hours of assistance throughout the research project.

Very special appreciation is expressed to my parents, Mr. and Mrs. Jih-Sung Chao, and my darling wife, My-Na Diec, for their unending support, encouragement, sacrifices and love.

TABLE OF CONTENTS

Chapter Page

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION AND REVIEW OF LITERATURE

Geranium (*Pelargonium hortorum*): Seedlings vs. Cuttings

Geraniums are perennial herbs or shrubs, mostly of Africa, and grow in the open in warm regions. The numerous horticultural forms are usually not distinguished in reports, but the following species or varieties are sometimes distinguished (16): (a) *P. domesticum* Bailey, Lady Washington geranium; (b) *P. graveolens* L'Her., rose geranium; (c) *P. hortorum* Bailey (X P. zonale Willd.), fish geranium; (d) *P. peltatum* Ait., ivy geranium.

Geraniums represent one of the most exciting commercial flower crops in the country. No other flower has shown a greater rate of increase in dollar value to commercial floriculture and better performance for purchasers during the last twenty years. In 1959, geraniums were the sixth most important flower crop in the United States. Chrysanthemums, bedding plants, roses, carnations, and foliage plants, in that order, ranked higher than geraniums (19). In many areas, it is the No. 1 pot-plant. Current economic trends indicate that the demand for geraniums is still on the increase. One of the chief reasons for its great popularity is its ability to grow and flower under adverse and varying conditions of temperature, soil, light and moisture. It is

very versatile in its usage and adapts well to the average homeowner's idea of what a plant should do; namely, flower all summer long without receiving much care.

Surely, a major development in geraniums is the newer seed-propagated strains. For the first time, seed-geraniums are seriously challenging cutting-propagated plants including the all important "4-inch in flower" market for April-May sales. The main thing hindering the expansion of the seed geranium market is that many growers just don't believe they will flower in May. Too many growers had unfortunate experiences with earlier varieties. In Spring 1977, hundreds of growers across the U.S. grew millions of seed geraniums-both as flats and 4's. They confirmed one point that flowering of seed geraniums is reliable (3).

The seed geranium has experienced a rapid rise in popularity in recent years and are now in seventh place among the 1979 season's major bedding plants (29). Impatiens, marigolds, begonias, geraniums from cuttings, vegetable plants, and petunias, in that order, ranked higher than seed geraniums. Seed strains are not all things to all growers. They still have mainly a single floret vs. the semi-doubles of most cuttings. Also, there is a tendency for some seeded cultivars to drop petals during shipment. But the economics came were obvious: seed propagation eliminates the fuel-costly growing of mother plants from September through March. Seed propagation permits scheduling large blocks of plants to flower at once and done properly, they will all flower uniformly. Also, seedling geraniums often perform better outdoors in the summer than the plants from cuttings (3, 4). Seed geraniums are relatively free of viruses or other pathogens that

plague most cutting types. This does not mean that seed geraniums are particularly resistant to diseases. It does mean starting with a healthy plant. There may be certain disease organisms present on or in the seed that infect the young seedlings, but seeds are usually surface-treated with fungicide.

Seed geraniums are not being pushed to replace cuttings but to develop a new market for geraniums as a bedding plant, along with petunias, impatiens, etc.

Seed-borne Diseases

Seedling diseases are important to the growers because of: (a) poor stands, (b) a decrease in production, (c) excess amount of seed being required, and (d) weakening of the plants, making it much easier for secondary organisms to infect. The control of disease during seed germination is one of the most important tasks of the propagator. The most universally destructive pathogens are those that cause "damping-off," which may cause serious losses of young plants. There are many fungal, viral, and bacterial diseases that are seedborne and are responsible for damping-off and other seedling diseases. In such cases, specific methods of control are required during propagation. Seed treatment with fungicides is the most practical and economically sound means for protection against soilborne or seedborne diseases. Fungicides used to treat seeds, are chloranil, thiram, ferbam, benomyl, captan, and zinc trichlorophenate. All are available under various trade names.

Even though geranium seeds are usually fungicide-treated, it would be of interest to know which, if any disease organisms are on or in the

seeds of 'Sooner Red' and 'Fire Flash' harvested in Baha, California and Costa Rica.[1] This is the area where much of the seed is produced.

Pythium ultimum Trow

Background

The genus Pythium was established by Pringsheim in 1858. During the past several decades, research on plant diseases causing economic loss in specific crops has stressed the role of Pythium spp. as root pathogens. The genus Pythium includes a number of readily recognized species with wide distributions and host ranges.

The disease that tops any geranium grower's list is commonly called stem rot or blackleg. It manifests itself by blackened, rotted areas of the stem. The blackleg disease of geraniums caused by species of Pythium was found by Ward in England in 1883 (19, 31). During the latter part of 1919, P. debaryanum Hesse sensu Middleton and three other fungi belonging to the same genus were isolated from blackened geranium stems in the agricultural greenhouses at Washington D.C., and were found capable of reproducing this condition when inoculated into healthy cuttings (9). According to Middleton (22), Pythium spp. that infected Pelargonium spp. were P. ultimum, P. splendens, P. debaryanum, P. vexans, P. megalacanthum, P. intermedium, P. mamillatum, and other related species. The disease appears first as a brown water-soaking at the base of the cutting or wounds on the young plants. The rotted

---

[1]Seeds for this study were donated by the Ball Seed Company, West Chicago, Illinois. 'Sooner Red' seeds were harvested in Baha, California, and 'Fire Flash' seeds were harvested in Costa Rica.

area rapidly enlarges and turns coal-black, progressing 3 or 4 inches up the stem from the base of the cutting (9).

Pelargonium cuttings were easily infected by Pythium spp. and often mentioned in the early literature (9, 10, 11), whereas little or no information was discussed relative to the blackleg disease of seed geraniums.

Pythium ultimum was first described by Trow (28) in 1901 as a pathogen in rotton cress-seedlings and first mentioned by Gill (22) in 1936 as a pathogen infecting Pelargonium hortorum. According to Middleton (22), P. ultimum is closely related to P. debaryanum which causes serious disease on a variety of plants including Pelargonium spp.. P. ultimum affects a large number of plant species of Aloe, Antirrhinum, Azalea, Begonia, Brassica, Calceolaria, Calendula, Citrus aurantium, Coleus, Dahlia, Euphorbia, Lilium, Pelargonium, and Saintpaulia. In addition to a wide host range, this pathogen also has a wide geographic distribution including areas such as the United States, the Union of South Africa, Canada, England, Philippine Islands, Australia, Rhodesia, France, New Zealand, Germany, and Denmark (22).

Pythium ultimum is perhaps the most common member of the genus in the United States and frequently reported throughout the world and is a major root pathogen affecting a large number of plant species, especially in the seedling stage (1, 13, 18, 20, 22, 25).

Infection

Most Pythium spp. infect mainly juvenile or succulent tissues. This restricts their parasitism to seedlings or the feeder roots or root tips of older plants, and to watery fruits or stem tissues. They

do not spread widely throughout host cells and are quickly followed by more aggressive or faster growing fungi. Juvenile or succulent host tissue is very susceptible to infection and Pythium spp. commonly infect seed and the radicals causing seed rot and pre-emergence damping-off. This disease is most noticeable in nursery beds, greenhouse flats, and row crops because symptons develop suddenly, killing large numbers of seedlings in local areas (13). At a later stage, when cells of stems and main roots have developed secondary wall thickenings, infection is restricted to feeder roots, causing seedlings to become stunted and chlorotic. This early root rot is an important cause of poor growth and yield in many agricultural crops, since plants frequently fail to recover even if conditions become unfavorable for further disease development. Susceptible feeder roots are almost constantly present under perennial plants, and become infected when environmental conditions are favorable.

Many histological studies of penetration have been made. Spencer & Cooper found that infection of cotton roots by germ tubes from zoospores occured in about 2 hours, while infection by mycelial fragments took about 12 hours (26). P. ultimum penetrates the roots of peach by means of infection pegs produced from appressoria. Subsequent growth of this fungus is both intercellular and intracellular. Hyphae of P. ultimum penetrate the root within 5 to 8 hours, mainly at junctions of epidermal cells, with intracellular invasion occuring into an adjacent cell. Colonization of the cortex occurs within 24 hours, and stelar invasion by 36 hours. Cell collapse and separation follow. Colonization of root cells is limited to those cells lacking secondary thickening of the cell walls (23).

Environmental Factors Influencing Disease Development

Disease development depends on environmental conditions, host susceptibility, and the presence of virulent Pythium spp. Soil temperature and moisture are the most important factors. Which factor is more important in any given instances is sometimes dependent on the Pythium species involved (13). Biesbock & Hendrix (8) found that P. vexans responded more to soil moisture than to soil temperature, particularly to a wet-dry cycle involving saturated soil. P. irregulare responded more to soil temperature than to soil moisture. Klisiewicz (17) found that certain Pythium spp. caused more damping-off and root rot at low temperatures, while others were more damaging at high temperatures. Generally, P. irregulare, P. ultimum and related species are more damaging at lower temperatures, while P. myriotylum, P. aphanidermatum, P. arrhenomanes, P. splendens and related species are more damaging at higher temperatures. According to Middleton (22), the minimum temperature for mycelial growth of P. ultimum on corn meal agar was 1°C, an optimum of 28°C, and a maximum of 37°C. Bateman (6) discovered that Poinsettia root rot due to P. ultimum was very severe when the soil moisture level was above 70%, but much less severe at values lower than 70%. P. ultimum saprophytically colonized 8-12% of dead, excised peach roots in soil at 30-90% moisture holding capacity. Griffin (12) found that P. ultimum grew well under conditions where the pore space was filled with either water or air. He concluded that toleration of high soil moisture and conditions of poor gas exchange were an ecological advantage to this fungus.

Survival

Pythium spp. survive in soil by saprophytic growth and by resistant resting structures. They are not vigorous competitors, and their saprophytic activities are greatly restricted (5). Generally, they grow saprophytically only under circumstances where other organisms either are not present or have greatly reduced activity due to environmental conditions.

The chief mechanism of survival for *Pythium* spp. is by means of zoospores and sporangia for short and intermediate periods, and oospores for longer periods. Stanghellini & Hancock (27) found that sporangia of *P. ultimum* persisted for 11 months in air-dried and moist field soil with little or no decrease in either the rate or percentage germination. *P. ultimum* has also been reported to survive at $-18^{o}C$ for 24 months, and in air-dried soil for 12 years (15).

Control Measures

As with many other plant problems, prevention of root rots is the best measure against infection. Most *Pythium* spp., once established in soil, produce oospores that persist for many years. Once established in the soil, these resting structures are virtually impossible to eliminate except with wide spectrum soil fumigants (13).

On a small scale, *Pythium* spp. can be eliminated from soil for greenhouse use by heating. The common method is by steam treatment or pasteurization. The latter method is preferred because it does not kill all organisms or drastically alter soil structure. Excessive heat treatment may so change the biotic flora as to render the soil unfit

for plant growth.

Fumigation with chloropicrin or methyl bromide, or combinations of the two, is now standard practice in many nursery and horticultural operations. An important advantage of fumigation is the control of weeds, many of which also harbor pathogens.

Captan, ferbam, thiram, and zineb have been tried to control Pythium root diseases with limited success in greenhouse tests. Soil drenching has also proved effective. Lesan (Dexon) (p-dimethyl-aminobenzenediazo sodium sulfonate) is active against several Pythium spp. Truban (ETMT, Terrazole) (5-ethoxy-3-trichloromethyl-1,2,4,-thiadiazole) was introduced in 1970. The 1978 Farm Chemical Handbook, indicated that Truban is used for control of Pythium and Phytophthora. It is available in combination with methyl thiophanate as the broad spectrum soil fungicide Banrot. Wheeler, Hine and Boyle (32) found that Terrazole had activity as great or greater than Lesan against Pythium spp.

Most pre- and some post-emergence damping-off may be controlled by seed treatment with a suitable fungicide or combinations of fungicides such as captan or thiram.

In the long run, control of Pythium diseases by resistant cultivars may be a promising and enduring approach to effective control. Due to the wide host range of many Pythium spp., this will be a difficult breeding job.

Research Objectives

The objectives of this study were:

1. To determine what organisms were associated with seed-borne diseases of *Pelargonium hortorum* cultivars 'Sooner Red' and 'Fire Flash';
2. To observe the effectiveness of two fungicides, Truban (Mallinckrodt Inc.) and RE 20615 (Chevron Chemical Co.), against *Pythium ultimum* inoculated on 'Sooner Red' and 'Fire Flash' plants at two different ages; and
3. To observe the effect of time of fungicide application on control of *Pythium ultimum*.

CHAPTER II

MATERIALS AND METHODS

Experiment 1: Isolation and Pathogenicity Test of Seed-borne Organisms

The geranium seed used in this experiment and the following experiments consisted of two cultivars 'Sooner Red' harvested in Baha, California, and 'Fire Flash' harvested in Costa Rica. These seeds were contributed by the Ball Seed Company, West Chicago, Illinois, and had not been treated with a fungicide when received. The experiment was conducted in the transfer room of the Plant Pathology Laboratory.

Isolation of Organisms from Seed

The seeds were divided into two portions. One portion was untreated to determine whether surface-borne pathogens were present. The other portion was surface disinfested to determine if pathogens were present within the seed.

The first portion of 150 seeds of each cultivar were soaked in running tap water for 45 minutes and then rinsed three times with sterile, distilled water. Three seeds were placed (plated-out) on each of 50 water agar (WA) plates.

The second portion of 150 seeds of each cultivar were soaked in running tap water for 45 minutes and then disinfested with a 1:4

dilution of 5.25% sodium hypochlorite (Clorox). The disinfestant was drained off and the seeds washed three times with sterile, distilled water. Three seeds were plated-out on each of 50 WA plates.

All plates were incubated at room temperature (25°C). After 4 weeks, some seeds and seedlings were surrounded with unknown organisms. Each organism was transferred by hyphal tips onto Potato-Dextrose Agar (PDA) for inoculum increase. Each PDA plate received one organism and was incubated at room temperature.

Pathogenicity Test

First Pathogenicity Test

Surface-disinfested seeds of the two cultivars were plated-out on PDA plates and germinated at room temperature. Each PDA plate received 10 evenly separated seeds of the same cultivar. The plates were examined with a low-power dissecting microscope at 24-hour intervals. Some seeds were free of contamination and some contained contaminating colonies which were carefully cut from the agar. Approximately 5 days later, seedlings which appeared normal and organism-free under the dissecting microscope were individually transplanted. Three seedlings of the same cultivar were moved with forceps and placed at regular intervals on a special agar in Bi-petri plates (30)(Figure 3, P. 26). One half of each Bi-petri plate was filled with 2% agar made with 2/3-strength Hoagland's solution (14). The Hoagland-agar plates (HAP) were placed horizontally at room temperature for 6-8 hours. This allowed the primary root to start down into the agar. The plates were then placed on edge to allow the seedlings to orient normally, and allow the

tops to grow upward into the agar-free half of the plate. The seedlings received 200 ft-c of cool white fluorescent light for 8 hours a day.

Four days after transplanting young seedlings to HAP, the organisms previously isolated from the seed were cut with a cork borer into several agar discs and one disc of inoculum was placed between the roots of 2 adjacent seedlings about 6 mm below the upper HAP surface. Each HAP plate received one kind of organism which was isolated from the same cultivar as the seedlings grown on the HAP plate. The mycelia grew into contact with the seedlings. Two weeks after inoculation, some organisms were found to be pathogenic, some were not. Those having pathogenicity were reisolated on PDA plates. Pure cultures were made at this time by hyphal tip transfer.

Second Pathogenicity Test and Identification

The pure cultures were tested again for pathogenicity using the same method and procedure described above. Each organism was inoculated on both seedling cultivars and 3 replications were made. Two weeks after inoculation, those again showing pathogenicity were collected and identified.

Pathogenicity Test on Different Seedling Stages

The seedlings of both cultivars were grown on HAP until 1, 2, 3, 4, 5, and 6-wk-old. The pathogenic organisms identified were used to inoculate these six seedling stages. In addition, three nonpathogenic organisms isolated from the same seed were used to inoculate the 1-wk-old seedlings. The inoculation procedure was the same as above.

Experiment 2: Comparison of Two Fungicides on Control of *Pythium* Blackleg on 14-wk-old Plants

Experimental Treatment

A total of 12 treatments with two cultivars were as follows:

'Sooner Red'

1. Control -- Non infested soil; no fungicides
2. Control -- Soil artificially infested with *Pythium ultimum*; no fungicides
3. Infested, Truban applied at transplanting
4. Infested, Truban applied one week after transplanting
5. Infested, RE 20615 applied at transplanting
6. Infested, RE 20615 applied one week after transplanting

'Fire Flash'

Treatments 7 through 12 were in the same sequence as shown above.

Seedling Culture

'Sooner Red' and 'Fire Flash' seed treated with Thiram were directly seeded with 1 seed per 7.62 cm (3-inch) plastic pot on January 31, 1979. The growing medium was Redi-Earth[1] which had been steam sterilized.

Germination was under an intermittent mist system and a 21°C

---

[1]Redi-Earth is a commercial peat-vermiculite growing medium, from W.R. Grace Company.

(70°F) soil temperature was maintained. After germination, the seedlings were moved to a greenhouse maintained at a night temperature of 17°C (62°F) and a day temperature of 21-24°C (70-75°F).

Plants were watered with tap water applied with a clean beaker so as to prevent splashing. The soil was allowed to become dry to the touch between waterings. After establishment in 7.62 cm pots, the seedlings were fertilized with 500 ppm of N, P_2O_5 and K_2O using 20-20-20 weekly.

The seedlings were utilized in the experiment when they were 14-wk-old.

Inoculum Preparation

A culture of Pythium ultimum was obtained from the University of Illinois, Urbana, Illinois.[2] The source was from beans from the Experiment Station at Geneva, New York. A pure culture was made by hypal tip transfer after the culture was received. The pure isolate was maintained on PDA and transferred monthly. The same isolate was used for all experiments.

For soil infestation, the fungus was grown on a medium of corn-meal and sand.[3] The pure isolate was grown in the medium in one-quart fruit jars for 12-14 days at room temperature. The inoculum then was dumped out and ground finely in a surface-sterilized grinder. This

---

[2]Courtesy of Dr. R. D. Neely, Plant Pathology Department.

[3]Corn-meal sand medium:

Water	1500 ml
Corn-meal	1000 g
Washed white sand	1000 g

finely ground inoculum was mixed well to give a uniform organism density.

Fungicides

The fungicides used were Truban (30 W.P.)[4] and a new experimental fungicide - RE 20615 (50 W.P.).[5] According to recommendations, the rates used were:

Truban - 2.3 g per 3.78 liter (8 oz/100 gal of water) and 90 ml per 11.43 cm plastic pot (3 oz/4.5" pot).

Re 20615 - 1.4 g per 3.78 liter (4.8 oz/100 gal of water) and 90 ml per 11.43 cm plastic pot (3 oz/4.5" pot).

Soil Infestation and Fungicide Application

Transplanting and infestation were done May 9, 1979. In transplanting the 14-wk-old plants from 7.62 cm pots to 11.43 cm pots, the root balls were handled with minimum damage. Each 11.43 cm pot received 120 g of steam-sterilized Redi-Earth and 60 g of prepared inoculum. The growing medium and inoculum were mixed thoroughly.

The application of each fungicide to the 11.43 cm pot was made immediately after transplanting and divided into two parts: forty-five ml of fungicide was applied first, 104 ml of tap water was added

---

[4]Truban:
Mallinckrodt Chemical Inc.
5-ethoxy-3-trichloromethyl-1,2,4-thiadiazole

[5]RE 20615:
Chevron Chemical Co.
2-chloro-N-(2,6-dimethylphenyl)-N-(tetrahydro-2-oxo-3-furanyl) acetamide

followed by another 45 ml of fungicide and finally another 104 ml of water. This split application was to improve the penetration of fungicides. The total amount of fluid the 11.43 cm pot received was 298 ml (10 oz) of which 90 ml was fungicide and 208 ml water. No leaching occurred.

The fungicides were applied at 4-week intervals following the rates and method described above.

Watering System

In order to avoid splashing water and possibly leaching the organism and fungicides through the bottom holes of the pot, a plastic saucer 17 cm in diameter holding about 267 ml water was placed under each 11.43 cm pot. Water was added to the saucer and the water was absorbed by capillary movement. The saucers were filled every other day and any water remaining in the saucer was dumped out 8 hours later. Plants were fertilized with 500 ppm of N, P_2O_5 and K_2O using 20-20-20[6] weekly, applied in the saucers.

Experimental Design

A randomized complete block design was used with one pot per treatment that was replicated twelve times.

Physical Arrangement

The study was conducted in the Horticulture Greenhouses. Twelve wire benches each 107 x 365 cm were used. Pots were spaced 54.8 x 76.2

---

[6]Peter Fertilizer Products, W. R. Grace & Co.

cm apart. The blocks ran from east to west along the length of the bench. Block 1 was closest to the cooling pads while block 12 was farthest from the pads (Figure 1). Border plants were placed on the east and west ends of the bench.

Environmental Conditions

The plants were transplanted and inoculated May 9, 1979 and the termination date of the experiment was June 20, 1979. During this period of time, the soil temperature was approximately 21-24°C (70-75°F), the night air temperature was about 20°C (68°F), and the day temperature was approximately 24-31°C (75-88°F). Light intensity was approximately 4000-4500 foot-candles during the brightest part of the day.

Phytotoxicity Test

Forty-eight plants were used to test phytotoxicity. Twenty-four plants of each cultivar were treated with Truban and another 24 plants of each cultivar were treated with RE 20615 at the rate and amounts per pot previously described.

Data Recorded

1. Top rating - A scale of 1-7 was used, 1 having the lowest disease severity and 7 having the highest (Figure 2). Features taken into consideration were: degree of chlorosis, bloom quality, amount of vegetative growth, and number of breaks. Top rating was taken twice, the first time was four weeks after inoculation and the second was six weeks after inoculation.

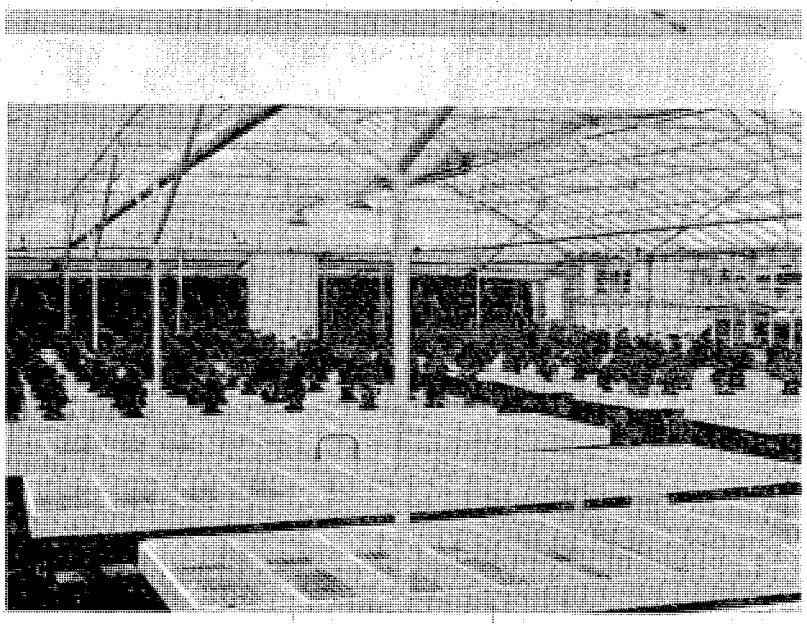
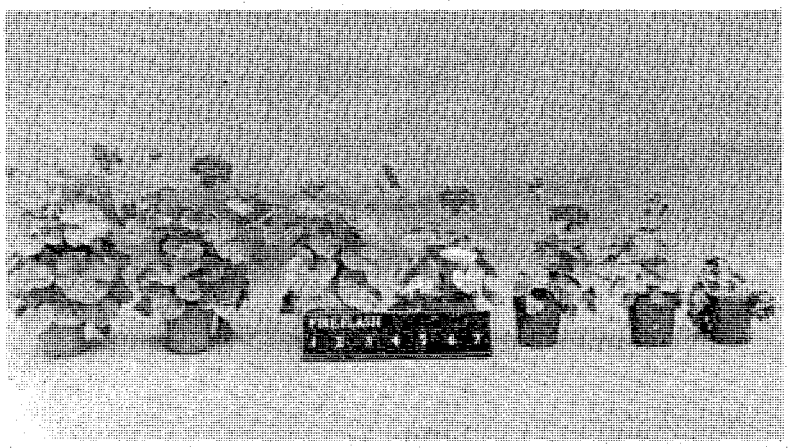


Figure 1. View of Overall Experiment for 14-wk-old Plants for Pythium - Fungicide Studies. Plants Were Set in A Randomized Complete Block Design With Border Plants on the East and West Sides of Each Bench.



1: Healthy
7: Most Severe
Cultivar: 'Fire Flash'

Figure 2. Top Rating Scale From 1-7

All data below were recorded six weeks after inoculation only.

2. Plant height (cm) - The height was measured from the soil line to the first top node of the highest branch.

3. Top fresh weight (g) - The above-ground plant parts were carefully handled without losing dead tissue still hanging on the plant. The fresh top was weighed as soon as it was cut off.

4. Ratio of the length of stem discoloration to the top height in percentage.

Other Data Recorded

1. Flower Bud Formation -- To observe whether flower buds formed after inoculation.

2. Reisolation -- Stems from which isolations were to be made were first scrubbed free of adhering soil, then rinsed in 75% alcohol. Bits of tissue at the infection margin were quickly excised with a flamed scalpel and placed on water agar plates which were incubated at room temperature. About 36 hours later, hypal tip transfer to PDA plates was made and after a week of growth the isolates were identified.

Experiment 3: Comparison of Two Fungicides on Control of *Pythium* Blackleg on 7-wk-old Plants

Except as indicated below all experimental procedures were the same as those employed in Experiment 2.

The age of the plants used was 7 weeks. Watering was done every two days. The transplanting and inoculation date was June 13, and the

termination date was July 26, 1979. During this period of time, the soil temperature was 22-31°C (72-88°F), the night air temperature was about 22°C (72°F), and day temperature range was 27-35°C (80-95°F). The light intensity was approximately 4500-5000 foot-candles during the brightest part of the day.

CHAPTER III

RESULTS AND DISCUSSION

Experiment 1: Isolation and Pathogenicity Test of Seed-borne Organisms

Results

After the second pathogenicity test, seven pathogenic isolates were obtained. Five were identified as Alternaria spp., one as Nigrospora sp., and one as Curvularia sp.. Curvularia sp. was isolated from 'Fire Flash' with no surface-disinfestion; Nigrospora sp. from 'Sooner Red' with surface-disinfestion; and Alternaria was isolated twice from 'Sooner Red', once with surface-disinfestion, once with no surface-disinfestion and three Alternaria isolates were obtained from 'Fire Flash', two with no surface-disinfestion and one with surface-disinfestion (Table I). In addition, five nonpathogenic organisms -- species of Aspergillus, Penicillium, Torula, Hormicium and Cladosporium were also isolated.

The three isolated seed-borne pathogens -- Alternaria, Nigrospora and Curvularia, were used to inoculate the six different seedling stages of both cultivars. These three isolates proved to be highly pathogenic to 1, 2, and 3-wk-old seedlings of both cultivars, but they had little or no effect on 4, 5, and 6-wk-old seedlings of both cultivars. Species of Aspergillus, Cladosporium and Torula were not

TABLE I

PATHOGENIC ORGANISMS ISOLATED FROM SEED

	Sooner Red		Fire Flash		
Organisms	SD[y]	NSD[z]	SD	NSD	Total
Alternaria	1[x]	1	1	2	5
Nigrospora	1				1
Curvularia				1	1

[x]Number of isolates

[y]Surface-disinfested (treated with 1:4 Clorox)

[z]Non-surface-disinfested

pathogenic on 1-wk-old seedlings of either cultivar (Figure 3).

Discussion

Both 'Sooner Red' and 'Fire Flash' had seed-borne pathogens regardless of the location where they were produced. According to the results, Alternaria might be in or on the seed, Curvularia on the seed, and Nigrospora in the seed. However, this may not be the case. Further study is needed to determine if the pathogens are carried internally. Alternaria spp. were isolated five times from seed, and appears to be a more prevalent seed-borne pathogen.

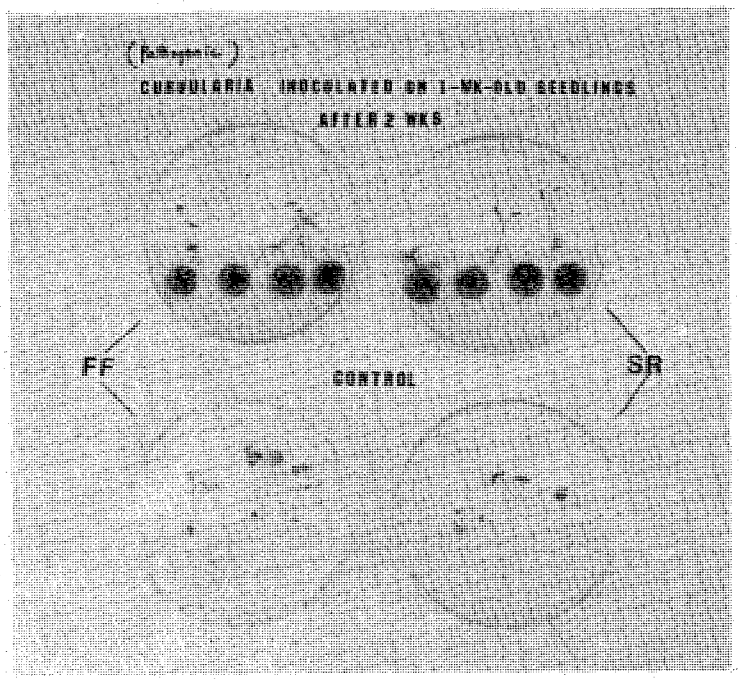
Alternaria, Nigrospora, and Curvularia were very pathogenic to 1, 2, and 3-wk-old seedlings, but appear to be less virulent as the seedling age increases. This means that they are weak pathogens but the fungicidal seed treatment is still needed to get a good germination rate.

Aspergillus, Cladosporium, and Torula caused no damage on 1-wk-old seedlings whereas Alternaria, Nigrospora, and Curvularia did. This means that pathogenicity was not associated with age of seedlings in these studies and that the former three organisms were not pathogenic.

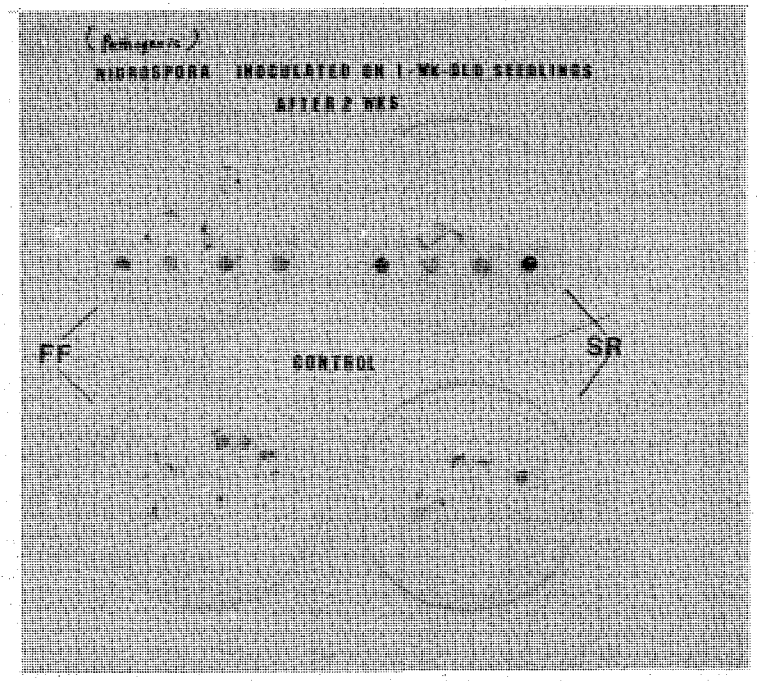
It should be pointed out that the results of isolations vary according to the techniques used. Other pathogens might have been isolated with different techniques.

Experiment 2: Comparison of Two Fungicides on Control of Pythium Blackleg on 14-wk-old Plants

According to the Analysis of Variance (AOV), the data for

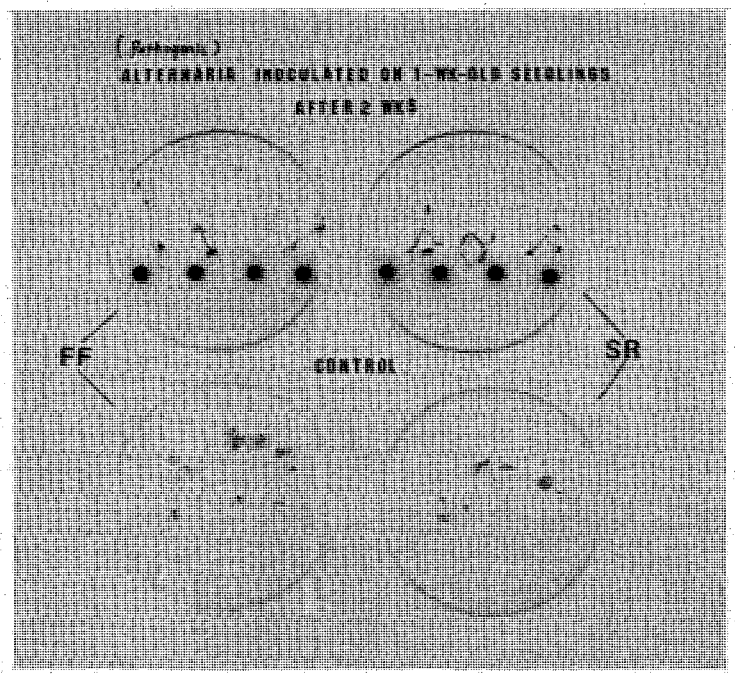


a). Inoculated with Curvularia on 1-wk-old Seedlings.
Upper Left: 'Fire Flash', Dead
Upper Right: 'Sooner Red', Dead
Lower Left: 'Fire Flash', Control
Lower Right: 'Sooner Red', Control

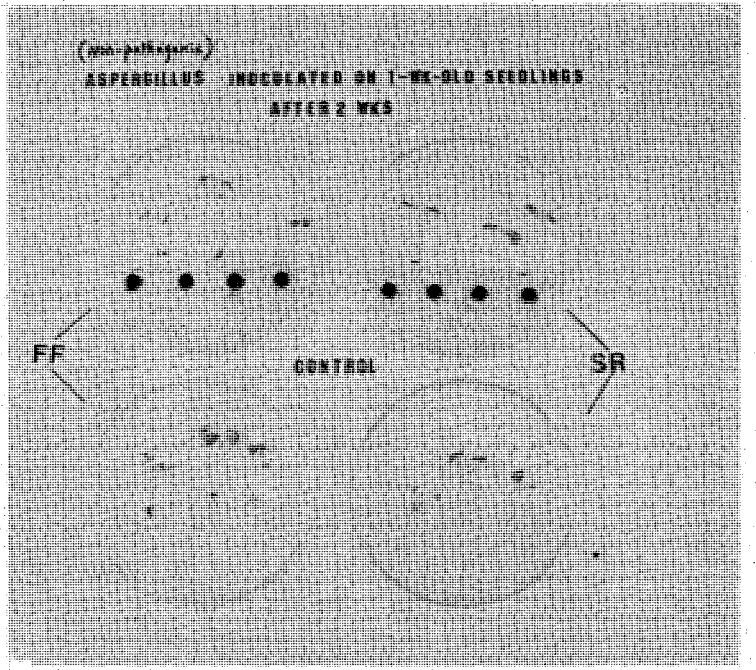


b). Inoculated with Nigrospora on 1-wk-old Seedlings.
Upper Left: 'Fire Flash', Dead
Upper Right: 'Sooner Red', Dead
Lower Left: 'Fire Flash', Control
Lower Right: 'Sooner Red', Control

Figure 3. Geranium Seedlings Growing in Bi-petri Plates on Agar Made With 2/3-Strength Hoagland's Solution (HAP) After a 2-Wk-Infection Period.

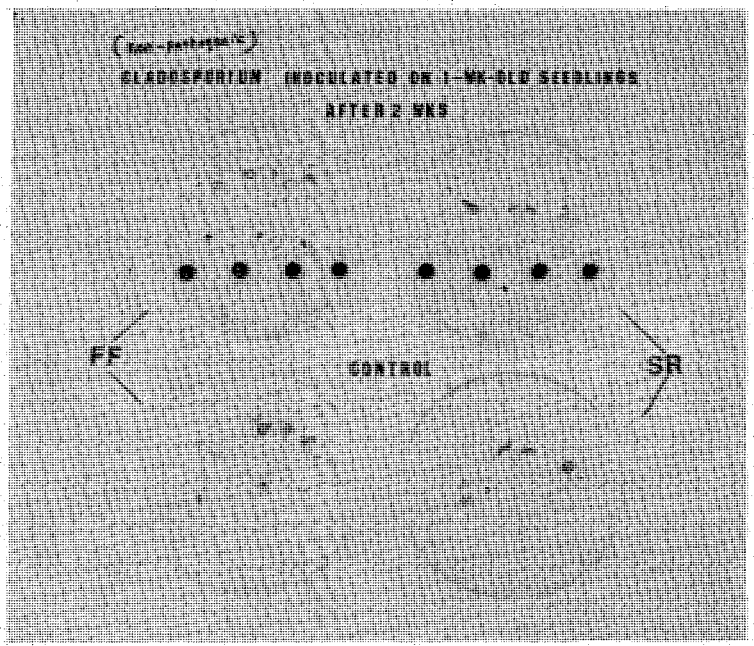


c). Inoculated with Alternaria on 1-wk-old Seedlings.
Upper Left: 'Fire Flash', Dead
Upper Right: 'Sooner Red', Dead
Lower Left: 'Fire Flash', Control
Lower Right: 'Sooner Red', Control

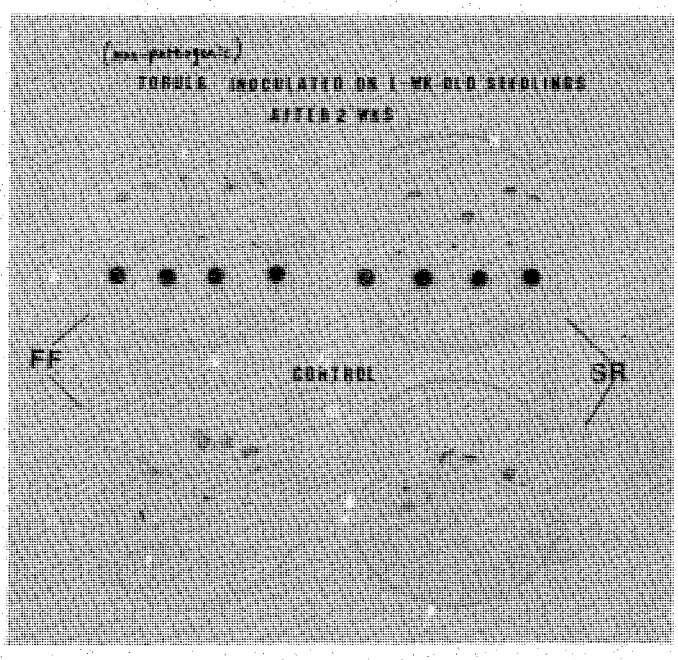


d). Inoculated with Aspergillus on 1-wk-old Seedlings.
Upper Left: 'Fire Flash', Healthy
Upper Right: 'Sooner Red', Healthy
Lower Left: 'Fire Flash', Control
Lower Right: 'Sooner Red', Control

Figure 3. (Continued)

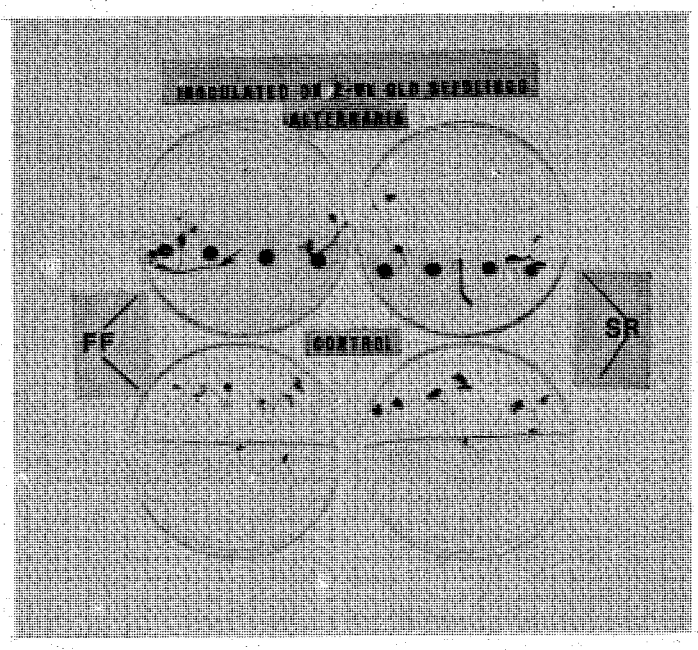


e). Inoculated with Cladosporium on 1-wk-old Seedlings.
Upper Left: 'Fire Flash', Healthy
Upper Right: 'Sooner Red', Healthy
Lower Left: 'Fire Flash', Control
Lower Right: 'Sooner Red', Control

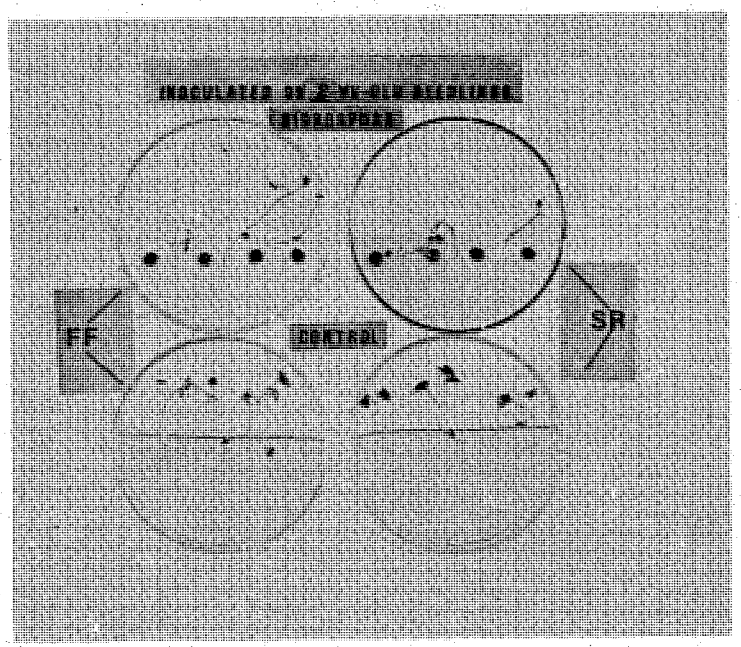


f). Inoculated with Torula on 1-wk-old Seedlings.
Upper Left: 'Fire Flash', Healthy
Upper Right: 'Sooner Red', Healthy
Lower Left: 'Fire Flash', Control
Lower Right: 'Sooner Red', Control

Figure 3. (Continued)

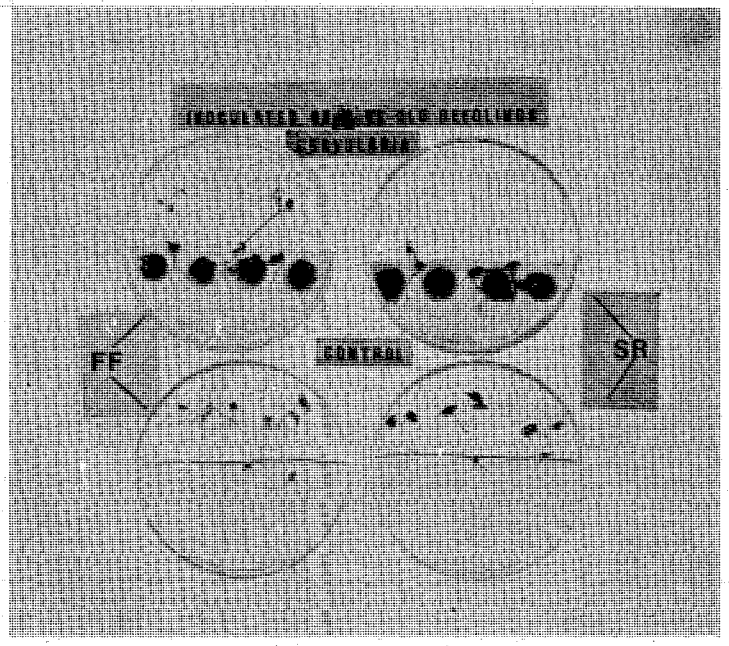


g). Inoculated with Alternaria on 2-wk-old Seedlings.
Upper Left: 'Fire Flash', Dead
Upper Right: 'Sooner Red', Dead
Lower Left: 'Fire Flash', Control
Lower Right: 'Sooner Red', Control

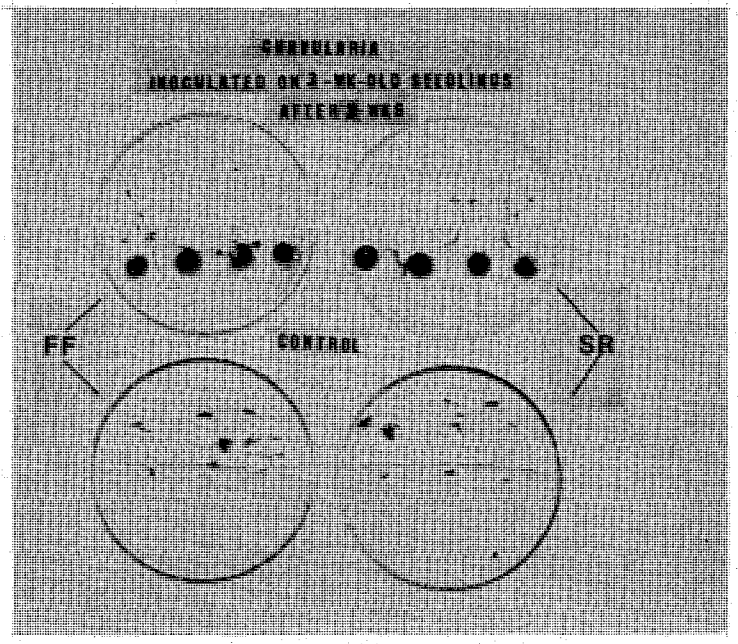


h). Inoculated with Nigrospora on 2-wk-old Seedlings.
Upper Left: 'Fire Flash', Dead
Upper Right: 'Sooner Red', Dead
Lower Left: 'Fire Flash', Control
Lower Right: 'Sooner Red', Control

Figure 3. (Continued)

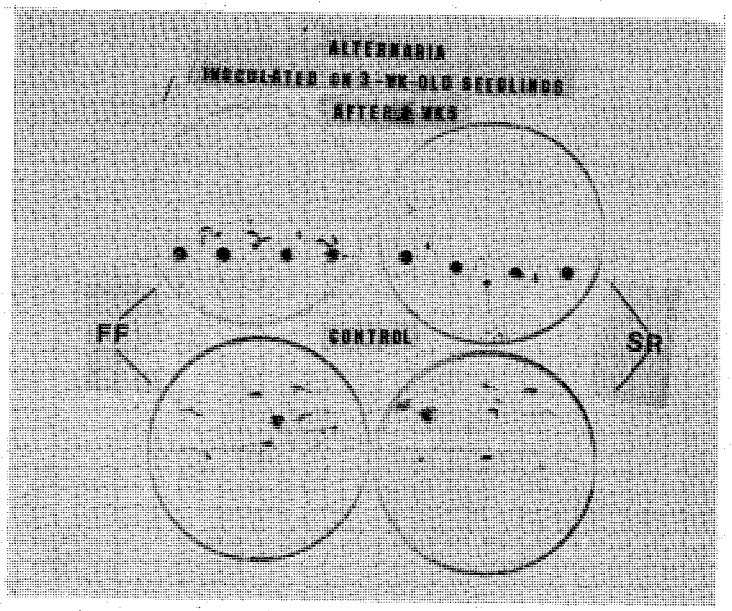


i). Inoculated with Curvularia on 2-wk-old Seedlings.
Upper Left: 'Fire Flash', Dead
Upper Right: 'Sooner Red', Dead
Lower Left: 'Fire Flash', Control
Lower Right: 'Sooner Red', Control

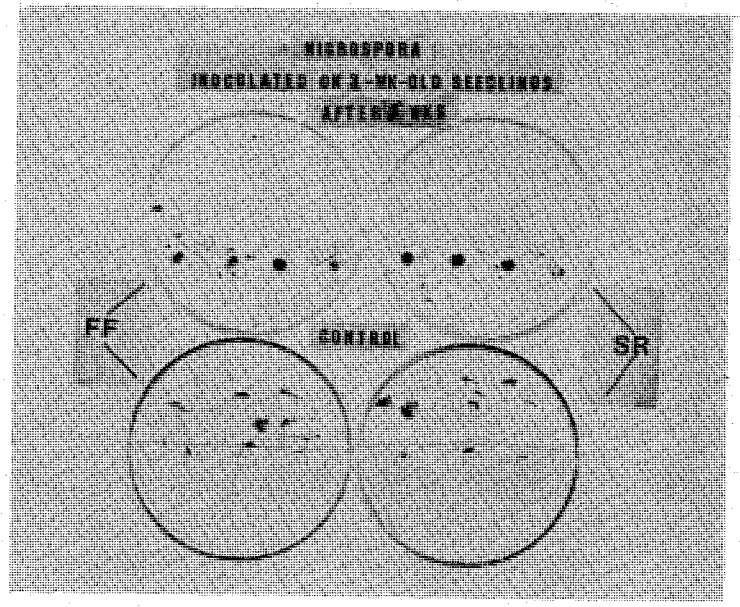


j). Inoculated with Curvularia on 3-wk-old Seedlings.
Upper Left: 'Fire Flash', Dead
Upper Right: 'Sooner Red', Dead
Lower Left: 'Fire Flash', Control
Lower Right: 'Sooner Red', Control

Figure 3. (Continued)

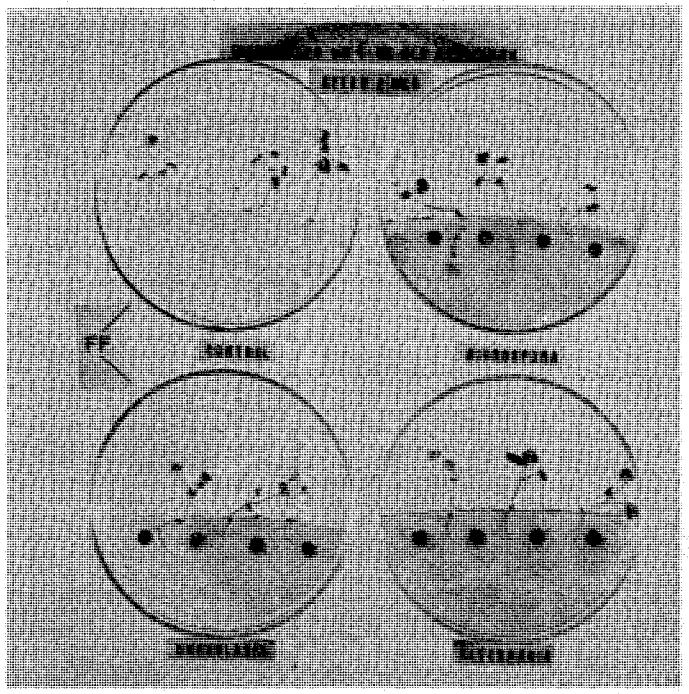


k). Inoculated with Alternaria on 3-wk-old Seedlings.
Upper Left: 'Fire Flash', Dead
Upper Right: 'Sooner Red', Dead
Lower Left: 'Fire Flash', Control
Lower Right: 'Sooner Red', Control

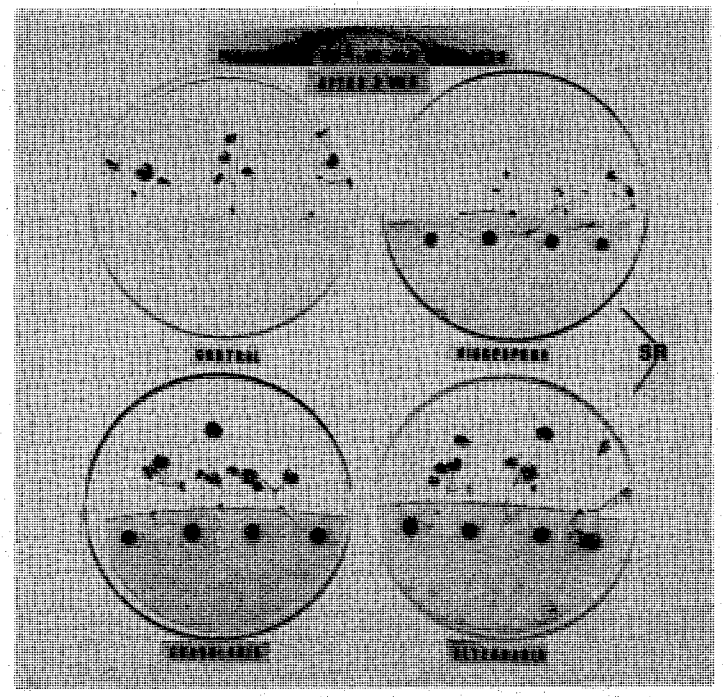


1). Inoculated with Nigrospora on 3-wk-old Seedlings.
Upper Left: 'Fire Flash', Dead
Upper Right: 'Sooner Red', Dead
Lower Left: 'Fire Flash', Control
Lower Right: 'Sooner Red', Control

Figure 3. (Continued)

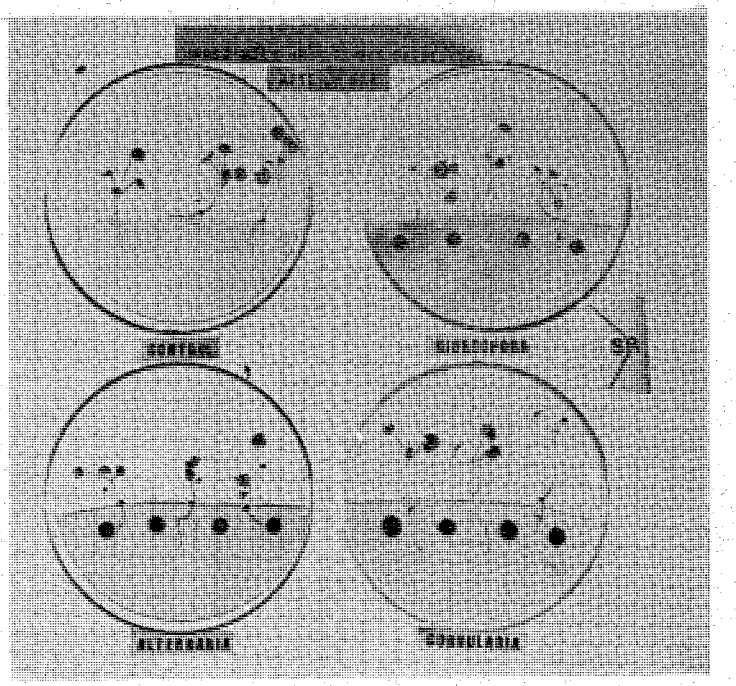


m). Four-wk-old Seedlings of 'Fire Flash' Inoculated with:
Nigrospora - One Dying, No Effect on the Others
Curvularia - No Effect
Alternaria - No Effect

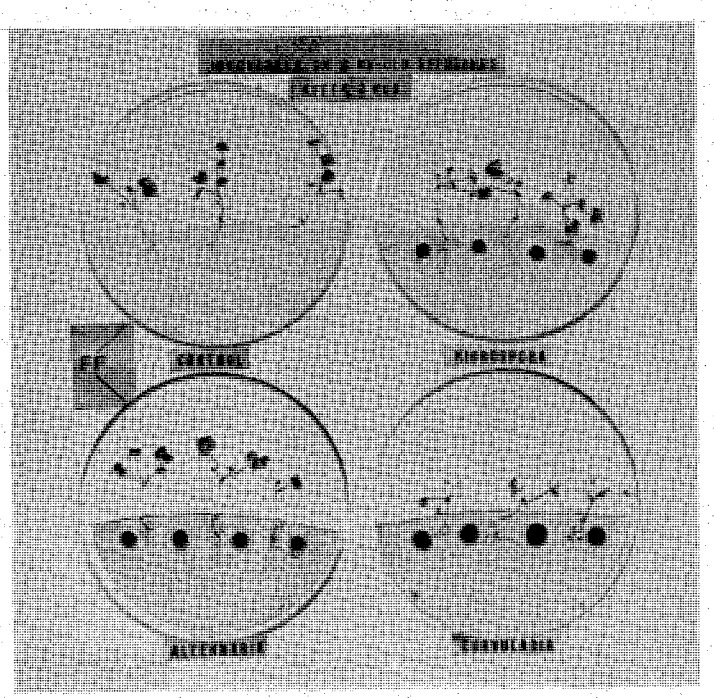


n). Four-wk-old Seedlings of 'Sooner Red' Inoculated with:
Nigrospora - One Dying, No Effect on the Others
Curvularia - No Effect
Alternaria - No Effect

Figure 3. (Continued)

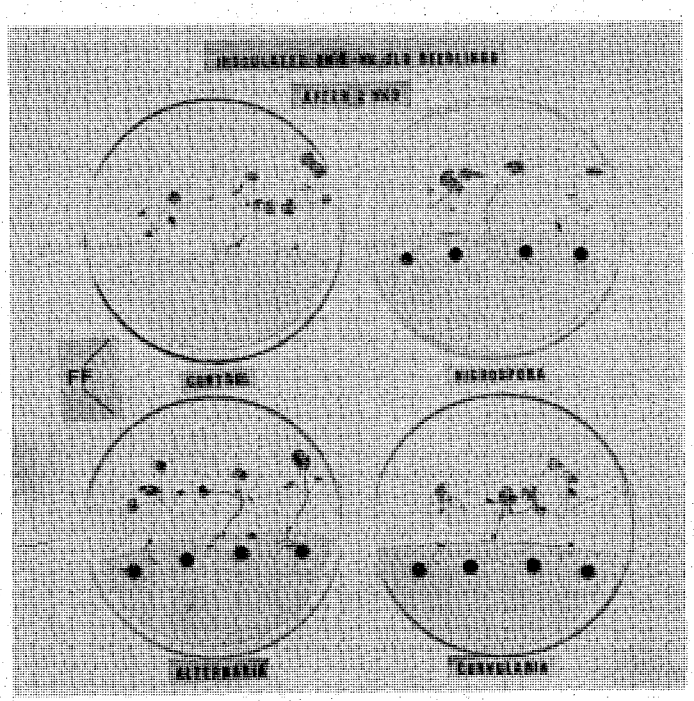


o). Five-wk-old Seedlings of 'Sooner Red'
Inoculated with:
Nigrospora - No Effect
Alternaria - No Effect
Curvularia - No Effect

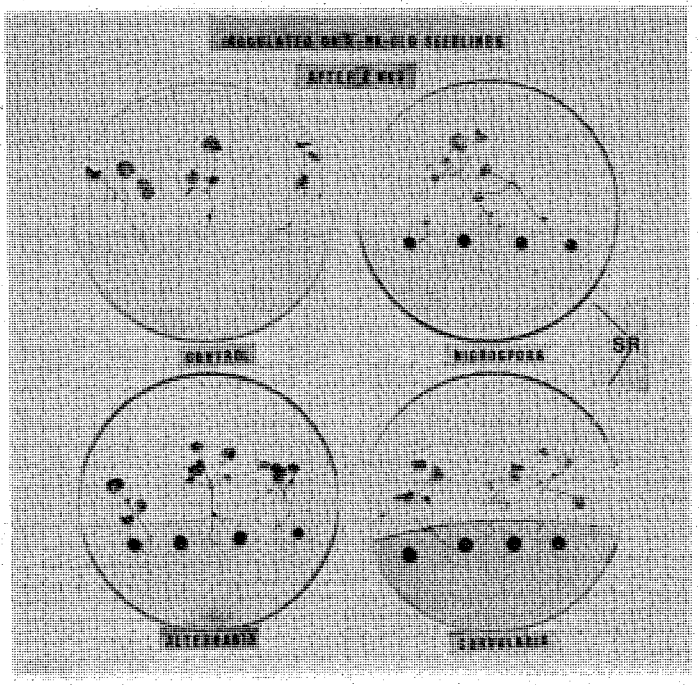


p). Five-wk-old Seedlings of 'Fire Flash'
Inoculated with:
Nigrospora - No Effect
Alternaria - No Effect
Curvularia - No Effect

Figure 3. (Continued)



q). Six-wk-old Seedlings of 'Fire Flash'
Inoculated with:
Nigrospora - No Effect
Alternaria - No Effect
Curvularia - No Effect



r). Six-wk-old Seedlings of 'Sooner Red'
Inoculated with:
Nigrospora - No Effect
Alternaria - No Effect
Curvularia - No Effect

Figure 3. (Continued)

experiment 2 showed two common results: the non-inoculated control was always significantly different from the inoculated control and the inoculated control was significantly different from the plants which were inoculated and received fungicide treatments (Appendix, Table VII-XII). It was evident in Figure 4 that the inoculated control was very severe and non-inoculated control was healthy for both cultivars.

Top Rating

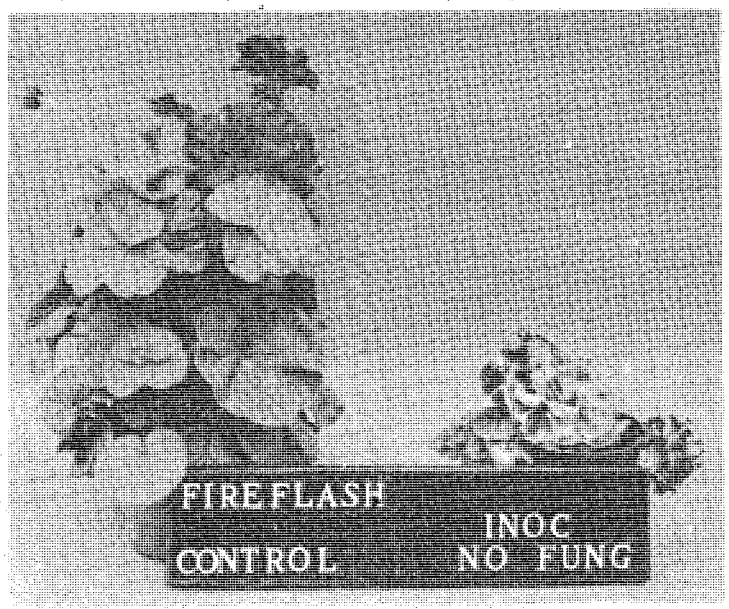
According to the AOV for the early top rating made 4 weeks after inoculation (Appendix, Table VII), type of fungicide and the time of fungicide application had a significant effect on the top quality (Table II). This indicated that better top growth was obtained with RE 20165 than Truban and that fungicides applied at transplanting were better than when applied 1 week later (Figure 5). There were no interactions among treatments.

According to the AOV for the late top rating made 6 weeks after inoculation (Appendix, Table VIII), the results were the same as those of the early top rating (Table III, Figure 6).

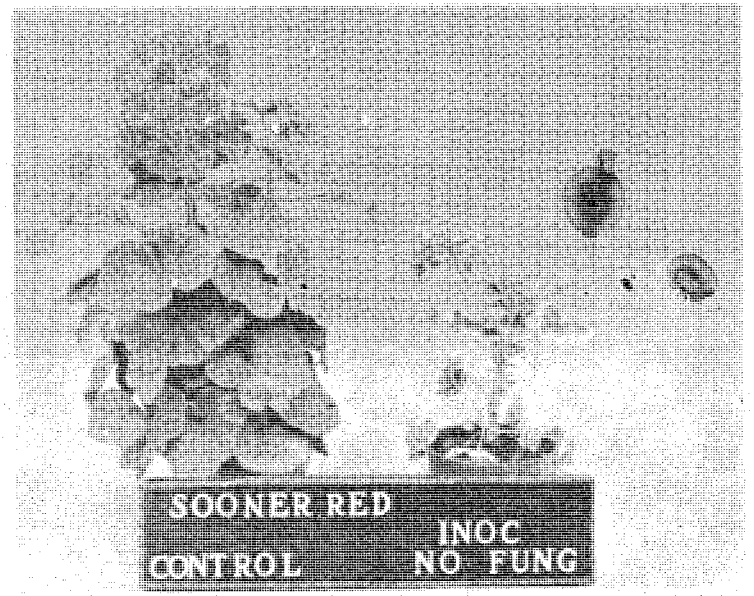
There was a significant difference between the early and the late top ratings (Appendix, Table IX). This indicated that over all treatments top quality at four weeks after inoculation was better than that six weeks after inoculation.

Plant Height

According to the AOV (Appendix, Table X), cultivar, type of fungicide and time of fungicide application had significant effects on height. This indicated that 'Sooner Red' was taller than 'Fire Flash',



a). Cultivar: 'Fire Flash'
Inoculated Control (right): Dying
Non-inoculated Control (left): Healthy



b). Cultivar: 'Sooner Red'
Inoculated Control (right): Dying
Non-inoculated Control (left): Healthy

Figure 4. Comparison Between Inoculated Control and Non-inoculated Control 6 Weeks After Inoculation for 14-Wk-Old Plants.

TABLE II

MEANS OF EARLY TOP RATING* FOR THE SECOND (14-WK-OLD) AND THE THIRD (7-WK-OLD) EXPERIMENTS

Treatment	Fire Flash		Sooner Red		Average		Totals	
	14-wk	7-wk	14-wk	7-wk	14-wk	7-wk	14-wk	7-wk
Control non-inoculated	1.000	1.000	1.000	1.166	1.000	1.083		
Control inoculated	4.208	4.250	3.916	5.041	4.062	4.645		
Average	2.604	2.625	2.458	3.103				
Truban at transplanting	2.708	3.666	3.291	3.833	3.000	3.750	3.239	3.854
1 wk later	3.291	3.750	3.666	4.166	3.479	3.958		
Average	3.000	3.708	3.479	4.000				
RE 20615 at transplanting	2.791	3.916	2.416	3.833	2.604	3.875	2.75	3.375
1 wk later	2.750	2.791	3.041	2.958	2.895	2.875		
Average	2.770	3.354	2.729	3.395				
Fungicides applied at transplanting	2.75	3.791	2.854	3.833	2.802	3.812		
1 wk later	3.020	3.270	3.354	3.562	3.187	3.416		

*Rating made 4 weeks after inoculation, based on a scale of 1-7, 1 healthy 7 most severe

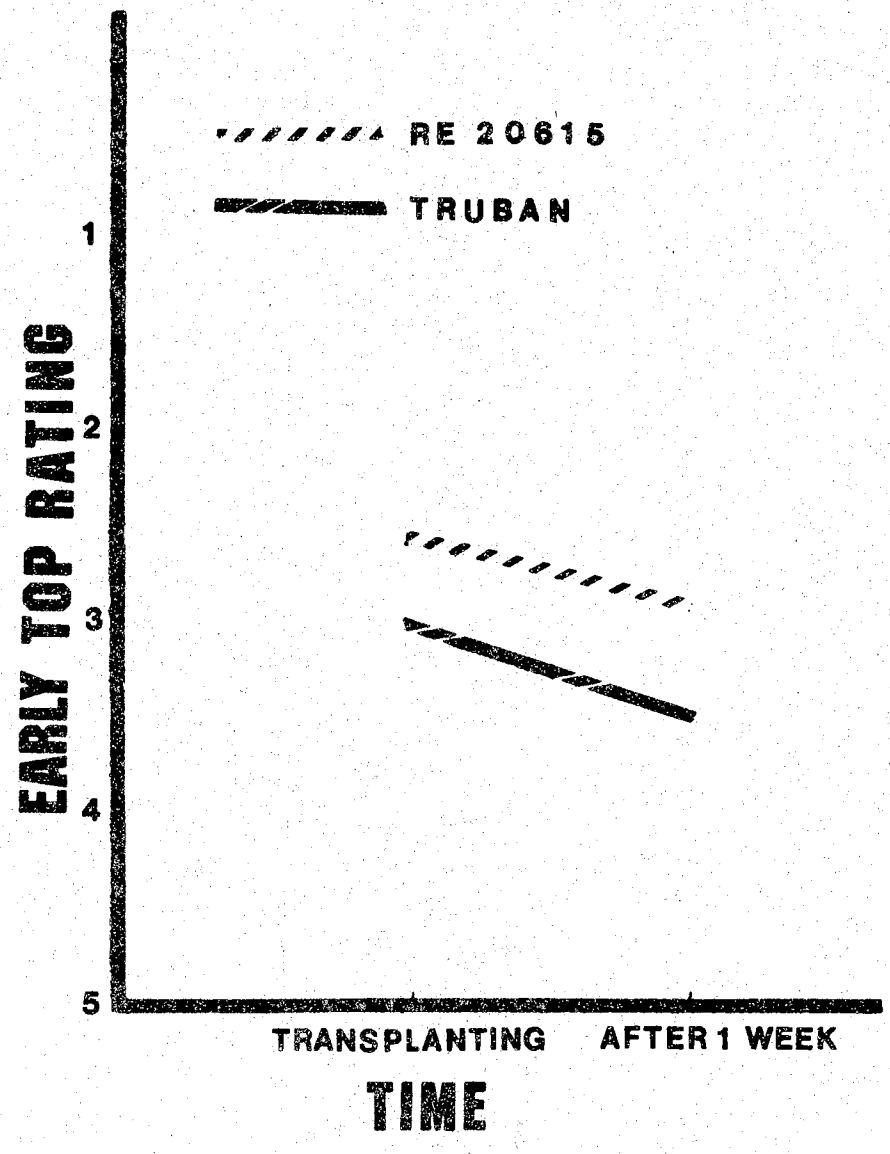


Figure 5. The Effect of Fungicides (Truban and RE 20615) and Timing of Application. Rating Made 4 Weeks After Inoculation for 14-wk-old Plants, Using a Scale of 1-7 with 1 Healthy 7 Most Severe.

TABLE III

MEANS OF LATE TOP RATING* FOR THE SECOND (14-WK-OLD) AND THE THIRD (7-WK-OLD) EXPERIMENTS

Treatment	Fire Flash		Sooner Red		Average		Totals	
	14-wk	7-wk	14-wk	7-wk	14-wk	7-wk	14-wk	7-wk
Control non-inoculated	1.241	1.000	1.183	1.166	1.212	1.083		
Control Inoculated	4.458	4.791	4.000	5.458	4.229	5.125		
Average	2.850	2.895	2.591	3.312				
Truban at transplanting	2.900	4.083	3.350	4.041	3.125	4.062	3.402	4.416
1 wk later	3.300	4.833	4.058	4.708	3.679	4.770		
Average	3.100	4.458	3.704	4.375				
RE 20615 at transplanting	2.708	3.291	2.391	3.666	2.550	3.479	2.800	3.125
1 wk later	2.833	2.666	3.266	2.875	3.050	2.770		
Average	2.770	2.979	2.829	3.270				
Fungicides applied at transplanting	2.804	3.687	2.870	3.854	2.837	3.770		
1 wk later	3.066	3.750	3.662	3.791	3.364	3.770		

*Rating made 6 weeks after inoculation, using a scale of 1-7, 1 healthy 7 most severe

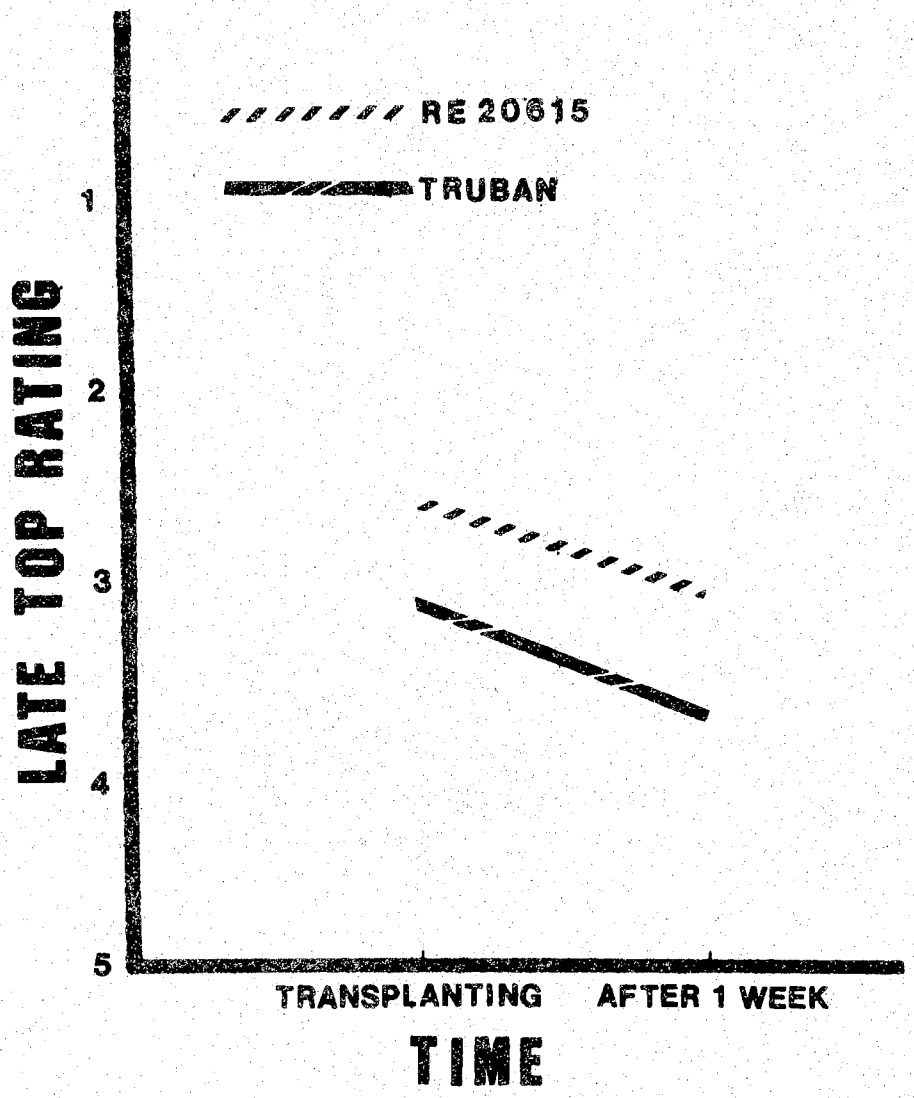


Figure 6. The Effect of Fungicides (Truban and RE 20615) and Timing of Application. Rating Made 6 Weeks After Inoculation for 14-wk-old Plants, Using a Scale of 1-7 with 1 Healthy 7 Most Severe.

RE 20615 was better than Truban relative to plant height and greater plant height occurred when fungicides were applied at transplanting than when applied one week later (Table IV).

There was an interaction between type of fungicide and cultivar (Figure 7). This indicated that 'Sooner Red' was more sensitive to RE 20615 than Truban while type of fungicide showed no effect on 'Fire Flash'.

Top Fresh Weight

According the the AOV (Appendix, Table XI), cultivar, type of fungicide and the time of fungicide application had significant effects on top weight (Table V, Figure 8). The results indicated that 'Fire Flash' gained more weight than 'Sooner Red', more top weight was obtained with RE 20615 and greater top weight was obtained when fungicides were applied at transplanting than when applied one week after transplanting. There were no interactions among treatments.

Ratio of Length of Stem Discoloration to Top Height in Percentage

According to the AOV (Appendix, Table XII), there was an interaction between cultivars and inoculated control. This indicated that when inoculating with P. ultimum with fungicide application, 'Fire Flash' was more diseased than 'Sooner Red'. There was no significant effects relative to type of fungicide, time of fungicide application and interaction between them (Table VI).

TABLE IV

MEANS OF PLANT HEIGHT (CM) FOR THE SECOND (14-WK-OLD) AND THE THIRD (7-WK-OLD) Experiments

Treatment	Fire Flash		Sooner Red		Average		Totals	
	14-wk	7-wk	14-wk	7-wk	14-wk	7-wk	14-wk	7-wk
Control non-inoculated	28.166	16.000	31.416	17.125	29.791	16.562		
Control inoculated	17.583	6.250	20.750	5.458	19.166	5.854		
Average	22.875	11.125	26.083	11.291				
Truban at transplanting	23.500	7.541	23.250	8.541	23.375	8.041		
1 wk later	20.500	7.750	20.416	7.225	20.458	7.487	21.916	7.764
Average	22.000	7.645	21.833	7.883				
RE 20615 at transplanting	23.166	7.391	28.916	8.566	26.041	7.979		
1 wk later	21.166	10.183	23.416	11.041	22.291	10.612	24.166	9.295
Average	22.166	8.787	26.166	9.804				
Fungicides applied at transplanting	23.333	7.466	26.083	8.554	24.708	8.010		
1 wk later	20.833	8.966	21.916	9.133	21.375	9.050		

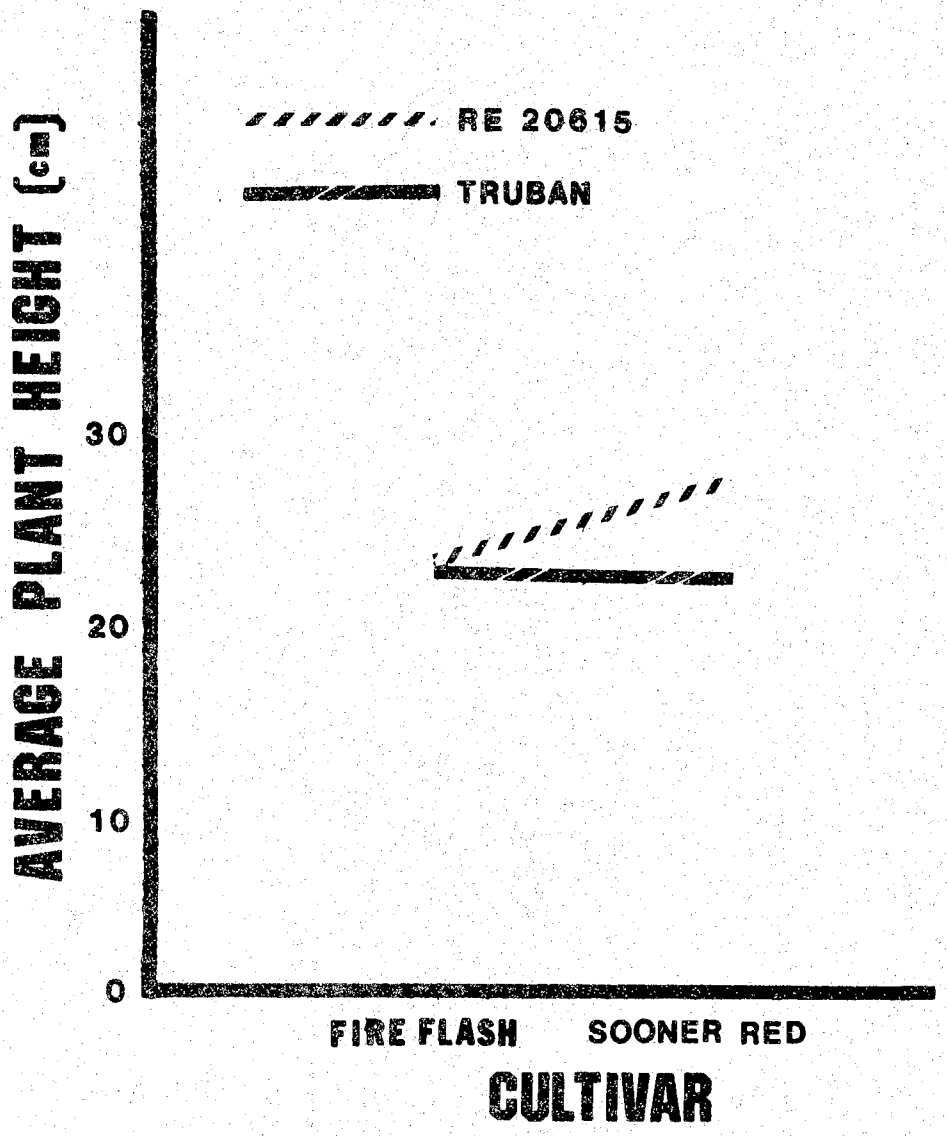


Figure 7. The Effect of Interaction Between Cultivars and Type of Fungicide in Controlling P. ultimum on 14-wk-old Plants. Two Unparallel Lines Indicate That There Was an Interaction.

TABLE V

MEANS OF TOP WEIGHT[x] (G) FOR THE SECOND (14-WK-OLD) AND THE THIRD (7-WK-OLD) EXPERIMENTS

Treatment	Fire Flash		Sooner Red		Average		Totals	
	14-wk	7-wk	14-wk	7-wk	14-wk	7-wk	14-wk	7-wk
Control non-inoculated	243.641	93.666	211.466	80.633	227.554	87.150		
Control inoculated	80.991	13.558	102.725	9.650	91.858	11.604		
Average	162.316	53.612	157.095	45.141				
Truban at transplanting	170.200	25.658	142.750	25.491	156.475	25.575	138.727	23.562
1 wk later	137.550	21.925	104.408	21.175	120.979	21.550		
Average	153.875	23.791	123.579	29.333				
RE 20615 at transplanting	180.116	29.191	181.783	26.875	180.950	28.033	170.168	35.583
1 wk later	169.841	43.241	48.933	43.025	159.387	43.133		
Average	174.979	36.216	165.358	34.950				
Fungicides applied at transplanting	175.158	27.425	162.266	26.183	168.712	26.804		
1 wk later	153.695	32.583	126.670	32.100	140.183	32.341		

[x]Top weight obtained 6 weeks after inoculation, grams of fresh weight of above ground parts

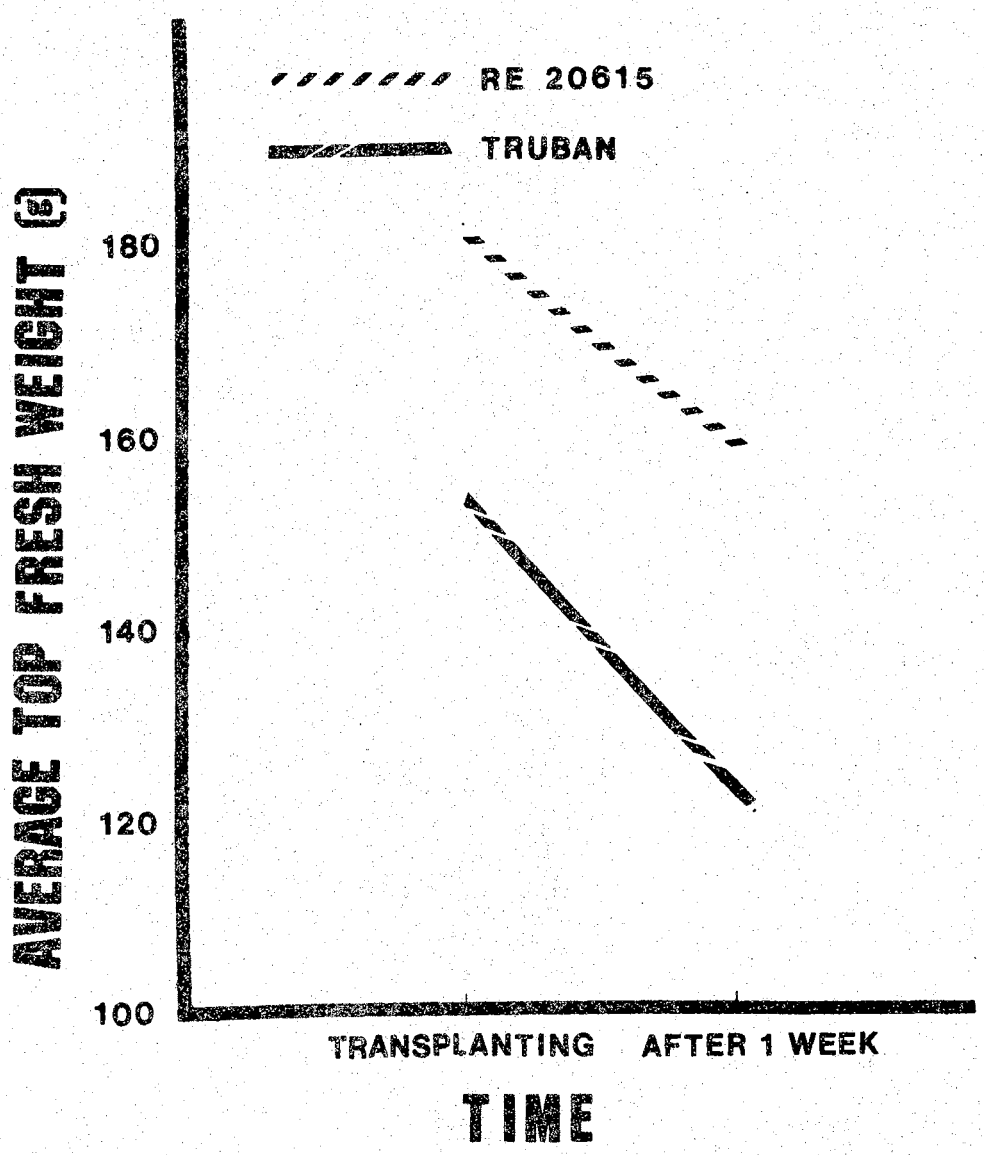


Figure 8. The Effect of Fungicides (Truban and RE 20615) and Timing of Application. Top Fresh Weight Obtained 6 Weeks After Inoculation (Grams of Fresh Weight of Above Ground Plant Parts for 14-wk-old Plant).

TABLE VI

MEANS OF RATIO FO LENGTH OF STEM DISCOLORATION TO PLANT HEIGHT FOR THE SECOND (14-WK-OLD) AND THE THIRD (7-WK-OLD) EXPERIMENTS

Treatment	Fire Flash		Sooner Red		Average		Totals	
	14-wk	7-wk	14-wk	7-wk	14-wk	7-wk	14-wk	7-wk
Control non-inoculated	0.000	0.000	0.000	0.000	0.000	0.000		
Control inoculated	25.650	38.366	8.516	59.408	17.083	48.887		
Average	12.825	19.183	4.258	29.704				
Truban at transplanting	0.641	22.133	1.991	34.708	1.316	28.420	2.495	31.847
1 wk later	2.650	31.641	4.700	38.908	3.675	35.275		
Average	1.645	26.887	3.345	36.808				
RE 20615 at transplanting	0.000	18.150	0.000	18.750	0.000	18.450	0.100	17.602
1 wk later	0.000	16.666	0.400	16.841	0.200	16.754		
Average	0.000	17.408	0.200	17.795				
Fungicides applied at transplanting	0.320	20.141	0.995	26.729	0.658	23.435		
1 wk later	1.325	24.154	2.550	27.875	1.937	26.014		

Phytotoxicity Test

No phytotoxic effects were observed on either cultivar due to fungicide application (on buffer row plants) during the entire experiment.

Reisolation

Pythium was not isolated from the non-inoculated controls, however, some species of *Aspergillus*, *Alternaria* and *Fusarium* were isolated from them. *Pythium* was reisolated from most of the inoculated controls. In addition, many *Fusarium*, *Rhizopus*, *Aspergillus* were found. For all fungicide-treated plants, *Pythium* was sometimes reisolated from stunted plants with or without discoloration. Several of isolates of species such as *Fusarium*, *Alternaria*, *Aspergillus* and *Rhizopus* were also obtained.

Flower Bud Formation

At time of inoculation with *Pythium* most of the 14-wk-old plants of both cultivars had flowered or had unopened buds.

Discussion

Even though *P. ultimum* affects root growth, a root-rating was not attempted due to certain difficulties in evaluation: the drastic reduction of root growth in controls when the plants became pot bound; continued slow growth of inoculated plant-roots; and difficulty in removing all the growing medium from the roots. However, the other growth evaluations made were sufficient to draw conclusions.

Top rating, top fresh weight, and plant height all indicated that RE 20615 was more effective in controlling P. ultimum than Truban and that fungicide application at transplanting gave better control of Pythium than when applied one week later. However, timing had no significant effect on stem discoloration.

All non-inoculated controls were healthy and all inoculated controls were severely diseased. Two applications of Truban or RE 20615 appeared to give a measure of protection which was significantly different from the inoculated controls. Neither fungicide tested afforded complete protection against infection by P. ultimum. By comparing the late top rating with the early one, it was evident that the second fungicide application (four weeks after the first application) did not do a good job. Since most fungicides act as protectants rather than eradicants, once Pythium has become well established it will be difficult to control. Therefore, the earlier the fungicide is applied the better the effect will be.

An ideal fungicide should be non-toxic to growing plants, effective in preventing the establishment of Pythium over the period of time required to produce a crop, and effect under conditions most favorable for development of the pathogen. In this case, RE 20615 was not toxic to the plants and was effective in controlling P. ultimum.

From empirical observations, it appeared that root rot was not always correlated with reductions in shoot height. Some plants were stunted but with no discoloration. In this experiment, the most conspicuous sympton caused by P. ultimum was stunting. Possible reasons for this were: 1). The plants when inoculated were 14 weeks old, infections were confined to the younger and more succulent feeder roots

causing stunting and chlorosis. 2). Fertilization -- Mellano et al found that in the absence of P. ultimum the tops of the fertilized plants were much larger than those of unfertilized plants, while in the presence of root rot, stunting of the top was always more severe in fertilized plants than in unfertilized plants (21). 3). If the fungus grew into the cells of the mature portions of the primary and secondary roots, hyphal growth first slowed and then stopped (21). 4). Stoppage of infection is probably due to a host resistance reaction manifested by the formation of a cork cambium completely across and within the stem, barring further progress of the hyphae after infection has already proceeded some distance from the point of inoculation (9). No further rapid growth took place, but, the plants remained turgid and dwarfed.

Soil moisture and temperature, important factors in disease development, were not investigated in this experiment. However, filling-up the saucers every other day caused the soil surface to remain relatively moist. According to Roncadori & McCarter (24), root necrosis and stunting are affected greatly by soil temperature. The soil temperature range for this experiment was 21-24^{o}C which was more favorable for infection (2) although the optimum temperature for P. ultimum growth in culture was 28^{o}C (22).

It is difficult to evaluate how much moisture and temperature contributed to the disease development in this experiment, but plots were well randomized and effects should have been spread over all treatments.

In addition to P. ultimum which was reisolated from inoculated plants, Fusarium, Alternaria, Aspergillus, and Rhizopus species also

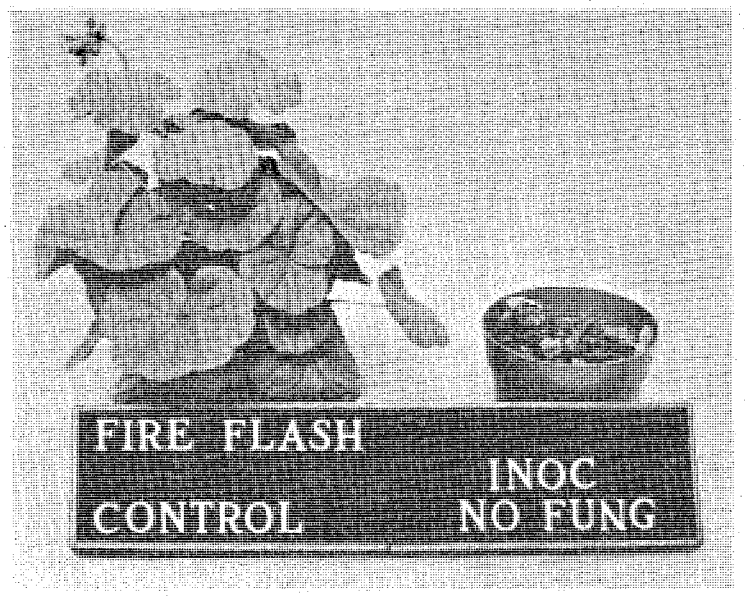
were isolated from non-inoculated controls and inoculated plants. According to Experiment 1, Alternaria and Aspergillus were seed-borne organisms which might not be eradicated by Thiram seed treatment and sill existed as the plants grew. Rhizopus was possibly a contaminant. Consequently, Alternaria, Aspergillus and Rhizopus apparently did not damage the established plants. Although Fusarium was associated with necrotic roots more often than other fungi, their importance in stunting is doubtful (24). Also, in a preliminary test made prior to this experiment in the same greenhouse, inoculating with Fusarium sp. on the same seedling cultivars that were 6-wk-old caused no pathogenicity. Therefore, only P. ultimum was associated consistently with root and stem rot and stunted plants.

Experiment 3: Comparison of Two Fungicides on Control of Pythium Blackleg on 7-wk-old Plants

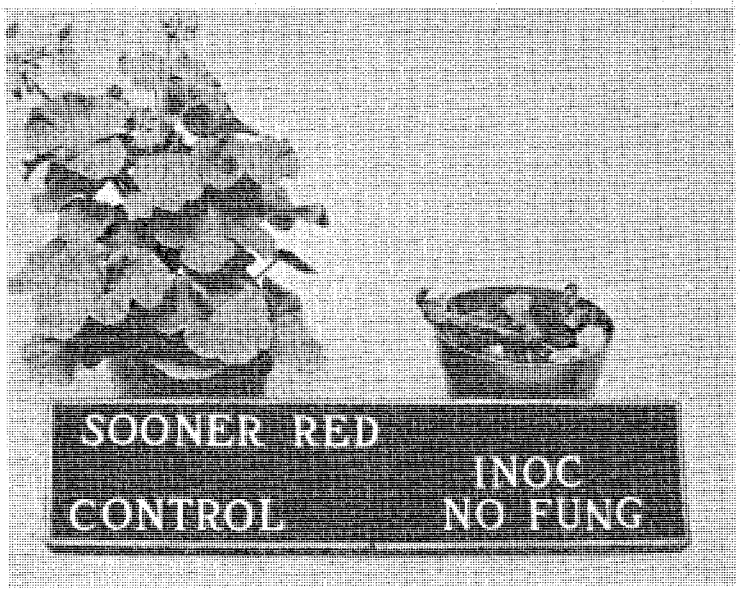
According to the AOV, all the data recorded below showed two common results: the non-inoculated control was significantly different from the inoculated control and inoculated control was significantly different from inoculated plants receiving fungicide treatments (Appendix, Table VII-XII). It was evident in Figure 9 that the inoculated control was very severely infected and the non-inoculated control was healthy.

Top Rating

The AOV for the early top rating (Appendix, Table VII) indicated that there were no significant differences among the treatments



a). Cultivar: 'Fire Flash'
Inoculated Control (right): Dead
Non-inoculated Control (left): Healthy



b). Cultivar: 'Sooner Red'
Inoculated Control (right): Dead
Non-inoculated Control (left): Healthy

Figure 9. Comparison Between Inoculated Control and Non-inoculated Control 6 Weeks After Inoculation for 7-Wk-Old Plants.

(Table II) while for the late top rating (Appendix, Table VIII), the type of fungicide had a significant effect on top quality, i.e., RE 20615 gave a better top quality than Truban (Table III). In addition, the AOV (Appendix, Table IX) showed that plants rated six weeks after inoculation (late top rating) were more diseased than when the plants were rated earlier (four weeks after inoculation).

Plant Height

The AOV (Appendix, Table X) indicated that there were no significant differences among treatments (Table IV).

Top Weight

According to the AOV (Appendix, Table XI), type of fungicide had a significant effect on top weight, i.e., RE 20615 was better than Truban in controlling the disease (Table V). In addition, there was an interaction between type of fungicide and application time (Figure 10). This interaction indicated that RE 20615 showed a better effect on top weight when applied one week later while Truban showed a slightly decreasing effect when applied one week later. The result of better top weight with later application of RE 20615 is not consistant with most of the other results in these experiments. More research should be done to clarify these results.

Ratio of Length of Stem Discoloration to Top Height in Percentage

The AOV (Appendix, Table XII) indicated that there were no significant differences among treatments (Table VI).

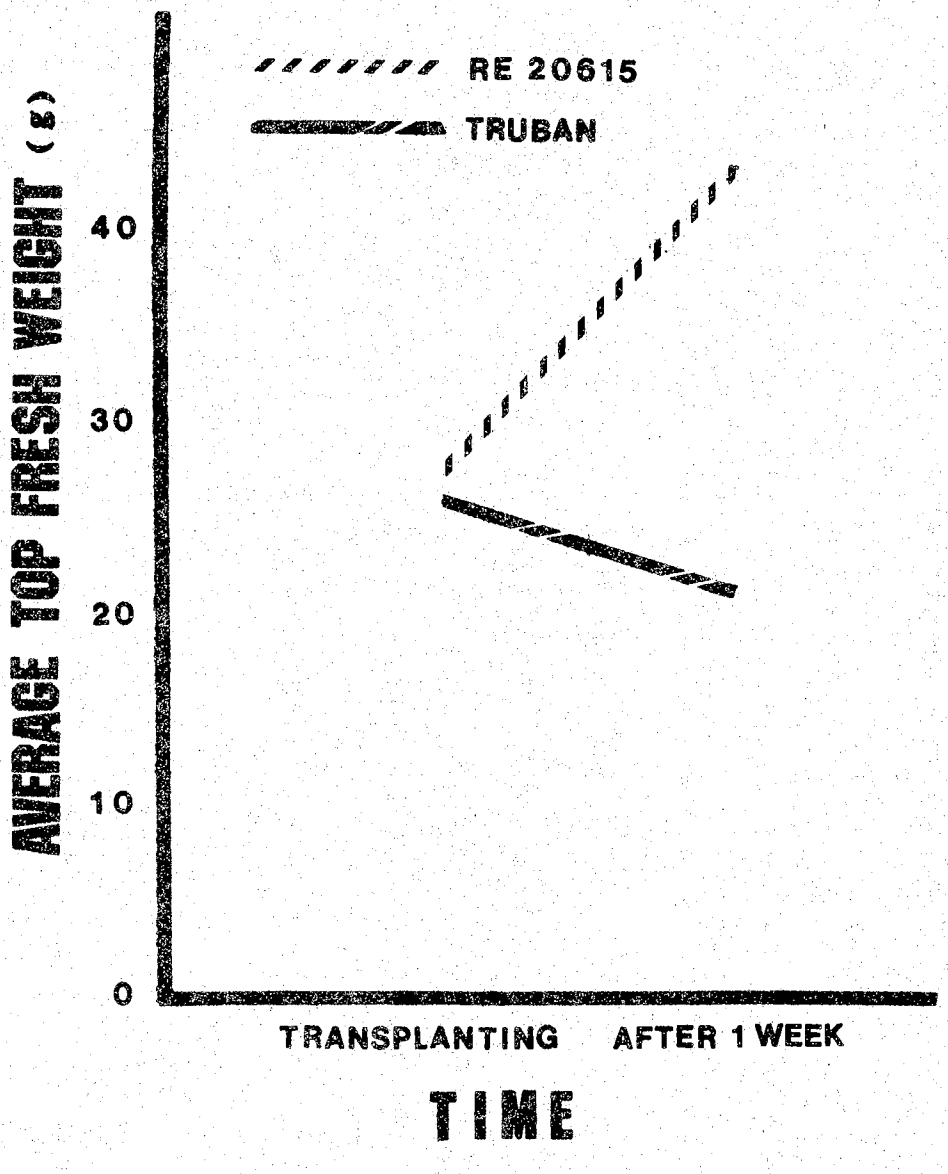


Figure 10. The Effect of Interaction Between Fungicide and Application Time for 7-wk-old Plants of Both Cultivars. Two Unparallel Lines Indicate That There Was an Interaction.

Phytotoxicity Test

There were no phytotoxic effects due to fungicides applied to buffer row plants during the entire experiment.

Reisolation

The results were the same as previously described for Experiment 2.

Flower Bud Formation

There was no flower bud formation when the inoculation was first made on 7-wk-old plants. Six weeks after inoculation, only 37 plants were without flower buds while the others (107 plants) had flower buds. Of the 37 without flower buds, 9 were inoculated controls and 28 were dead about three weeks after inoculation. These 28 dead plants did not have enough time to form buds.

Discussion

According to the results obtained above, only the late top rating and top weight showed a significant difference for type of fungicide, i.e., RE 20615 was better than Truban. There was no time difference for fungicide application for each datum recorded. This might be due to the following reasons:

1. Since the plants when inoculated were 7-wk-old (half the age of the plants used in the previous experiment), they were more susceptible to *P. ultimum* which tends to infect mainly juvenile or succulent tissues.

2. The Pythium inoculum used here was the same amount as Experiment 2. This inoculum was probably too much for the 7-wk-old plants and fungicides applied at transplanting could not reduce Pythium sufficiently and the fungus still had the ability to damage the young plants.

3. For geraniums, the optimum day temperature is 18-21°C on cloudy days and 21-24°C on bright days and optimum night temperature is 16-18°C (14). However, in this experiment, both night temperature (22°C) and day temperature (27°-35°C) were not so favorable for the geranium growth whereas the soil temperature (22°-31°C) was still favorable for Pythium growth. Accordingly, these higher temperatures possibly weakened the plants. In most cases, where optimum temperature has been reported for disease development by Pythium on its various hosts, disease had been most severe at temperatures unfavorable for growth of the host plant with little relation to the temperatures favoring fungal growth (7).

Therefore, when fungicides were applied at transplanting, enough Pythium inoculum survived to cause damage to the younger and weakened plants. The degree of damage was probably no less than that of plants with fungicides applied one week later so that there was no significant difference for the timing of fungicide application.

In addition, there was no delay in flower bud formation. Most plants had formed flower buds six weeks after inoculation. Even the most stunted and severely diseased plants had buds (Figure 11).

Statistically, we could not compare the two different growth stages of seedling geraniums due to the different experimental time, however, the 7-wk-old plants seemed more susceptible to P. ultimum than the 14-wk-old plants.

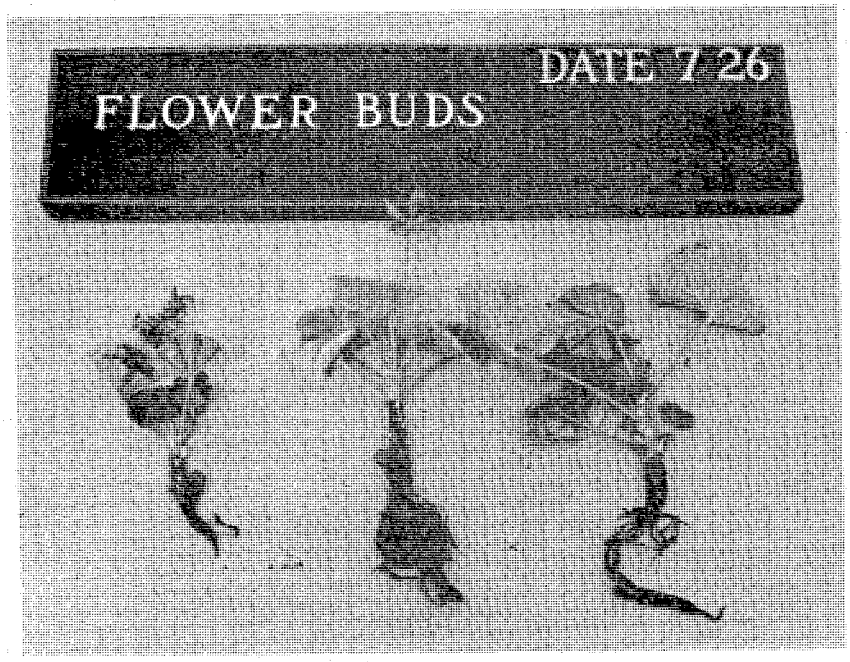


Figure 11. Flowers and Flower Buds Seen Even in Stunted Severely Rotted Plants for 7-Wk-Old Plants.

CHAPTER IV

PRINCIPAL CONCLUSIONS

1. Organisms isolated in this study were present in or on the seed of 'Fire Flash' and 'Sooner Red' geraniums. Isolates of Alternaria, Curvularia, and Nigrospora species were pathogenic to 1, 2, and 3-wk-old seedlings in laboratory tests but caused little or no damage on 4, 5, and 6-wk-old seedlings. Alternaria was the most prevalent in or on the seed. Isolates of Aspergillus, Torula, Cladosporium, Penicillium and Hormicium species were not pathogenic to the very young geranium seedlings.

2. The plants inoculated with P. ultimum developed severe symptoms of stunting, stem discoloration, the upper leaves of many plants were chlorotic, many lower leaves were dead, and eventual death resulted in many cases.

3. The experimental fungicide RE 20615 was more effective than Truban in most cases and no phytotoxicity occurred.

4. For 14-wk-old plants, both fungicides applied at transplanting were more effective than when applied one week later, but no significant effect of timing was observed on 7-wk-old plants. This was probably because the younger plants were damaged rapidly.

5. P. ultimum infection did not delay flower bud formation.

6. In addition to P. ultimum which was reisolated from inoculated plants, other fungi such as Fusarium, Alternaria, Aspergillus and

Rhizopus species were also isolated from all plants. These fungi apparently caused no damage on the geraniums.

7. For 14-wk-old plants, there were significant differences on cultivars for top weight and plant height. 'Fire Flash' gained more weight but was shorter than 'Sooner Red'. Neither cultivar appeared to be more resistant to *P. ultimum* than the other.

LITERATURE CITED

1. Adegbola, M. O. K., D. J. Hagedorn. 1969. Host-parasite relations in Pythium bean blight. Phytopathology 59:1484-87.

2. Arndt, C. H. 1943. Pythium ultimum and the damping-off of cotton seedlings. Phytopathology 33:607-10.

3. Ball, Vic, ed. 1977. Seed geraniums. Grower Talks. 41(5):1-13.

4. _____. 1978. Production schedules for hybrid geraniums. Grower Talks. 42(7):18-21.

5. Barton, R. 1961. Saprophytic Activity of Pythium mamillatum in Soils. II. Factors Restricting P. mamillatum to Pioneer Colonization of Substrates. Trans. Br. Mycol. Soc. 44:105-18.

6. Bateman, D. F. 1961. The effect of soil moisture upon development of poinsettia root rot. Phytopathology 51:445-51.

7. _____. and A. W. Dimock. 1959. The influence of temperature on root rots of poinsettia caused by Thielaviopsis basicola, Rhizoctonia solani, and Pythium ultimum. Phytopathology 49:641-47.

8. Biesbrock, J. A. and F. F. Hendrix. 1959. Influence of soil water and temperature on root necrosis of peach caused by Pythium spp. Phytopathology 60:880-82.

9. Braun, H. 1924. Geranium stem rot caused by Pythium complectens n. sp. J. Agr. Res. 29:399-419.

10. _____. 1925. Comparative studies of Pythium debaryanum and two related species from geranium. J. Agr. Res. 30:1043-62.

11. Buddin. W. and E. M. Wakefield. 1924. 'Black Leg' of Pelargonium cuttings. Gard. Chron (III) 75:25, illus.

12. Griffin, D. M. 1963. Soil physical factors and ecology of fungi. II. Behavior of Pythium ultimum at small soil water suctions. Trans. Br. Mycol. Soc. 46:368-72.

13. Hendrix, F. F. and W. A. Campbell. 1973. Pythium as plant pathogens. Ann. Rev. Phytopath. 11:77-91.

14. Hoagland, D. R. and D. I. Arnon. 1950. The water-culture method for growing plants without soil. Calif. Agr. Expt. Station. Circular 347.

15. Hoppe, P. E. 1966. Pythium species still viable after 12 years in airdried muck soil. Phytopathology 56:1411.

16. Index of plant diseases in the United States. 1960. Agriculture Handbook. No. 165. USDA. P. 159.

17. Klisiewicz, J. M. 1968. Relation of Pythium spp. to root rot and damping-off of safflower. Phytopathology 58:1384-86.

18. Kraft, J. M. and D. W. Burke. 1971. Pythium ultimum as a root pathogen of beans and peas in Washington. Plant Dis. Rep. 55:1056-60.

19. Mastalerz, J. W., ed. 1971. Geraniums. A Penn State Manual. 2nd ed. Pennsylvania Flower Growers. University Park, Pennsylvania. PP. 123-24, 218-20.

20. McCarter, S. M. and R. W. Roncadori. 1971. Influence of low temperature during cotton seed germination on growth and disease susceptibility. Phytopathology 61:1426-29.

21. Mellano, H. M., D. E. Munnecke, and R. M. Endo. 1970. Relationship of seedling age to development of Pythium ultimum on roots of Antirrhinum majus. Phytopathology 60:935-42.

22. Middleton, J. T. 1943. The taxonomy, host range and geographic distribution of genus Pythium. Torrey Bot. Club Mem. 20:1-171.

23. Miller, C. R., W. M. Dowler, D. H. Peterson, and R. P. Ashworth. 1966. Observations on the mode of infection of Pythium ultimum and Phytophthora cactorum on young roots of peach. Phytopathology. 56:46-49.

24. Roncadori. R. W. and S. M. McCarter. 1972. Effect of soil treatment, soil temperature, and plant age on Pythium root rot of cotton. Phytopathology 62:373-76.

25. Sleeth, B. 1953. Winter Haven decline of citrus. Plant Dis. Rep. 37:425-26.

26. Spencer, J. A. and W. E. Cooper. 1967. Pathogenesis of cotton by Pythium species: zoospores and mycelium attraction and infectivity. Phytopathology 57:1332-38.

27. Stanghellini, M. E. and J. G. Hancock. 1971. The sporangium of Pythium ultimum as a survival structure in soil. Phytopathology 61:157-164.

28. Trow, A. H. 1901. Observation on the biology and cytology of Pythium ultimum n. sp. Ann. Bot. 15:269-312.

29. Voigt, A. O. 1979. Flower marketing information. The Penn State Univ. and the USDA Coorperating. P. 1.

30. Wadsworth, D. F. 1966. "Etiology of spring dead spot, a root rot complex of Bermudagrass." Ph.D. Dissertation. University of California, Davis.

31. Ward, H. M. 1883. Observation on the genus Pythium (Pringsh). Quart. Jour. Micros. Sci. (n.s.) 23:485-515, illus.

32. Wheeler, J. E., R. B. Hine, and A. M. Boyle. 1970. Comparative activity of Dexon and Terrazole against Phytophthora and Pythium. Phytopathology 60:561-62.

APPENDIX

TABLE VII

ANALYSIS OF VARIANCE: EARLY TOP RATING FOR THE SECOND (14-WK-OLD) AND THE THIRD (7-WK-OLD) EXPERIMENTS

Source	Df	Sum of Squares		Mean Squares		Observed F		Required $F_{.05}$
		14-wk	7-wk	14-wk	7-wk	14-wk	7-wk	
No Fungicide								
Cultivar (CV)	1	0.25	2.75	0.25	2.75	0.32	0.88	3.92
Fungus	1	112.54	152.30	112.54	152.30	141.12*	48.97*	
CV*Fungus	1	0.25	1.17	0.25	1.17	0.32	0.38	
With Fungicides								
CV	1	1.15	0.67	1.15	0.67	1.44	0.21	
Fungicide	1	5.75	5.51	5.75	5.51	7.21*	1.77	
CV*Fungicide	1	1.63	0.38	1.63	0.38	2.04	0.12	
Time	1	3.57	3.76	3.56	3.76	4.47*	1.21	
CV*Time	1	0.32	0.38	0.32	0.38	0.38	0.12	
Fungicide*Time	1	0.21	8.76	0.21	8.76	0.26	2.82	
CV*Fungicide*Time	1	1.15	0.00	1.15	0.00	1.44	0.00	
With Inoculation								
Control vs Others	1	21.88	20.42	21.88	20.42	27.34*	6.56*	
Error	121	96.51	376.35	0.80	3.11			

*Significant at 5% level

TABLE VIII

ANALYSIS OF VARIANCE: LATE TOP RATING FOR THE SECOND (14-WK-OLD) AND THE THIRD (7-WK-OLD) EXPERIMENTS

Source	Df	Sum of Squares		Mean Squares		Observed F		Required $F_{.05}$
		14-wk	7-wk	14-wk	7-wk	14-wk	7-wk	
No Fungicide								
Cultivar (CV)	1	0.80	2.08	0.80	2.08	0.82	0.54	3.92
Fungus	1	109.20	196.02	109.20	196.02	111.43*	50.91*	
CV*Fungus	1	0.48	0.75	0.48	0.75	0.49	0.19	
With Fungicides								
CV	1	2.63	0.26	2.63	0.26	2.68	0.07	
Fungicide	1	8.70	40.04	8.70	40.04	8.87	10.41*	
CV*Fungicide	1	1.78	0.84	1.78	0.84	1.82	0.22	
Time	1	6.66	0.00	6.66	0.00	6.80	0.00	
CV*Time	1	1.68	0.09	1.68	0.09	1.71	0.02	
Fungicide*Time	1	0.01	12.04	0.01	12.04	0.02	3.13	
CV*Fungicide*Time	1	0.29	0.01	0.29	0.01	0.29		
With Inoculation								
Control vs Others	1	24.43	35.21	24.43	35.21	24.93*	9.15*	
Error	121	118.63	465.30	0.98	3.85			

*Significant at 5% level

TABLE IX

ANALYSIS OF VARIANCE: EARLY AND LATE TOP RATINGS FOR THE SECOND (14-WK-OLD) AND THE THIRD (7-WK-OLD) EXPERIMENTS

Source	Df	Sum of Squares		Mean Squares		Observed F		Required $F_{.05}$
		14-wk	7-wk	14-wk	7-wk	14-wk	7-wk	
Cultivar (CV)	1	0.97	4.13	0.97	4.13	0.57	0.63	3.92
Treatment	5	257.59	439.23	51.52	87.85	30.52*	13.34*	2.29
CV*Treatment	5	10.79	3.56	2.15	0.71	1.28	0.10	
Error	121	204.28	796.63	1.69	6.58			
Period[1]	1	1.29	2.44	1.29	2.44	14.29*	6.03*	3.91
Error	132	11.94	53.32	0.09	0.40			

[a]Early Top Rating vs. Late Top Rating

*Significant at 5% level

TABLE X

ANALYSIS OF VARIANCE: PLANT HEIGHT (CM) FOR THE SECOND (14-WK-OLD) AND THE THIRD (7-WK-OLD) EXPERIMENTS

Source	Df	Sum of Squares		Mean Squares		Observed F		Required $F_{.05}$
		14-wk	7-wk	14-wk	7-wk	14-wk	7-wk	
No Fungicide								
Cultivar (CV)	1	123.52	0.33	123.52	0.33	7.16*	0.02	3.92
Fungus	1	1354.69	1376.02	1354.69	1376.02	78.59*	84.21*	
CV*Fungus	1	0.02	11.02	0.02	11.02		0.67	
With Fungicides								
CV	1	88.17	9.44	88.17	9.44	5.11*	0.58	
Fungicide	1	121.50	56.27	121.50	56.27	7.05*	3.44	
CV*Fungicide	1	104.16	3.64	104.16	3.64	6.04*	0.22	
Time	1	266.67	25.94	266.67	25.94	15.47*	1.59	
CV*Time	1	16.67	5.09	16.67	5.09	0.96	0.31	
Fungicide*Time	1	4.17	60.96	4.17	60.96	0.24	3.73	
CV*Fungicide*Time	1	20.17	2.19	20.17	2.19	1.16	0.13	
With Inoculation								
Control vs Others	1	288.15	137.47	288.15	137.47	16.72*	8.41*	
Error	121	2085.56	1977.66	17.24	16.34			

*Significant at 5% level

TABLE XI

ANALYSIS OF VARIANCE: TOP WEIGHT (G) FOR THE SECOND (14-WK-OLD) AND THE THIRD (7-WK-OLD) EXPERIMENTS

Source	Df	Sum of Squares		Mean Squares		Observed F		Required $F_{.05}$
		14-wk	7-wk	14-wk	7-wk	14-wk	7-wk	
No Fungicide								
Cultivar (CV)	1	327.08	861.06	327.08	861.06	0.14	2.84	3.92
Fungus	1	22096.31	68486.07	220960.31	68486.07	91.42*	225.57*	
CV*Fungus	1	8718.32	249.79	8718.32	249.79	3.60	0.82	
With Fungicides								
CV	1	9560.04	17.85	9560.04	17.85	3.96*	0.06	
Fungicide	1	23725.88	3468.01	23725.88	3468.01	9.81*	11.42*	
CV*Fungicide	1	2564.73	3.92	2564.73	3.92	1.06	0.01	
Time	1	19533.92	735.93	19533.92	735.93	8.08*	2.42	
CV*Time	1	1198.51	3.45	1198.51	3.45	0.49	0.01	
Fungicide*Time	1	1164.83	2194.59	1164.83	2194.59	0.48	7.23*	
CV*Fungicide*Time	1	427.57	10.80	427.57	10.80	0.17	0.04	
With Inoculation								
Control vs Others	1	75215.25	6199.21	75215.25	6199.21	31.12*	20.42*	
Error	121	292443.79	36735.80	2416.89	303.60			

*Significant at 5% level

TABLE XII

ANALYSIS OF VARIANCE: RATIO[x] FOR THE SECOND (14-WK-OLD) AND THE THIRD(7-WK-OLD) EXPERIMENTS

Source	Df	Sum of Squares		Mean Squares		Observed F		Required $F_{.05}$
		14-wk	7-wk	14-wk	7-wk	14-wk	7-wk	
No Fungicide								
Cultivar (CV)	1	880.65	1328.25	880.65	1328.25	6.18*	1.00	3.92
Fungus	1	3502.08	28679.85	3502.08	28679.85	24.61*	21.61*	
CV*Fungus	1	880.65	1328.25	880.65	1328.25	6.18*	1.00	
With Fungicides								
CV	1	21.66	637.57	21.66	637.57	0.15	0.48	
Fungicide	1	137.76	4870.65	137.76	4870.65	0.97	3.67	
CV*Fungicide	1	13.50	545.30	13.50	545.30	0.09	0.41	
Time	1	39.27	159.65	39.27	159.65	0.28	0.12	
CV*Time	1	1.82	49.30	1.82	49.30	0.01	0.04	
Fungicide*Time	1	27.95	438.62	27.95	438.62	0.19	0.33	
CV*Fungicide*Time	1	0.14	35.77	0.14	35.77		0.03	
With Inoculation								
Control vs Others	1	4784.23	11209.53	4784.23	11209.53	33.63*	8.45*	
Error	121	17214.87	160558.70	142.27	1326.93			

[x]Length of stem discoloration to top height in percent

*Significant at 5% level

VITA

Hua-Fu Chao

Candidate for the Degree of

Master of Science

Thesis: FUNGICIDAL INVESTIGATIONS WITH PYTHIUM ULTIMUM AND STUDIES WITH SEED-BORNE PATHOGENS OF CULTIVARS OF PELARGONIUM HORTORUM BAILEY

Major Field: Horticulture

Biographical:

Personal Data: Born in Taipei, Taiwan, Republic of China, January 3, 1951, the eldest son of Mr. and Mrs. Jih-Sung Chao.

Education: Graduated from Taipei Municipal Chien-Kuo High School, Taipei, Taiwan, in 1969; received the Bachelor of Science degree in Biology from Tunghai University in 1973; completed the requirements for the Master of Science degree in Horticulture at Oklahoma State University in December, 1979.

Experience: Work-study at Horticulture Greenhouse, November 1978-April 1979; Graduate Research Assistant, Department of Horticulture, Oklahoma State University, September 1979 - December 1979.