STUDIES ON DAMPING-OFF OF ALFALFA CUTTINGS IN THE GREENHOUSE

by

PAUL GYAMERA ADDOH

B. S., (Agric.) Purdue University, 1959

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Botany and Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1961

TABLE OF CONTENTS

INTRODUCTION

In plant breeding work (hybridization, polycrossing and increasing desired clonal lines) where genetic factors are to be maintained, asexual propagation is nearly always necessary. Fortunately alfalfa plants can be propagated asexually by rooting stem cuttings.

Petioles of alfalfa leaves with the leaflets intact have been found to root when placed in running water, vermiculite, sand or soil under moist conditions. Advantage is taken of this fact to test alfalfa plants for resistance to a number of pathogens by using alfalfa leaves. The trifoliolate leaves give the added advantage that three replications of a treatment can be made with a single leaf.

Since 1942 when Tysdal et al. (58) suggested the use of stem sections for asexual propagation of alfalfa plants, both public and commercial breeders have made frequent use of this technique, both in testing for seed and forage yield. This procedure has made it possible for growers to select desirable plants in summer or fall and vegetatively increase their clonal lines several fold, run field trials more rapidly, and to obtain several clonal lines in one season as well as all year round. Over the years alfalfa breeding stations have vegetatively propagated alfalfa plants in the greenhouse but with difficulties (18, 22, 25, 32, 68). However, this has been done only with considerable difficulty due to damping-off, failure to root, and other factors.

The methods employed in vegetative propagation have centered around the following techniques: the use of a portion of a single internode and a single node with the trifoliolate leaf attached; the use of running water,

moist vermiculite, sand and soil for rooting media; the use of growth regulators and rooting chemicals to induce vegetative growth and root formation; and the incorporation of chemical and sanitary measures to prevent and to control diseases among cuttings.

At the Kansas Agricultural Experiment Station where many clonal lines are tested and screened annually in breeding programs, desirable plants are selected in the field in late summer or fall. Stems are transferred to the greenhouse in water troughs and cut into portions containing single nodes with entire leaves attached. These are rooted in moist sand in aluminum pans with a surface area of two square feet. The sand beds are kept moist until harvesting which is usually in three to five weeks.

Until 1948 the rooting medium used at this station consisted of unsterilized, fine grade, washed sand (22) taken from the Kansas river in Manhattan, Kansas. Loss of cuttings (unable to root and diseased) during this time often ran as high as 90 to 100 percent in individual pans. Later the sand used for rooting medium was sterilized at 15 lbs steam pressure for four to five hours. The use of sterilized pans, clean asbestos-covered benches, Pano-drench and Captan as fungicides, was also incorporated into the rooting programs.

In the fall of 1959 when this study was begun, and in 1960 while it was still in progress, losses at the breeding station were still running as high as 80-90 percent and often even 100 percent in individual flats despite the use of fungicides, more sanitary precautionary measures and improved techniques.

As of the time this study was begun, adequate methods of making

cuttings and rooting them in suitable media under sanitary conditions were apparently well understood as indicated from reports of workers both in Canada and in the United States (18, 22, 25, 32, 68).

However, high percentage losses from damping-off, both before and after the cuttings had rooted, were commonplace (18, 22, 25, 32, 68) and seemed to be the general and greatest trouble at this and other stations.

Disease organisms Rhizoctonia sp., Ascochyta imperfecta Peck, and Fusarium sp., had been suggested as the cause of disease in cuttings (22, 25, 32). The general feeling was that Rhizoctonia sp. splashed from greenhouse benches onto the rooting medium during the rooting period, or brought into the greenhouse with the plants, was probably the main cause of damping-off among cuttings. Control measures suggested had been tried at this station without much success thus far.

This study was initiated, therefore, to: (1) find a chemical control for Rhizoctonia sp.; (2) investigate further the cause or causes of damping-off among cuttings and; (3) find chemical controls for other possible causal organisms.

REVIEW OF LITERATURE

Tysdal and Westover (57) have described extensively the problems that faced alfalfa breeders around 1937. Breeding for resistance to nematodes, bacterial wilt and other diseases seemed to center around the incorporation of genetic materials from the original Turkistan variety originating in southeast Asia. Cooperative works through conferences and contributions from workers around the world were also recorded.

Propagation of the crop plants by means of vegetative cuttings was mentioned by Tysdal et al. (58) as a means of increasing clonal lines of hybrid alfalfa for commercial production. Garner (21) also described the use of cuttings and layers for propagating pome and stone fruits. Elling (18) at Minnesota, made a detailed study of this mode of propagation by testing the efficiency of using plants of different ages, stem cuttings from various parts of the plant, different rooting media and auxins. He suggested that the use of stem cuttings of portions of single internodes and single nodes, with the leaves attached, from succulent, vigorously growing stems, consistently gave better rooting results than cuttings made from older stems. Auxins of indole butyric and indole acetic acids used in his trials did not significantly increase the rooting ability of alfalfa cuttings. He also found that unigro was superior to sand or running water as a rooting medium. Garner (21) similarly suggested that cuttings rich in carbohydrates should be used, and, that stems which have shed at least some of their leaves should be avoided, if possible.

While propagation by means of cuttings has been a boon to alfalfa breeders, various workers (18, 22, 25, 32, 68) have directly or indirectly reported difficulties encountered in rooting alfalfa stem sections. White (68) at Saskatoon, Canada, indicated that by the usual methods of planting stem sections of two nodes of mature stems in sand, an average of 30-40 percent of the cuttings rooted, and, that it was almost impossible to propagate some plants by this method. He reported, however, that 85-97 percent of cuttings made from single internodes and single nodes rooted in running water.

The pathological aspects of rooting alfalfa cuttings had not been

reported or investigated until Grandfield et al. (22) made their study about 1948. They reported that Rhizoctonia sp., Ascochyta imperfecta Peck, and Fusarium sp. were the principal isolates recovered from diseased cuttings rooted in sand beds. From pathogenicity tests in which plants and sand beds were infested with the organisms, they concluded that Ascochyta imperfecta was the most pathogenic of the three isolates, and probably the most difficult to control. Since then other researchers (22, 25, 32) have described similar losses of cuttings caused by Rhizoctonia sp., and have amply described the symptoms and development of the disease. Rhizoctonia spp., have also been encountered in greenhouse nurseries and in the field (1-3, 7, 13, 15-17, 19, 20, 22, 23, 25, 28, 32, 34-39, 41, 43, 44, 48-54, 60-63, 65, 66). Geographic distribution, host range, pathogenic differences between strains and environmental factors that influence growth and pathogenicity of the species have also been described (1-3, 7, 8, 13, 16, 17, 19, 20, 25, 28, 30, 32, 35-39, 41-43, 48-64, 60-63, 65, 66).

Elmer (19) studied parasitism of potatoes by Rhizoctonia spp., for 12 years in Kansas and concluded that relatively cool temperatures and high moisture in July and August (critical periods in growing potatoes in Kansas) favored the persistence of Rhizoctonia sclerotia and increase of infection, while the reverse conditions favored the persistence of the mycelial stage. Kernkamp et al. (32) reported that at 60-65° F. alfalfa cuttings rooted poorly and cuttings were killed in 23 days by Rhizoctonia solani Kuehn. At 70-75° F. some roots were formed but the cuttings were killed in 16 days, while at 80-85° F. the cuttings formed no roots and were killed in 3 days. Smith (53) worked with 17 isolates of Rhizoctonia

spp., and reported that isolates from alfalfa were pathogenic to both leguminous and non-leguminous plants but isolates from non-leguminous plants were rarely pathogenic to alfalfa. It is the consensus of opinion, however, that Rhizoctonia spp., brought into the greenhouse with the plants, or splashed from the surfaces of greenhouse benches into rooting media, are the main cause of damping-off of alfalfa cuttings (22, 25, 32).

Control measures against Rhizoctonia spp., and other soil pathogens have involved the use of chemicals as dusts, vapors, and sludges (1, 9, 23, 25, 28, 34, 35, 41, 43, 48, 52, 60, 64). In recent years Captan, Panogen-Drench, Ceresan, Phaltan, PCNB (Pentachloro nitro benzene), MAS (Methyl arsine sulfide), and Dexon are generally used (1, 4, 23, 25, 28, 32, 41).

Kernkamp et al. (32) indicated that Phygon, applied dry to stems of cuttings before planting was the chemical treatment in their trials that most satisfactorily prevented the introduction of Rhizoctonia spp. into the rooting media for which they preferred vermiculite rather than sand as a rooting medium. Haskett el al. (25) on the other hand considered 300 ppm of Captan 50W, applied at the rate of 1000 cc per square foot of sand, and steam sterilization of sand as the best treatment to control damping-off of alfalfa cuttings caused by Rhizoctonia sp. PCNB, MAS, and Ceresan at the rate of 2-6 oz per 100 lb seeds, or seed furrow treatment of 25 ppm of dry soil weight, has been reported by Leach et al. (35) as satisfactory control against Rhizoctonia sp., and Pythium spp.; mixtures of PCNB with other fungicides improved their effectiveness against Rhizoctonia sp. especially. Brown (4) used dusts of 100 percent PCNB M275 to control R. solani.

In nature, R. solani seems to be controlled by antagonistic organisms two of which are Trichoderma spp., and Bacillus subtilis Cohn emed. Prazmowski (15, 24, 61, 65, 66). Sandford (50), however, reported that R. solani was often more virulent in natural soil than in sterilized soil. This would suggest that the virulence of R. solani in soil is synergistic when it is either in association with other organisms or with food materials that are destroyed by soil or sand sterilization.

The importance of other parasitic organisms and the damage they cause to alfalfa cuttings cannot be overlooked. Of these Ascochyta imperfecta, Pythium spp., Fusarium spp., and, to some extent Colletotrichum spp., and other leaf spot fungi need special mention (5, 6, 8, 10-14, 22, 24-27, 29-31, 33, 39-41, 45-47, 55, 56, 59, 67, 69).

Many workers (8, 10, 13, 14, 22, 29, 31, 39, 45-47, 55, 59) have reported the importance of A. imperfecta in the production of alfalfa for hay, forage, and seed. Cormack (12), Peterson and Melchers (45), and Kernkamp and Hemerick (31), have reported similarly the symptoms and effects of this organism on alfalfa. It causes leaf spots, stem lesions, floral sterility and seed abortion. Infection begins in spring with increasing severity through summer. The time it produces pycnidia (summer and fall) coincides with the time alfalfa stems are brought into the greenhouse for making cuttings (10, 22, 25, 45). It also overwinters in crop debris, stem and leaf lesions and in the soil for 2 years or more. Cormack (12) reported that it even persists in- and outside of seeds as small sections of mycelium. Peterson and Melchers (45) further stated that the inoculum for the late cuttings originated mainly from infected crop residues from the first cutting. There is ample indication, therefore, that it is also a very destructive pathogen of the crop.

Kernkamp and Hemerick (31) while studying blackstem of alfalfa caused by Ascochyta imperfecta found that none of the chemicals used in their trials could control the organism. It is no wonder that Haskett et al. (25) concluded that A. imperfecta was undoubtedly the most difficult pathogen among alfalfa cuttings to control chemically. Wallen and Hoffman (64), however, controlled A. pisi on broad beans with an acetone solution of Captan 75 applied to the roots. More Captan was found in the foliage when applied in this manner than when a water suspension was used. It might be feasible to use a similar treatment to control A. imperfecta in the field and thereby avoid carrying it to the greenhouse with cuttings.

The importance of Pythium spp., Fusarium spp., and Colletotrichum species have been considered by numerous workers and the control measures against them are similar to those of Rhizoctonia spp. (24, 25, 34, 35, 41, 43, 66). Pythium spp., are world wide in distribution and are one of the principal causes of damping-off and root rots of seedlings and mature plants (40). Pythium debaryanum Hesse has been found to be the most pathogenic species to alfalfa in Iowa and Manitoba (5, 38). Gregory et al. (24) found P. debaryanum and P. ultimum Trow the most pathogenic of 5 species tested on alfalfa. Halpin and Hanson (26) in testing for the pathogenicity of 5 Pythium species on alfalfa, sweet clover, red clover and Ladino white clover found P. debaryanum, P. irregulare, and P. ultimum the most pathogenic species. Selected species of these and other genera associated with alfalfa were, therefore, included in the present studies with a view to finding out more about their possible relation to the vegetative propagation of the alfalfa crop in the greenhouse.

Alternaria spp., supposedly saprophytes are also often associated with alfalfa plant materials (12). Information concerning the relation (of the last four organisms) to alfalfa cuttings in the greenhouse is, however, very meager.

MATERIALS AND METHODS

Methods used in Testing with Alfalfa Cuttings

Source of Plants. On October 4, 1959, and November 11, 1959, a total of 14 alfalfa plants of clone 50-1266 were taken into the greenhouse from the Kansas State University alfalfa breeding nursery at the Ashland farm. These plants which originated from the variety Buffalo, were used in the subsequent greenhouse studies during the winter of 1959-1960 as well as the following year.

On November 1, 1960, 9 additional plants of clone C-84 selected from Turkistan alfalfa, variety Nemastan, and 7 plants of clone 30-1108 were dug up from the same nursery. The former were resistant to nematodes, bacterial wilt and spotted alfalfa aphids but very susceptible to leaf spots and other fungal diseases. The latter had similar qualities but were less susceptible to leaf spots. These plants were placed in 6-inch pots and filled with field soil. They were taken to the greenhouse and grown for use.

In testing for the relative pathogenicity of fungal species to cuttings and in determining the kind of fungal pathogens present in sterilized and unsterilized sand, seedlings were found to give the fastest

results. However, both cuttings and seedlings were used in this study.

Removal of Stock Plants from the Field and Transplanting in the Greenhouse. The top growth and/or winter killed stems were cut off the selected plants as close as possible to the ground with a pair of shears. The plants were then carefully dug up with a spade and pick axe, taking as much of the original soil as possible with the roots. Great care was taken to protect the crown buds and the root system. After transplanting in the greenhouse, they were sprayed with a mixture of three tablespoonsful of manzate fungicide or one half tablespoonful of Captan 75, and two teaspoonsful of malathion per gallon of tap water about every 1-2 weeks as a preventive measure against greenhouse pests and fungal diseases. The soil was loosened and fertilized with hyponex or vigero as often as necessary. Plants were watered daily during the summer and spring and every 1-2 days during the fall and winter.

Rooting Medium. Fine grade, washed "masons" sand from the Kansas river that had been heaped outside the greenhouse was used for the rooting medium. It was covered and sterilized in covered 4-gallon aluminum pails in an autoclave at 15 lb steam pressure for 4-5 hours. Sand containers used for rooting the cuttings were sterilized the same way.

Planting and Watering Cuttings. Cuttings were made by cutting off 3-4 week old top growths (usually before blooming) with a pair of shears from the greenhouse plants. The cuttings were washed under running tap water and placed in a trough of water under shade. Cuttings of 1/2 - 1 inch portions of single internodes and single nodes with their entire trifoliolate leaves attached were made with a sharp single-edged razor blade and placed directly into a second trough of water under shade. The

upper cuts were made as close as possible to the nodes without injuring the petioles, stipules or buds. Water in the troughs was changed frequently. When more than one fungus species was involved in any one experiment, cuttings for each fungal treatment were placed in a separate water trough. This way, contamination of the cuttings with different fungal materials was avoided.

Two types of containers were used to root the cuttings and make the treatment tests. Aluminum loaf pans 9 5/8" x 5 1/2" x 2 3/4", with a surface area of one-third square foot were found to be the most satisfactory. Eight holes, about 3/16 inches in diameter, were punched in the bottom of these pans to allow for drainage. The pans were then filled with 1 3/4 - 2 kg of sand. In addition to these, large aluminum pans with a 2 sq. ft. surface area were divided into 1/3 sq. ft. sections with aluminum foil and occasionally used in place of the loaf pans.

Two deep furrows, 2 inches apart and about 1 1/2 inches from the walls of the pans were made in the sand with a metal spatula. Ten cuttings (unless otherwise stated) were planted in each row. Cuttings were then watered or chemically treated with a 1.7 liter metal can with a spout 3 inches long or, with a 360 cc plastic laundry sprinkler equipped with a screw cap bearing 7 small holes. As soon as the sand beds were planted and watered they were placed on wooden racks in a growth chamber.

One of two types of growth chambers were available for use. The first (Figure 1), and the one generally used, consisted of a wooden chamber (130" x 52" x 72" in front--52" at the back) situated on top of a soil filled greenhouse bench 36" high. It was covered in front and on

one side with a translucent, thin plastic material reinforced with fine wire gauze. The third side was wooden, while the rear wall was glass, separating it from the adjourning greenhouse to the west. The top of the chamber consisted of part of the glass roof of the greenhouse. The front portion had two doors (38" x 26") with spring snap locks.

A one-ton General Electric room air conditioner was placed inside 30" from the floor of the chamber. It was regulated with a Fenwall thermostat to maintain a temperature of 19-20° C. and equipped with a series of aluminum baffles inclined at 45° to the north to prevent direct light from the south (but not light from the north) from entering the chamber. This cooling system had the disadvantage of blowing air-borne organisms into the chamber. Also, the temperature could not be raised when it fell below the set range on cold nights and days.

The second growth chamber consisted of an iron frame (64" x 36" x 36") with a translucent plastic cover all around and covered on top with heavy cloth or cheese cloth as desired (Figure 2). It was set on a gravel covered bench 36" high in the greenhouse.

Both chambers were sprayed with malathion and then with water to eliminate greenhouse pests and dust particles inside of them. They were then allowed to dry before using.

Inoculation of Sand Beds with Desired Organisms. Inoculations were made in one of the following ways: (1) by "planting" 3-1 sq. cm. units of fungal inoculum, grown on 20 percent potato dextrose agar under laboratory conditions, in the sand beds at three equidistant places between the two rows of planted cuttings; (2) by mixing tiny units of the fungal inoculum with the sand before placing it in the containers.

Fig. 1. Air-conditioned growth chamber (in the background), with both doors opened to show cuttings growing in sand beds.

Fig. 2. Growth chamber with translucent plastic cover around it.

Foreground. A set-up of sand beds in loaf pans in which cuttings are being rooted.

Background. Two water-mist sprayers inside the chamber, as part of the greenhouse equipment.

Top. Cheese cloth covering the top of the chamber to prevent direct light from reaching the plants.

Inoculation of Potted Plants with Desired Organisms. The required amount of fungal inoculum was blended with water in a Waring blender for sixty seconds. Potted plants were then inoculated with the fungal suspension by using an atomizer. A list of the organisms used in testing the alfalfa cuttings and seedlings is presented in Table 1.

Harvesting Cuttings and Rating the Amount of Infection. Cuttings were harvested after the rooting period by carefully digging them up with a metal spatula and washing the roots in a trough of tap water.

In tests involving only Rhizoctonia, the cuttings were rated as follows:

A. Plants that are living and rooted.

B. Plants that are living, rooted and not infected.

In other tests involving a number of fungal species, cuttings were rated as follows:

1. Plants that are living, rooted and not infected.
2. Plants that are living, rooted but with minor infection.
3. Plants that are living, rooted but with 50 percent stem infection.
4. Plants dead and not rooted.

Isolation of Organisms from Diseased Plants and Maintaining Cultures. After the cuttings had been harvested they were kept in separate, moist, paper towels in plastic dishes. These were stored in a refrigerator for a day or so, when necessary, until isolations could be made.

When preparing to make isolations, selected plants were washed in distilled water, using a pair of forceps to handle them, and blotted dry. Small sections were cut from these plants with a pair of sterile scissors

and sterilized in 25 percent chlorox for 60-90 seconds in the case of stems, and 15 seconds for roots. The dissected pieces were plated on acidified 20 percent potato dextrose agar (P.D.A.) in petri plates. One or two drops of 25 percent lactic acid was added to each poured plate before solidification. Plates were incubated, kept inverted in plastic chambers to incubate at room temperature and illuminated. Isolates were examined within 5-10 days. Organisms not readily identified were transferred to 20 percent P.D.A. slants for later identification. Stock cultures were maintained on 20 percent P.D.A. slants kept at room temperature and illumination for 5-10 days or until adequate growth was made and then stored in a refrigerator.

Organisms used in testing cuttings are listed in Table 1.

Methods Used in Testing with Alfalfa Seeds

Source of Seeds. The seeds used in these experiments were Cody alfalfa, breeders lot, obtained from California in June, 1957. They had an average of 85 percent germination when grown on sterilized, moist, filter paper in petri plates or in sterilized sand under laboratory conditions.

Seed Treatment Prior to Sowing. Before using the seeds, they were surface sterilized in 1/1000 mercury chloride solution for one minute, then washed in distilled water for 20 minutes, spread on paper towels on a clean laboratory table and covered with plastic dishes until they were completely dry. Seeds were then gently scarified by mechanical means for

one minute and stored in fresh envelopes. Shrivelled, small and wrinkled seeds were discarded. Only clean and apparently healthy seeds were used in testing. When treated this way, they had an average of 92 percent germination on sterile,moist, filter paper in petri plates or in sterilized sand.

Sowing and Watering of Seeds. Seeds were sown by making four furrows two inches apart and 1/4 inch deep in the sand beds along with the width of the containers. The furrows were made with a wooden stake by pressing it to a depth of 1/4 inch in the sand. Twenty-five seeds were dropped from a small envelope into each furrow and distributed uniformly along the entire length with a camel's hair brush. The seeds were covered, watered and placed in the growth chamber.

Inoculation of Sand Beds and Seedlings with Desired Organisms. The fungal inoculum was prepared by blending the required amount of inoculum (grown on 2 percent P.D.A. in petri plates) with water in a Waring blender for sixty seconds. Sand beds were inoculated by using separate laundry sprinklers for each fungus, and covering the inoculum lightly with sand. Seedlings were inoculated with the same preparations applied with separate atomizers for each one.

Harvesting Seedlings and Rating The Amount of Infection. Seedlings were harvested by digging them up with a metal spatula. They were washed by the roots in a trough of water, examined and classified as (1) healthy or (2) diseased.

Isolation of Organisms from Diseased Seedlings and Maintaining Cultures. The procedures used in isolating organisms from diseased cuttings

were also used here except that stem and root sections were sterilized for 15 seconds.

Organisms used in testing seedlings were the same as those used in testing cuttings (See Table 1).

RESULTS

Chemical Control of Sand- and Stem-Borne Damping-Off Organisms of Alfalfa Cuttings

The studies discussed in this section were designed to learn if Rhizoctonia solani as well as other organisms isolated from diseased alfalfa cuttings could be controlled in sand beds by chemical treatments.

Chemical Control of Rhizoctonia solani. On November 30, 1959, 36 loaf pans were filled with sterilized sand and each planted with 20 cuttings made from 3-week old stems of clone 50-1266. Plants were placed in the air-conditioned growth chamber and allowed to grow. They were watered every 1-2 days.

On the third day 30 of the sand beds were inoculated with Rhizoctonia solani (A54R) which had been previously isolated from alfalfa roots. Three 1 sq. cm. pieces of P.D.A. covered with a 3-day old growth of this strain were spot inoculated at equidistant points between the two rows of planted cuttings and allowed to grow and infect the cuttings.

On the morning of December 8, 1959, while the sand beds were still fairly moist, some of the plants were found wilting (damping-off). Six replications of the treatments listed in Table 2 were, therefore, made at this time and randomized.

Table 1. List of organisms used in testing with alfalfa cuttings and seedlings.

Organism	Designation	Source
1. *Rhizoctonia solani* Kuhn	A54R	Isolated from alfalfa roots and supplied by Dr. P. C. Duffield
2. *Alternaria tenuis* Nees	*Alternaria*	Isolated by author from diseased alfalfa cuttings
3. *Pythium debaryanum* Hesse	*Pythium*	Obtained from Iowa State College and supplied by Dr. P. C. Duffield
4. *Ascochyta imperfecta* Peck	*Ascochyta*	Isolated by author from diseased alfalfa cuttings
5. *Colletotrichum graminicolum* (Ces.) G. W. Wilson	*Colletotrichum*	Isolated by author from diseased alfalfa cuttings
6. *Colletotrichum* sp.	*Colletotrichum*	Isolated by author from diseased alfalfa cuttings
7. *Fusarium roseum* (*Avenaceum*) (Fries) Saccardo Syn. *F. subulatum* Appel. and Wollenweber	*Fusarium* (Pink)	Isolated by author from diseased alfalfa cuttings
8. *Fusarium solani* (Martius) Appel and Wollenweber Syn. *F. alluviale* Wollenweber and Reinking	*Fusarium* (Yellow)	Isolated by author from diseased alfalfa cuttings
9. *Fusidium* sp.	(*Cephalosporium*) in early experiments	Isolated by author from diseased alfalfa cuttings
10. *Ascochyta* sp.	*Ascochyta* (Air)	Air sampled by Dr. C. L. Kramer

The chemical treatments were repeated twice more at two-week intervals on December 22, 1959, and January 5, 1960.

Table 2. Chemical treatments for the control of Rhizoctonia solani (A54R) among cuttings.

Treatments	Concentration of chemical	Amount used per pan
1. Cuttings + A54R + Panogen-Drench 0.4	1 cc/1-33 liters distilled water	100 cc
2. Cuttings +A54R + Captan 75	0.4 gm/liter distilled water	100 cc
3. Cuttings + A54R + Dithane D-14	2 cc/liter distilled water	100 cc
4. Cuttings + A54R + D-113	0.6 cc/liter distilled water	100 cc
5. Cuttings + A54R	--	--
6. Cuttings (Control)	--	--

A week after the last chemical treatment, the plants were harvested, examined, and rated according to the degree of infection (Figures 3, 4). The results of these are presented in Table 3.

Prevention of Damping-Off of Alfalfa Cuttings by Rhizoctonia solani by Increased Dosage of Inoculum. In order to check the limit of effectiveness of the chemicals used, an experiment using increased amounts of inoculum of Rhizoctonia solani (A54R) were run. On January 16, 1960, four-day old plates of Rhizoctonia

Table 3. Classification of plants according to the degree of infection.

Replication	Treatments: Captan 75 + A54R A	Captan 75 + A54R B	Pano-Drench + A54R A	Pano-Drench + A54R B	Dithane + A54R A	Dithane + A54R B	D-113 + A54R A	D-113 + A54R B	A54R only A	A54R only B	Cuttings (control) A	Cuttings (control) B
1.	20	17	18	18	11	5	3	2	4	3	19	19
2.	20	20	18	9	4	0	3	0	0	0	18	15
3.	13	11	19	18	13	3	10	1	10	8	19	17
4.	12	6	14	11	10	0	1	0	9	5	18	15
5.	20	18	19	17	12	0	8	0	0	0	15	9
6.	17	14	17	11	5	0	1	1	5	0	18	11
Total percent	85	71.7	87.5	70	46	6.7	21.7		23.3		89.1	
average							3.3		13.3		71.7	

A. Plants that are living and rooted.

B. Plants that are living, rooted and not infected.

A54R = Rhizoctonia solani (A54R).

solani (A54R), grown on P.D.A., were each divided into tiny pieces and one-tenth of each plate mixed thoroughly with the sterilized sand in each of 20 loaf pans. The pans were watered and placed in the air-conditioned growth chamber. Twenty other loaf pans (as controls) were filled with sterilized sand, watered and placed in the same growth chamber.

On the third day each of the 40 sand beds was planted with four-week old cuttings made from stems of clone 50-1266. Four replications of the following treatments were made and randomized:

Fig. 3. Cuttings in sand beds at the time of harvesting showing the effects of treatments.

Fig. 4. Representative sample of harvested, rooted cuttings showing the effects of treatments.

1. Captan 75 + Cuttings
2. Captan 75 + R. solani + Cuttings
3. Panogen-Drench + Cuttings
4. Panogen-Drench + R. solani + Cuttings
5. Dithane + Cuttings
6. Dithane + R. solani + Cuttings
7. D-113 + Cuttings
8. D-113 + R. solani + Cuttings
9. R. solani only
10. Cuttings only

The concentrations and amounts of chemicals used were the same as in the experiment above. The chemical treatments were repeated twice more at two-week intervals on February 2 and February 16, 1960.

One week after the last chemical application, February 23, the plants were dug up and examined for fungal infection (Figures 5, 6). The effect of treatments on fungal infection is presented in Table 5.

Prevention of Damping-Off of Alfalfa Cuttings by Rhizoctonia solani in Heavily Infested Sand by Increased Dosage of Chemicals and Inoculum. To further test the limits of effectiveness of the chemicals used, an experiment using even larger amounts of inoculum of Rhizoctonia solani (A54R) were run. In addition, increased dosages of the chemicals were used to study their effects on the cuttings. For this purpose 20 loaf pans were filled with sterilized sand on March 20, 1960, watered and placed in the growth chamber with plastic sides. The chamber was covered on top with a heavy white cloth. One plate each of four-day old Rhizoctonia

Fig. 5. Cuttings in sand beds at the time of harvesting showing the effect of treatments.

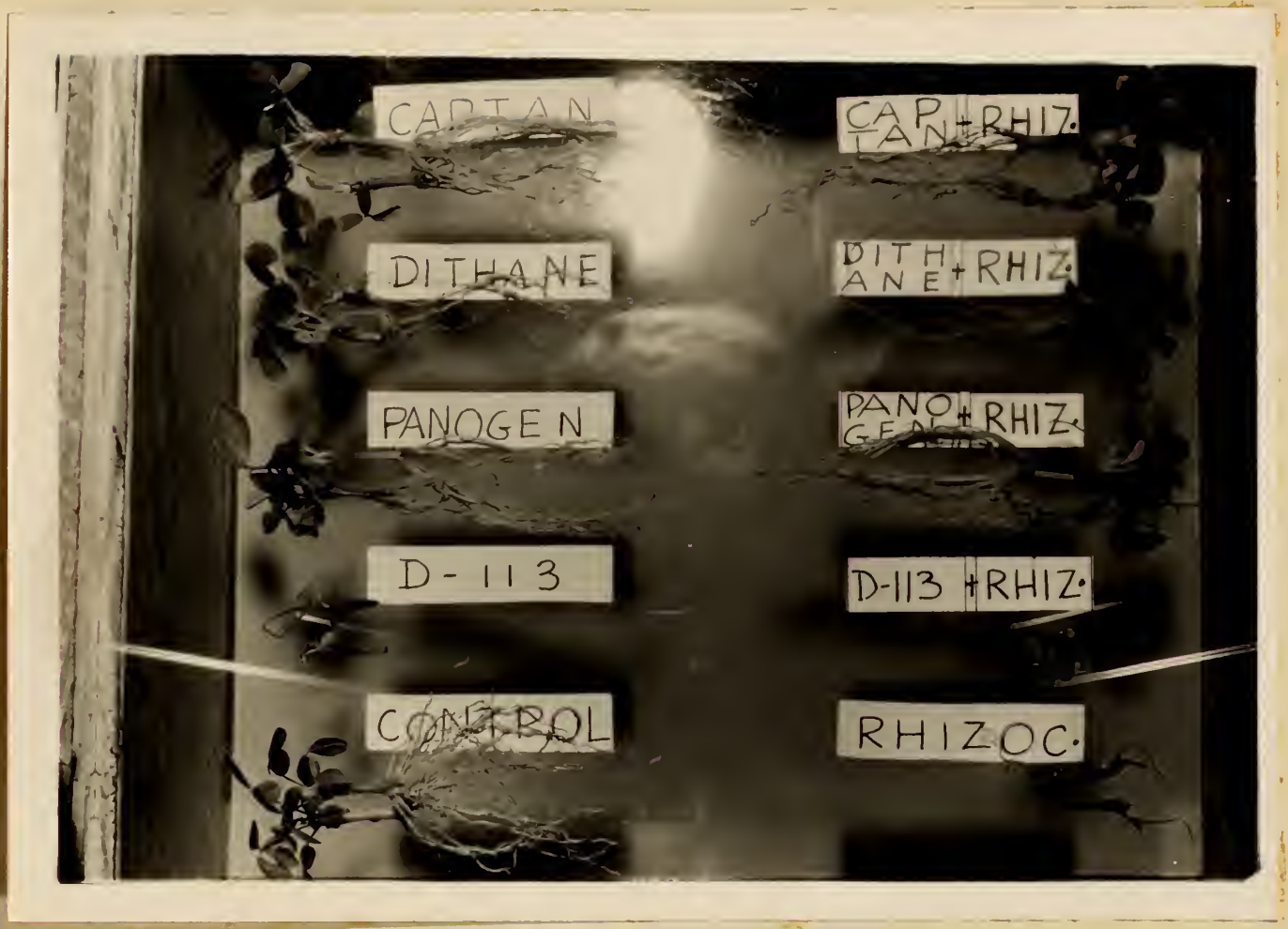


Fig. 6. Representative samples of harvested, rooted cuttings showing the effect of treatments.

Table 5. Classification of plants according to degree of infection.

Treatments	Rep 1		Rep 2		Rep 3		Rep 4		Total	Total
	A	B	A	B	A	B	A	B	%A	%B
Captan 75	20	19	20	17	20	17	18	14	97.5	83.75
Captan 75 + R	19	17	14	12	20	17	20	8	91.25	67.5
Panogen-Drench	20	18	20	11	20	15	20	17	100	76.25
Panogen D + R	18	5	20	9	18	13	18	13	92.5	50.0
Dithane	3	3	6	3	10	7	14	4	41.25	21.5
Dithane + R	3	2	8	3	10	5	12	10	41.25	25.0
D-113	0	0	3	0	6	0	2	0	13.75	0
D-113 + R	6	0	6	0	12	0	7	0	28.75	0
R-only	4	2	11	1	1	0	0	0	20.5	3.75
Cuttings only	20	14	19	17	20	13	19	14	97.5	72.5

A = Plants that are living and rooted.

B = Plants that are living, rooted and not infected.

(R = Rhizoctonia solani (A54R) four days old on P.D.A.).

solani (A54R) was cut into little pieces and thoroughly mixed with the sand in each of another 20 loaf pans. The pans were watered and placed in the growth chamber to incubate. They were all kept moist for the next ten days.

Since the temperature of the growth chamber varied with the temperature of the rest of the greenhouse, a study of it had to be made. It was recorded from two thermometers, one hung from the middle of the roof of the chamber, and the other placed in one of the sand beds in the middle,

every morning (8:00 - 9:00 a.m.), afternoon (1:00 - 2:00 p.m.) and night (10:00 p.m.), during the entire growth period. The average temperature for these periods was 21-25° C, 28-30° C., and 18-19° C, respectively.

After the ten-day incubation period, four randomized replications of each of the following treatments were made by applying the chemicals in two parts at an interval of eight hours and allowing them to stand overnight. The treatments are summarized in Table 6.

Table 6. Chemical treatments for the prevention caused by Rhizoctonia solani among cuttings.

Treatment	Concentration of chemicals used	Amount of chemical applied per pan
Captan 75	0.4 gm/liter distilled water	300 cc
Pano-Drench .4	1 cc/1 1/3 liter distilled water	300 cc
Dithane D-14	2 cc/liter distilled water	300 cc
D-113	0.6 cc/liter distilled water	300 cc
Captan 75 + R. solani	0.4 gm/liter distilled water	300 cc.
Pano-Drench .4 + R. solani	1 cc/1 1/3 liters distilled water	300 cc
Dithane D-14 + R. solani	2 cc/liter distilled water	300 cc
D-113 + R. solani	0.6 cc/liter distilled water	300 cc
R. solani only		
No treatments		

On the next day 20 cuttings were made from three-four week old stems of clone 50-1266 and planted in each sand bed. Twenty-one days after planting the plants were harvested and rated as described above and the results are presented in Table 7.

Table 7. Classification of plants according to the degree of infection.

Treatments	Rep 1		Rep 2		Rep 3		Rep 4		Totals	
	A	B	A	B	A	B	A	B	% Total A	% Total B
Captan 75	19	10	19	10	20	17	20	18	97.5	68.75
Captan 75 + *R. solani*	1	0	3	1	7	4	5	4	20	11.25
Panogen	19	15	20	15	20	19	20	17	98.75	82.5
Panogen + *R. solani*	9	3	5	1	5	1	3	1	27.5	7.5
Dithane	1	0	16	11	12	10	9	2	47.5	28.75
Dithane + *R. solani*	0	0	3	1	1	1	3	1	8.75	3.75
D-113	3	2	3	1	2	0	1	0	11.25	3.75
D-113 + *R. solani*	4	2	6	4	5	1	7	5	27.2	15
R. solani only	0	0	0	0	0	0	0	0	0	0
No treatments (Control)	20	17	16	13	20	18	20	20	95	85

A = Plants that are living and rooted.

B = Plants that are living, rooted, and not infected.

When 3 ppm (active ingredient) of Panogen-Drench was applied at the rate of 100 cc per pan, very little phytotoxic effects were observed on the plants. In the absence of Rhizoctonia solani, 100 percent of the plants survived and 76.25 percent plants without infection were obtained. In the presence of R. solani, (1/10 plate or less of inoculum per pan), an average of 90 percent of the plants survived and 60 percent of the plants were without infection.

When the same concentration of Panogen was applied at the rate of 300 cc per pan, slight yellowing of leaves was observed. Plants were a little stunted and had fewer roots than plants that had no chemical or fungal treatment. In the absence of R. solani, 98.75 percent of the plants survived with 82.5 percent being without infection. In the presence of R. solani, (one plate per pan), only 27.5 percent survived and 7.25 percent were without infection.

When 300 ppm (active ingredient) of Captan was applied at the rate of 100 cc per pan, the plants which were not infected were very vigorous (compared with control plants). Generally, plants were uniform in growth and strongly rooted with roots 2-6 inches long and averaging 8 roots per plant. In the absence of Rhizoctonia solani, 97.5 percent of the plants survived and 83.75 percent were without infection. In the presence of R. solani, (1/10 plate or less of inoculum per pan), an average of 88 percent of the plants survived and about 70 percent were without infection.

When the same concentration of Captan was applied at the rate of 300 cc per pan, and in the absence of R. solani, 97.5 percent survived and 68.75 percent were without infection. In the presence of R. solani, (one plate per pan), only 20.0 percent survived and 11.25 percent were uninfected.

When 280 ppm (active ingredient) of Dithane D-14 was applied at the rate of 100 cc per pan, the plants showed acute phytotoxic effects (yellowing and whitening of petioles, and margins of leaflets) 2-3 days after treatment. Surviving plants had weak, pale green leaves, one or two strong, long roots together with numerous, short, and fibrous roots that bore no nodules. About 1/4 inch to 1/ 2 inch from the base of the surviving plants were green, but shrivelled, soft and dead, and the roots emerged directly from the stem ends just above the dead regions. Occasionally, roots emerged from the nodes near the sand line when the rest of the basal portion had died.

When Dithane was applied at the rate of 300 cc per pan, acute phytotoxic effects showed up on the plants overnight. Similar but severer effects than described above were observed. The few plants that still survived were dead from the base up to the node. Such plants could undoubtedly not be used as transplants. Similar but severer symptoms than Dithane treatment were observed in the D-113 treatment.

In tests using Rhizoctonia solani without chemical treatments, the following two kinds of symptoms were observed: (1) a sudden, severe wilting of plants which later turned water-soaked, pale to dark brown and finally died off with the leaves still attached; and (2) gradual invasion of plants by the fungus, causing browning of stems, gradual stunting and finally death of the plants. Diseased stems usually had elliptical or oval, dark brown lesions which in severe cases merged together rapidly, encircled the stems and killed the plants. Symptoms of the first kind usually showed up in three days. Most of the damping-off, however, occurred during the first 7 to 14 days. Few plants ever rooted before being killed.

About 92 percent of the plants were damped-off or badly infected within 2-3 weeks.

At the rate of one plate of R. solani per pan, infection became severer and practically all the cuttings were damped-off within 5-10 days.

In the controls, which were neither inoculated or treated with chemicals, plants were vigorous and normal. On the whole 90.5 percent of the plants survived while 76.4 percent were disease free. Those that were diseased usually were split or decayed at the base or infected from the upper cut end. Few plants were rotted up to the node from either direction, a few others were unable to root, or dead.

Thus at low rates of inoculation with Rhizoctonia solani, Captan and Panogen-Drench gave the best control of damping-off. Captan and Panogen had little effect on R. solani, however, when one plate of inoculum was used per 1/3 sq. ft. of sand.

Chemical Treatment of Sand Infested With Damping-Off Organisms. On November 10, 1960, ten sand beds in which practically all the alfalfa cuttings planted in them had damped-off were obtained from the Kansas State University alfalfa breeding greenhouse. The purpose of this experiment was first to find out what pathogens were infesting the cuttings and sand and secondly, to find a chemical control for the possible pathogens.

About 100 gm of sand was taken from each sand bed and mixed in sterile dishes. Isolations were then made by (1) mixing 5 gm of sand from each 100 gm sample with 10 cc of sterile water and pipetting 1 ml of the suspension into duplicate plates of acidified 20 percent P.D.A., (2) by sprinkling small amounts of the mixed sand on another pair of acidified P.D.A. plates and, (3) by plating sterilized sections of diseased

cuttings taken from each sand bed on a pan of acidified P.D.A. plates.

The plates were examined within 3 days. As many different isolates as possible were transferred to P.D.A. slants and petri plates for further identification. Stock cultures were also kept in a refrigerator. The results of these isolations are presented in Table 8.

On November 17, 1961, the remainder of the sand was mixed together. Part of it was then sterilized. Sixty loaf pans were filled with unsterilized sand. Four replications of the treatments presented in Table 9 were then made and allowed to stand overnight.

On the following day 20 cuttings were made from four-week old stems of clone 50-1266, planted in each sand bed, watered and placed in the air-conditioned growth chamber.

Plants were harvested four weeks later, examined, rated and the results presented in Table 10. Isolations were then made from diseased stems. The results of these are also presented in Table 8.

Effects of Chemicals on Plants. There was general yellowing among all the plants, and especially the chemically treated ones, about three-four days after the treatments. For this reason the chemical applications were not repeated. Vapam and Crag Mylone, had caused acute bleaching, yellowing of leaves, and, in most cases, total death of plants by the seventh day. Manzate, Thiram, Spergon, Ferbam and Semesan had phytotoxic effects of acute yellowing and killing of plants. By the end of the second week the effects of the chemicals were very characteristic. In the order of their decreasing phytotoxic effects, Thiram, Manzate, Ferbam, Spergon and Semesan treated plants were either acutely yellowed and stunted or dead.

Table 8. Isolates recovered from sand and diseased cuttings prior to experimentation.

Method of Isolation	Isolates
Sand dilution	Fusarium roseum ((Fusarium (Pink))
	Fusarium solani ((Fusarium (Yellow))
Sprinkled sand	Fusarium roseum ((F. (Pink))
	Fusarium solani ((F. (Yellow))
	Alternaria spp.
Diseased cuttings	Alternaria spp.
	Ascochyta imperfecta
	Fusarium roseum
	Fusarium solani
	Fusidium spp.

At the rates the various chemicals were applied, Crag Mylone and Vapam were the most phytotoxic of them all. Phytotoxic symptoms caused by these chemicals showed up in a few days and cuttings treated with these chemicals were either partially or completely killed in one-two weeks. Captan 75 and Panogen which had before given satisfactory results were here found to be slightly phytotoxic to the cuttings. Of the other treatments, Thiram, Manzate, Ferbam, Spergon and Semesan showed acute

Table 9. Chemical treatments for the prevention of damping-off of cuttings caused by sand-borne fungal pathogens

Treatments	Concentration of chemicals	Amount used per pan
Captan 75	0.5 gm/liter distilled water	100 cc
Captan dieldrin	0.5 gm/liter distilled water	100 cc
Actidione Captan	0,5 gm/liter distilled water	100 cc
Manzate	0.5 gm/liter distilled water	100 cc
Dyrene 50W	0.5 gm/liter distilled water	100 cc
Phaltan	0.5 gm/liter distilled water	100 cc
Spergon	0.5 gm/liter distilled water	100 cc
Thiram	0.5 gm/liter distilled water	100 cc
Ferbam	0.5 gm/liter distilled water	100 cc
Tersan OM	0.5 gm/liter distilled water	100 cc
Semesan	0.5 gm/liter distilled water	100 cc
Crag Mylone 85W	1 gm/liter distilled water	100 cc
Vapam	3 cc/liter distilled water	100 cc
Pano-Drench .4	1 cc/1 1/3 liters distilled water	100 cc
Unsterilized sand (only)		
Sterilized sand (only)		

Table 10. Classification of plants according to the degree of infection.

Treatments	Total % Alive	Total % Healthy	Total % (Badly Infected or Dead)
Captan 75	50.0	36.25	63.75
Captan dieldrin	57.5	43.75	56.25
Actidione Captan	57.5	40.0	60.0
Manzate	26.25	13.75	86.25
Dyrene	56.25	35.0	65.0
Phaltan	63.75	46.25	53.75
Spergon	31.25	15.0	85.0
Thiram	30.0	1.25	98.75
Ferban	18.75	7.5	92.5
Tersan OM	57.5	31.25	68.75
Semesan	48.75	27.5	72.5
Crag Mylone	0	0	100.0
Vapam	0	0	100.0
Panogen	65.0	35.0	65.0
Unsterilized Sand	80.0	46.25	53.75
Sterilized Sand	87.5	73.75	26.25

phytotoxic effects. The least phytotoxic chemicals of them all were as follows in order of increasing phytotoxicity: (1) Phaltan, (2) Dyrene (3) Tersan OM (4) Actidione Captan and (5) Captan dieldrin.

The actual cause of the yellowing was not known. It is possible, however, that (1) the age of the plants, (2) their sugar content, (3) the effects of the chemicals themselves, or (4) the amount of light in the growth chamber could have been contributing factors. The most salient of the factors was probably the interaction between the plants and the chemicals. The fact that there was general yellowing in the treated as well as non-treated (check) pans, however, makes the remaining factors possibilities.

From this experiment, however, the best treatments were: (1) sterilized sand with 87.5 percent survived plants and 73.75 percent healthy plants; (2) unsterilized sand with 80 percent survived and 46.25 percent healthy plants; (3) Phaltan with 63.75 percent survived and 46.25 percent healthy plants; (4) Captan-dieldrin with 57.5 percent survived and 43.75 percent healthy plants; (5) Actidione-Captan with 57.5 percent survived and 40 percent healthy plants; (6) Captan 75 with 50 percent survived and 36.25 percent healthy plants; (7) Panogen with 65 percent survived and 35 percent healthy plants; (8) Dyrene with 56.25 percent survived and 35 percent healthy plants (Figures 6a, 6b).

Chemical Control of Damping-Off Among Cuttings Made From Stems Inoculated with Selected Fungi. The purpose of this experiment was to find out whether or no

FIGURE 6a

Effects of chemicals on rooting and survival of cuttings after harvesting.

FIGURE 6a

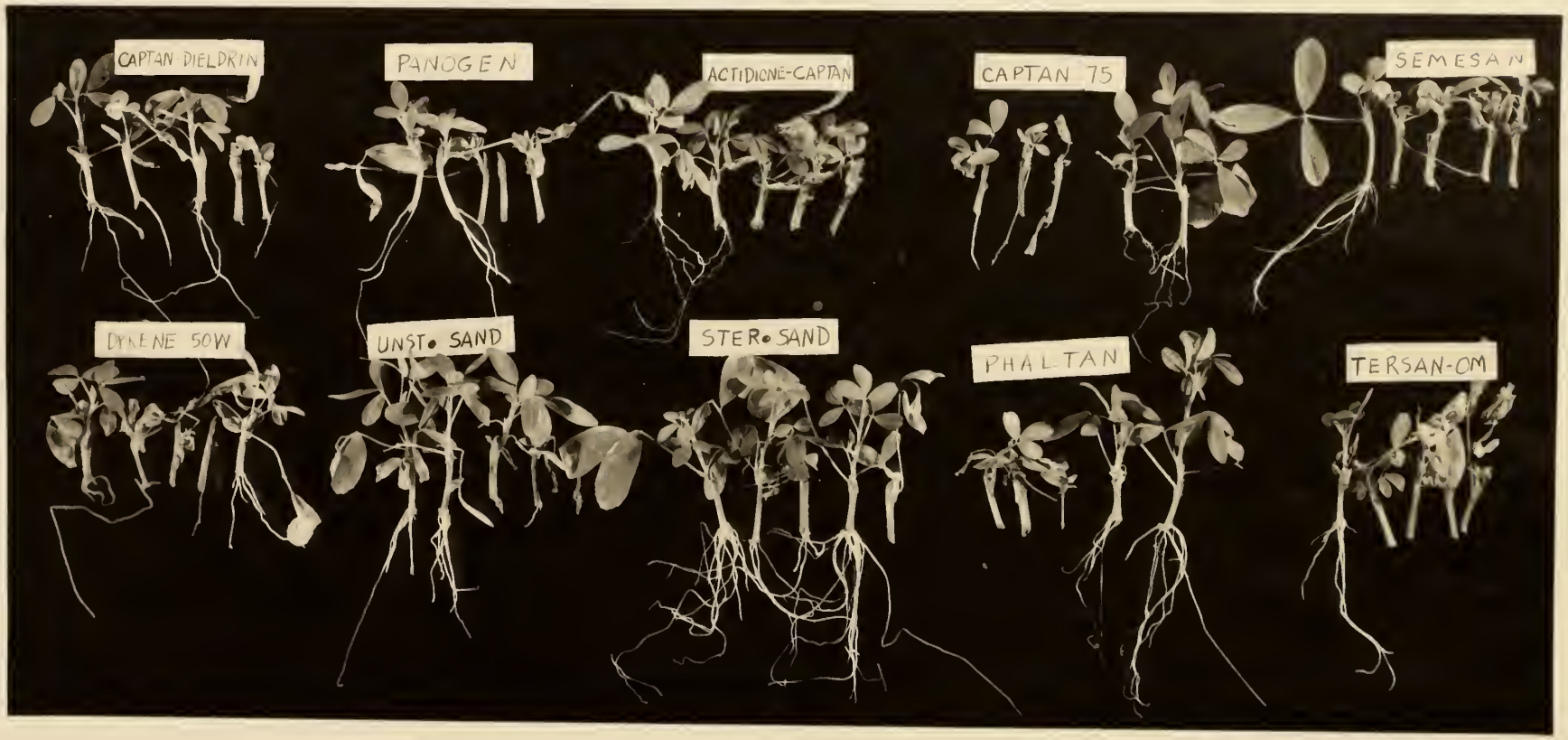
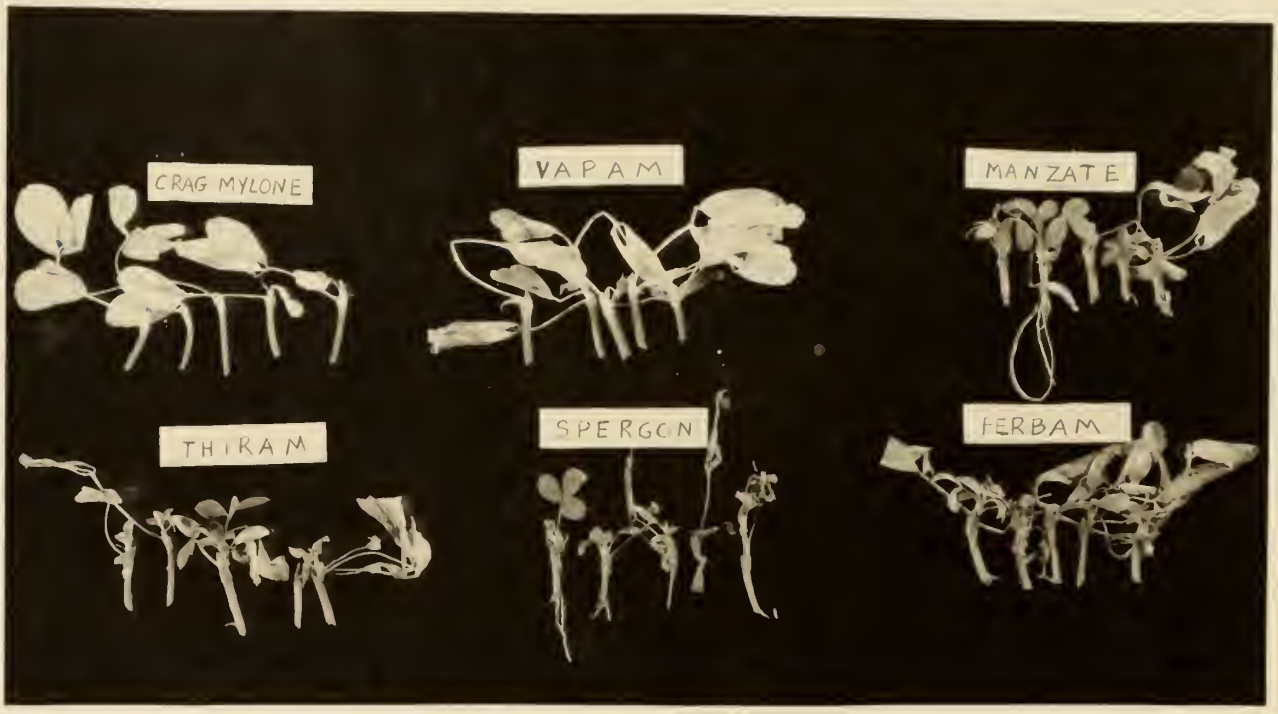

CAPTAN-DIELDRIN
PANOGEN
ACTIDIONE-CAPTAN
CAPTAN 75
SEMESAN
DYRENE 50W
UNST. SAND
STER. SAND
PHALTAN
TERSAN-OM

FIGURE 6b.

Effects of chemicals on rooting
and survival of cuttings after harvesting.

FIGURE 6b



damping-off of cuttings by stem-borne organisms could be chemically controlled.

On March 14, 1961, two plates each of ten-day old fungus inoculum grown on 2.0 percent P.D.A., were blended in a Waring blender with 100 cc distilled water. Seven potted plants of clone 30-1108 with four week old top growths were selected and given the following treatments by spraying the inoculum with an atomizer.

Fungal treatments

1. Rhizoctonia solani (A54R)
2. Pythium debaryanum
3. Colletotrichum graminicolum
4. Fusarium roseum
5. Fusarium solani
6. Ascochyta imperfecta
7. No inoculum (Control)

The plants were then placed in a humid and moist chamber for three days. They were then taken out and kept in a shade for five days.

Ten cuttings were then made from each potted plant and planted in each of six sterilized sand beds in loaf pans. Three replications of the following chemical treatments were then made and randomized. The chemical treatments were repeated three more times at seven day intervals.

Recording the temperature and relative humidity of the moist chamber. The moist chamber in which the plants were incubated was the same as that used by Grandfield et al. (22), Peterson and Melchers (45).

After the fourth chemical application and four weeks after planting, the plants were harvested, examined and rated as before. The total average results are presented in Table 13.

Table 11. Chemical treatments.

Chemical treatment	Concentration	Amount per pan
1. Captan 75	0.5 gm/liter 20 percent acetone solution	100 cc
2. Phaltan	0.5 gm/liter distilled water	100 cc
3. PCNB	0.5 gm/liter distilled water	100 cc
4. Dexon	0.5 gm/liter distilled water	100 cc
5. Panogen-Drench	1 ml/1 1/3 liters distilled water	100 cc
6. No chemical treatment		

From the inoculation studies, the most virulent of the fungal isolates was Ascochyta imperfecta. Infection caused by this isolate was so severe that it was doubtful whether the cuttings would survive or root at all. A very high percentage of cuttings made from the infected stems, however, survived and rooted. On the one hand, the number of infection sites or diseased spots seemed to increase on the stems below soil level without inhibiting the rooting ability of the plants. This increase in infection sites without actually inhibiting rooting or the survival of the plants caused most of them to be rated as "minor infection" (73.3 percent). On the other hand, few cuttings that were badly infected before planting succumbed to the black stem disease (12, 25, 31, 45, 47, 59) and either partially survived (50 percent stem infection) or completely died off within the four week growth period. An average of 73.3 percent of the cuttings made from heavily A. imperfecta infested stems could be used as transplants (with minor infection), and only 26.6 percent were either partially or completely dead and could not be used.

Table 12. Effect of chemical treatments on cuttings made from stems inoculated with fungi.

Treatment	Effects on plants
1. Captan 75	Acute phytotoxicity resulting in acute yellowing of leaves and destruction of the young buds. Symptoms increased with repeated treatments. By the time the third application was made, 90 to 100 percent of the plants had already died.
2. Phaltan	No effects observed
3. PCNB	No effects observed
4. Dexon	Acute phytotoxicity resulting in blackening and death of 80 to 100 percent of plants after the first week and 100 percent of the plants by the end of the third week.
5. Panogen-Drench	Stunting of plants and slight phytotoxicity.
6. Check	None

Table 13. Classification of Plants according to the degree of infection with the six fungi.

Fungal Treatments	Rhizoctonia solani				Pythium debaryanum				Colletotrichum graminicolum				Fusarium roseum				Fusarium solani				Ascochyta imperfecta				Check			
	Classification																											
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Captan 75			100				3.3	96.7			3.3	96.7			100				100				100		10		3.3	86.7
Phaltan	83.3	3.3	13.3		90	6.6	3.3		96.6		3.3		50	50				100			56.6	13.3	30		60	40		
PCNB	90		10		83.3		16.6		96.6	3.3			20	60	20		36.6		63.4		80	16.6	3.3		86.6	13.4		
Dexon			100		90		10				100				100				100			13.3	86.6				100	
Panogen-Drench	73.3		26.6		36.7		63.3		76.6	6.6	16.6		63.3		36.7		43.3		56.7		6.6		93.4		26.6	63.3		10
Check	40		60		100				100		13.3		86.7				96.6		3.3		73.3	16.6	10		70	23.3		6.6

1 = Total percent healthy plants.

2 = Total percent plants with minor infection.

3 = Total percent plants with 50 percent stem infection.

4 = Total percent plants dead or not rooted.

The only chemical which seemed to control Ascochyta imperfecta damping-off slightly was PCNB. Eighty percent of the plants were recovered with minor infection and 20.0 percent with major infection. In the Phaltan treatment, which had no phytotoxic effects, 56.6 percent of the plants were recovered with minor infection. In the rest of the chemical treatments from 93.4 percent to 100 percent of the plants had damping-off before harvesting.

Because of the acute phytotoxic effects of Dexon and the acetone solution of Captan 75 on the plants, their efficiency in controlling Ascochyta damping-off of cuttings could not be estimated. On the other hand, Panogen-Drench with little phytotoxic effects did not control the disease, it probably weakened the plants to a point where they had to succumb to both the fungal disease and chemical effects.

When the stems were infested with Rhizoctonia solani, 40 percent of the rooted plants were recovered with minor infection and 60 percent were completely dead. Corresponding figures for PCNB treatment were 90.0 percent and 10 percent, Captan 75 and Dexon with 0 and 100 percent each. Eighty-three and three-tenths percent of the plants from the Phaltan treatment had minor infection and 16.6 percent had major infection; 73.3 percent of the plants from the Panogen treatment had minor infection and 26.6 percent had major infection. Thus PCNB, Phaltan and Panogen each satisfactorily controlled damping-off caused by R. solani.

When the plants were infested with Pythium debaryanum, 100 percent of the rooted cuttings were recovered with minor infection. Ninety percent of the plants treated with Phaltan had minor infection and 10 percent had major infection; 83.3 percent of the plants treated with PCNB had minor

infection and 16.6 percent had major infection; 36.7 percent of the Panogen treated plants had minor infection while the remainder died.

When the plants were infested with Colletotrichum graminicolum all the plants were recovered with minor infection. Ninety-six and six-tenths percent of the plants were recovered with minor infection from both the Phaltan and PCNB treatments, and 100 percent plants with major infection from both Dexon and Captan 75 treatments.

Thirteen and three-tenths percent healthy plants and 86.7 percent plants with minor infection were recovered from the cuttings made from plants infested with Fusarium roseum. Corresponding values were 50.0 percent healthy and 50.0 percent plants with minor infection when treated with Phaltan; 20.0 percent healthy and 60.0 percent plants with minor infection when treated with PCNB: and 63.3 percent plants with minor infection when treated with Panogen. As usual, no surviving plants were recovered from both Captan and Dexon treatments.

With the Fusarium solani treated plants, 96.6 percent healthy and 3.3 percent plants with minor infection were recovered. When treated with PCNB, 36.6 percent healthy and 63.4 percent plants with minor infection were obtained. One hundred percent plants with minor infection were obtained when treated with Phaltan, and 43.3 percent healthy and 56.7 percent dead plants when treated with Panogen. No surviving plants were recovered from both Captan and Dexon treatments.

When cuttings were made from uninoculated plants and treated with the chemicals, the following results were obtained; 86.6 percent healthy and 13.4 percent plants with minor infection with PCNB treatment, 60 percent healthy and 40.0 percent plants with minor infection with Phaltan

treatment, 26.6 percent healthy and 63.3 percent plants with minor infection with Panogen treatment, 10.0 percent plants with minor infection with Captan treatment, and 70.0 percent healthy and 23.3 percent plants with minor infection when untreated with any chemicals.

The order of effectiveness of the treatments against the various fungi is summarized below in Table 14.

Thus of the chemicals, PCNB and Phaltan were the best to use. They had no phytotoxic effects on the plants. They were satisfactory treatments against Rhizoctonia solani and Ascochyta imperfecta. In most of the remaining cases they were only surpassed by the control (check) treatments.

Of the fungal isolates, the most virulent were Ascochyta imperfecta and Rhizoctonia solani. The amount of infection on the A. imperfecta inoculated plants was so heavy that to control its spread was impossible. The fact that 73.3 percent of the cuttings thus made rooted with comparatively minor infection, however, indicates that A. imperfecta did not inhibit the rooting of the cuttings for practically all the cuttings rooted in the controls (90.0 percent of them, not counting the 10.0 percent dead ones). As before, R. solani was considered the most important fungus to be encountered among alfalfa cuttings.

These observations seemed to support as well as contradict the observations made by Haskett et al. (25) at this station, even though we may be comparing the pathogenicity of two different isolates of A. imperfecta.

Fusarium roseum, Fusarium solani and Colletotrichum graminicolum could, therefore, be considered as weak pathogens under the conditions of this experiment. The only comment that could be made about F. roseum

Table 14. Summary of effectiveness of treatments.

Fungal Isolate	Order of effectiveness of treatments	Total av. % healthy plants	Total av. % plants with minor infection	Total av. % plants with major infection
Rhizoctonia solani	1. PCNB	—	90.0	10.0
	2. Phaltan	—	83.3	16.7
	3. Panogen	—	73.3	26.7
	4. Check	—	40.0	60.0
Pythium debaryanum	1. Check	—	100.0	0
	2. Phaltan	—	90.0	10.0
	3. PCNB	—	83.3	16.7
	4. Panogen	—	36.7	63.7
Colletotrichum graminicolum	1. Check	—	100.0	0
	2. PCNB/Phaltan	—	96.6	3.4
	3. Panogen	—	76.6	23.4
Fusarium roseum	1. Phaltan	50.0	50.0	0
	2. Check	13.3	86.7	0
	3. PCNB	20.0	60.0	20.0
	4. Panogen	—	63.3	36.7
Fusarium solani	1. Check	96.6	3.4	0
	2. PCNB	36.6	63.4	0
	3. Phaltan	—	100.0	0
	4. Panogen	43.3	—	56.7
Ascochyta imperfecta	1. PCNB	—	80.0	20
	2. Check	—	73.3	26.7
	3. Phaltan	—	56.6	43.4
Check	1. PCNB	86.6	13.4	0
	2. Phaltan	60.0	40.0	0
	3. Check	70.0	23.3	6.7
	4. Panogen	26.6	63.4	10.0

was that it seemed to require an alternation of slight drying and wetting for maximum growth and infection.

Chemical Control of Damping-Off of Alfalfa Cuttings Planted in Sand Beds Artificially Inoculated with Selected Fungal Isolates. The purpose of this experiment was first to find out which of the fungal isolates used in previous experiments, when present in the sand beds would cause damping-off of alfalfa cuttings and secondly to control by chemical means the damage the pathogen(s) might otherwise cause to the cuttings planted in the infested sand beds.

On April 6, 1961, large aluminum pans, divided into one-third square foot sections with aluminum foil, were filled with sterilized sand.

They were then inoculated with small pieces of seven day old fungal inoculum grown on P.D.A. The control treatments were not inoculated. The sand beds were placed in the air-conditioned growth chamber and allowed to incubate for five days.

Cuttings were then made from three to four week old stems of clone 50-1266, planted in the sand beds, and treated with the respective chemicals which were repeated weekly.

Two replications of the following fungal and chemical treatments were thus made and randomized.

Fungal treatments

1. Rhizoctonia solani (A54)	1/4 plate/1/3 sq. ft.
2. Pythium debaryanum	1/2 plate/1/3 sq. ft.
3. Colletotrichum graminicolum	1/2 plate/1/3 sq. ft.
4. Fusarium roseum	1/2 plate/1/3 sq. ft.
5. Fusarium solani	1/3 plate/1/3 sq. ft.

6. Ascochyta imperfecta 1/2 plate/1/3 sq. ft.

7. Check

Table 15. Chemical treatments.

Chemical	Concentration	Amount per replication
1. Captan 75	0.4 gm/liter water	100 cc
2. Phaltan	0.5 gm/liter water	100 cc
3. PCNB	0.5 gm/liter water	100 cc
4. Dexon	0.3 gm/liter water	100 cc
5. Panogen-Drench	1 cc/1 1/3 liters water	100 cc
6. Check	water	water

At the rates the chemicals were applied, Dexon was the only one that was phytotoxic to the plants. Most of the plants under this treatment died off within a week or so, a little longer than when (0.5 gm. concentration) was used before. They never had a chance to root. The few that escaped serious injury had very poorly developed roots or none at all. Panogen seemed to have slight adverse effects also. Most of the plants classified (total percent dead and not rooted) under this treatment were plants that had no roots. The effectiveness of the chemicals in preventing damping-off by the various fungi is summarized in Table 17. Plants were harvested, examined and rated as before, three weeks after planting and one week after the third chemical application.

Table 16. Symptoms on cuttings during the growth period.

Treatment	Symptoms
Rhizoctonia solani	About 90 percent of the cuttings had damped-off in the controls at the end of the first week and practically all of them by the end of the second and third weeks.
Pythium debaryanum	Plants were all stunted and pale green in color. Most of the damping-off occurred during the first 1 1/2 weeks.
Colletotrichum graminicolum	Disease development was slow as before. Few plants had died, however, by the end of the second and third weeks.
Fusarium roseum	Disease development was low as before. Few plants had died, however, by the end of the second and third weeks.
Fusarium solani	Plants were normal and vigorous. No signs of infection were observed until plants had been harvested.
Ascochyta imperfecta	Disease development was slow as before. Few plants had died, however, by the end of the second and third weeks.
Check	Plants were just normal and vigorous. No signs of infection observed until harvesting.

From this experiment it was found that Captan 75 and Panogen-Drench were the best treatments against Rhizoctonia solani. Captan 75 gave 70.0 percent complete control and 2.5 percent partial control, while Panogen-Drench gave 62.5 percent control and 15.0 percent partial control. Phaltan and PCNB gave fairly satisfactory control of 80 percent and 60 percent, respectively, on the basis of total average percent transplantable

plants. (Total average percent healthy + total average percent minor infection.) With R. solani uncontrolled by any chemical, only 2.5 percent of the plants escaped infection.

By similar reasoning, Captan 75 gave 60 percent complete control and 25.0 percent partial control against Pythium debaryanum while Panogen-Drench gave 65 percent and 10 percent complete and partial control respectively. PCNB was slightly superior to the check treatment with 35.0 percent and 10 percent complete and partial control as against 25.0 percent healthy plants with the organism uncontrolled.

With Colletotrichum graminicolum in the sand, the best preventive measure was obtained with PCNB, Panogen-Drench and Phaltan. 67.5 percent complete control and 20.0 percent partial control, were obtained with PCNB treatment as against 15 percent complete and 40 percent partial control in the check treatment. Corresponding value for Panogen-Drench, Phaltan and Captan 75 were 52.5 percent and 32.0 percent, 57.5 percent and 0.0 percent, 37.5 percent and 22.5 percent respectively.

The best treatments against Fusarium roseum were obtained with Panogen-Drench 82.5 percent and 12.5 percent complete and partial control respectively, 70.0 percent and 7.5 percent with Captan 75, 60.0 percent and 25.0 percent with Phaltan, 50.0 percent and 37.5 percent with PCNB. Corresponding results from the controls were 40.0 percent and 27.5 percent.

Panogen-Drench and Captan 75 were practically equally effective against Fusarium solani as they were against Fusarium roseum. Phaltan and PCNB were not only ineffective against F. solani but they seemed to enhance infection by the organism

The only real control against Ascochyta imperfecta was obtained with Captan 75. Sixty percent control and 10 percent partial control were obtained with Captan 75 as against 45.0 percent and 12.5 percent in the control. PCNB was only slightly superior to the control treatment with 45.0 percent control and 25.0 percent partial control of infection.

When cuttings were made from uninoculated plants and planted in sterilized sand, (check-check treatment), 95 percent healthy plants and 2.5 percent plants with minor infection were obtained. This treatment was superior to all the chemical treatments. The next best treatments were with PCNB and Phaltan in which 90 percent healthy and 7.5 percent plants with minor infection, and 82.5 percent healthy and 7.5 percent plants with minor infection were obtained, respectively.

The order of effectiveness of the treatments against the various fungi is summarized in Table 18.

On the whole, therefore, Captan 75, Panogen-Drench, PCNB and Phaltan were satisfactory treatments against the fungi. Captan and Panogen-Drench were specific against damping-off by Rhizoctonia solani and Pythium debaryanum.

PCNB, Panogen-Drench and Phaltan were satisfactory against Colletotrichum graminicolum; Panogen-Drench, Captan 75, Phaltan and PCNB were satisfactory against Fusarium roseum; Panogen-Drench and Captan 75 were satisfactory against Fusarium solani; while Captan 75 was considered the only satisfactory treatment against Ascochyta imperfecta. No practical advantage was obtained by treating cuttings planted in sterilized sand with the chemicals. In fact, they all seemed to injure the cuttings rooted in this manner slightly.

Table 17. Classification of plants according to the degree of infection with the six sand-borne fungi.

Fungal Treatments	Rhizoctonia solani				Pythium debaryanum				Colletotrichum graminicolum				Fusarium roseum				Fusarium solani				Ascochyta imperfecta				Check			
	Classification																											
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Captan 75	70	2.5	7.5	20	60	25	5	10	37.5	22.5	40	70	7.5	12.5	10	72.5	12.5	10	5	60	10	5	25	72.5	20			7.5
Phaltan	52.5	27.5	2.5	17.5	15	15	15	55	57.5	10	32.5	60	25	15		47.5	20		32.5	42.5	10	10	37.5	82.5	7.5	5		5
PCNB	47.5	12.5	5.0	35	35	10	25	30	67.5	20	5	7.5	50	37.5	2.5	10	45	12.5	30	12.5	45	25		30	90	7.5	2.5	
Dexon			2.5	97.5			100			10	90		17.5	12.5	70		18.5		82.5		12.5	5	82.5	45	10			45
Panogen-Drench	62.5	15	7.5	15	65	10	15	10	52.5	32.5	15		82.5	12.5	5	80	5	2.5	12.5	27.5	10		62.5	72.5	27.5			
Check	2.5		2.5	95	25			75	15	40	20	25	40	27.5	15	17.5	60	10	30	45	12.5	12.5	30	95	2.5	2.5		

1 = Total percent healthy plants (not infected).

2 = Total percent plants with minor infection.

3 = Total percent plants with 50 percent stem infection.

4 = Total percent plants dead or not rooted.

Table 18. Summary of effectiveness of treatments against the six sand-borne fungi.

Fungal isolate	Order of effectiveness of treatments	Total av. % healthy plants	Total av. % plants with minor infection	Total av. % badly infected plants
Rhizoctonia solani	1. Captan 75	70.0	2.5	27.5
	2. Panogen-Drench	62.5	15.0	22.5
	3. Phaltan	52.5	27.5	20.0
	4. PCNB	47.5	12.5	40.0
	5. Check	2.5	—	97.5
Pythium debaryanum	1. Captan 75	60.0	25.0	15.0
	2. Panogen-Drench	65.0	10.0	25.0
	3. PCNB	35.0	10.0	55.0
	4. Check	25.0	—	75.0
Colletotrichum graminicolum	1. PCNB	67.5	20.0	12.5
	2. Panogen-Drench	52.5	32.0	15.5
	3. Phaltan	57.5	—	42.5
	4. Captan 75	37.5	22.5	40.0
	5. Check	15.0	40.0	45.0
Fusarium roseum	1. Panogen-Drench	82.5	12.5	5.0
	2. Captan 75	70.0	7.5	22.5
	3. Phaltan	60.0	25.0	15.0
	4. PCNB	50.0	37.5	12.5
	5. Check	40.0	27.5	32.5
Fusarium solani	1. Panogen-Drench	80.0	5.0	15.0
	2. Captan 75	72.5	12.5	15.5
	3. Check	60.0	—	40.0
	4. Phaltan	47.5	20.0	32.5
	5. PCNB	45.0	12.5	42.5
Ascochyta imperfecta	1. Captan 75	60.0	10.0	30.0
	2. PCNB	45.0	25.0	30.0
	3. Check	45.0	12.5	52.5
	4. Phaltan	42.5	10.0	47.5
	5. Panogen-Drench	27.5	10.0	62.5
Check	1. Check	95.0	2.5	2.5
	2. PCNB	90.0	7.5	2.5
	3. Phaltan	82.5	7.5	10.0
	4. Panogen-Drench	72.5	27.5	0.0
	5. Captan 75	72.5	20.0	7.5

Isolation Studies

The purpose of these studies was to determine: (1) the organisms that infest the sand beds; (2) the organisms that infect the cuttings and seedlings and (3) the organisms that are not controlled on cuttings by the chemicals used.

In order to determine the kinds and relative numbers of organisms that infest the rooting media, the following experiment was set up. A lot of sand was mixed and divided into two parts. One half was sterilized and the other half was kept in containers. Twenty-four pans were filled with sterilized sand and another 24 with unsterilized sand. Twenty cuttings were made from three to four week old stems of clone 50-1266. These were sterilized, with 1/1000 solution of bichloride of Mercury for 1/2 - 1 minute, washed in distilled water, placed in 1/1000 indole-butyric acid solution for 10 to 15 seconds and planted in the sand beds. Four replications of the following treatments were made and randomized:

1A - Sterilized sand + cutting + tap water

2A - Unsterilized sand + cuttings + tap water

1B - Sterilized sand + cuttings + distilled water

2B - Unsterilized sand + cuttings + distilled water

1C - Sterilized sand exposed (not watered)

2C - Unsterilized sand exposed (not watered)

1D - Sterilized sand exposed + tap water

2D - Unsterilized sand exposed + tap water

1E - Sterilized sand exposed + distilled water

2E - Unsterilized sand exposed + distilled water

1F - Sterilized sand covered (not watered)

2F - Unsterilized sand covered (not watered)

The sand beds were placed in the air-conditioned growth chamber and the appropriate sand beds were kept moist at all times. Isolations were made from the sand beds the first day and every week for five weeks. A photograph of this experiment after five weeks growth is presented in Figure 7.

About 100 gm. of sand was taken from four replications of each treatment and mixed together in a sterile petri plate. Three-four gm. lots of sand were weighed out from each sand sample and mixed thoroughly with 8 ml. of sterile water in each of three test tubes. Duplicate plates of acidified 2.0 percent P.D.A. were mixed with 0.25 ml. of the sand suspension each before solidification. A third plate was mixed with 0.5 ml. of the suspension. After solidification, they were incubated, inverted, in plastic chambers and examined in five to seven days. Isolates that were not readily identified were transferred to fresh P.D.A. slants and plates for further identification.

Isolations were also made from the diseased cuttings after harvesting. At the end of the fifth week, the plants were harvested, examined and rated according to the degree of infection. The results are presented in Table 19. Photographs of the test plants are presented in Figures 7 and 8.

This experiment was repeated by using seedlings instead of cuttings. A dilution plate method of isolation from sand was also employed in place of the one used previously.

FIGURE 7

A set up of sand beds used for isolation purposes. (Left) Cuttings sterilized with bichloride of Mercury, treated with indole butyric acid and planted in sand beds (five weeks of growth).

1A - Sterilized sand + cuttings + tap water

2A - Unsterilized sand + cuttings + tap water

1B - Sterilized sand + cuttings + distilled water

2B - Unsterilized sand + cuttings + distilled water

1C - Sterilized sand exposed not watered

2C - Unsterilized sand exposed not watered

1D - Sterilized sand exposed + tap water

2D - Unsterilized sand exposed + tap water

1E - Sterilized sand exposed + distilled water

2E - Unsterilized sand exposed + distilled water

1F - Sterilized sand covered (not watered)

2F - Unsterilized sand covered (not watered)

FIGURE 7

Fig. 8. Cuttings sterilized with Mercury chloride, treated with indole butyric acid and planted in sand beds. (Harvested after five weeks of growth.)

1A. Sterilized sand + cuttings + tap water

2A. Unsterilized sand + cuttings + tap water

1B. Sterilized sand + cuttings + distilled water

2B. Unsterilized sand + cuttings + distilled water

Table 19. Classification of plants (Summary) according to the degree of infection when cuttings were sterilized.

Treatment	% Total Plants Surviving	% Total Plants Healthy	% Total Plants Slight - 50% stem Infection	% Total Plants 100% loss
1A	96.25	92.5	3.75	3.75
2A	95.0	92.5	2.5	5.0
1B	98.75	98.75	0	1.25
2B	96.25	95	1.25	3.75

1A - Sterilized sand + cuttings + tap water

2A - Unsterilized sand + cuttings + tap water

1B - Sterilized sand + cuttings + distilled water

2B - Unsterilized sand + cuttings + distilled water

A composite of 2 100 gm sample of sand was taken from the four replicated sand beds of each treatment and mixed in sterile plates. Twenty grams of the sand was weighed out and placed in 200 cc. of sterile water in a flask. From this, further dilutions of 1/100 and 1/1000 were made. One ml. of the suspension was then pipetted and mixed with a plate of cooled acidified, 2.0 percent P.D.A. before solidification. Plates were then incubated, inverted in plastic dishes. They were examined after five to seven days. Isolations were made the first day the experiment was begun and every week for five weeks. Table 20 shows the classification of the seedlings according to the degree of infection.

Isolations were also made from diseased seedlings after two weeks and

Table 20. Classification of seedlings after harvesting.

Treatments	Weeks after sowing 1 : % Surviving	Weeks after sowing 3 : % Healthy	Weeks after sowing 3 : % Diseased
1A	86	85	2.5
2A	85	81	9
1B	76	75	6
2B	82.5	79	7.5

1A - Sterilized sand + cuttings + tap water

2A - Unsterilized sand + cuttings + tap water

1B - Sterilized sand + cuttings + distilled water

2B - Unsterilized sand + cuttings + distilled water

again at the end of the fifth week. When the seedlings were harvested.

The total number of organisms that were isolated from the sand beds are presented in Table 21.

In order to determine what organisms infect both cuttings and seedlings, isolations were made from diseased plant materials. The kind and relative numbers of the organisms isolated from cuttings and seedlings are presented in Tables 22 and 23 respectively.

In order to determine what organisms are not controlled by the various chemicals used in these studies isolations were made from diseased plants. The organisms recovered are presented in Table 24.

The isolates recovered from the sand beds included a number of fungal organisms. The most common of these were: (1) Alternaria spp.,

Table 21. Organisms found infesting sand beds in the greenhouse.

Isolates	Kinds of sand bed											
	1A	1B	1C	1D	1E	1F	2A	2B	2C	2D	2E	2F
Alternaria spp.	109	138	7	12	2	—	66	90	10	8	8	4
Aspergillus spp.	27	24	25	104	23	—	14	19	24	30	47	1
Aspergillus niger	13	2	—	1	1	—	4	2	2	2	4	1
Ascochyta sp.	—	—	—	—	—	—	—	—	—	—	—	1
Botrytis spp.	2	—	4	2	5	—	—	1	—	1	—	—
Chaetomium spp.	—	—	—	—	—	—	—	1	2	6	1	—
Chaetophoma spp.	—	—	—	—	—	—	—	—	—	—	7	—
Cladosporium spp.	19	101	12	17	43	—	250	14	8	3	12	4
Cephalosporium spp.	6	—	4	—	1	—	—	—	—	5	—	7
Curvularia spp.	—	1	—	2	—	—	1	2	1	1	—	—
Cylindrocephalum sp.	—	—	—	—	—	—	1	—	—	—	—	—
Epicoccum spp.	—	2	1	1	1	—	—	—	—	—	—	2
Fusarium spp.	3	5	2	4	—	—	2	1	—	—	—	—
Fusarium roseum	1	—	—	—	—	—	1	2	—	—	—	—
Fusidium spp.	—	3	3	1	1	—	3	4	2	5	8	—
Gliocladium spp.	—	—	—	—	—	—	1	—	—	—	1	—
Helminthosporium spp.	54	7	3	8	—	—	—	3	1	4	1	4
Nigrospora spp.	—	—	—	—	—	—	—	—	1	—	—	1
Phycomycetes (Unidentified)	—	1	—	—	—	—	1	—	1	—	—	—
Phoma spp.	—	—	—	—	—	—	—	—	1	1	—	4
Paecilomyces spp.	—	—	—	—	—	—	—	1	—	2	—	1
Penicillium spp.	26	44	26	79	45	6	121	153	36	112	55	33
Pullularia spp.	—	4	7	1	3	—	—	4	2	3	2	—
Pythium spp.	—	—	—	—	—	—	—	—	1	—	2	1
Rhizopus spp.	1	1	1	—	—	—	1	—	2	2	2	2
Rhizoctonia solani	—	—	—	—	—	—	1	1	1	2	1	2
Schizotrichella spp.	—	—	—	—	—	—	1	—	—	1	2	—
Scopulariopsis spp.	1	—	2	—	1	—	—	1	—	—	—	—
Spicaria spp.	—	—	—	1	—	—	—	2	—	—	—	1
Sordaria spp.	—	—	—	—	—	—	—	1	—	1	—	—
Sterile (Unidentified)	—	9	2	—	—	—	5	2	2	2	—	2
Thielavia spp.	3	1	—	1	—	—	1	2	1	—	—	—
Trichothecium spp.	—	1	—	1	—	—	—	—	—	—	—	—
Trichoderma viride	1	3	—	3	1	—	12	7	7	5	4	12
Verticillium sp.	—	—	—	—	—	—	1	—	—	—	—	—
Yeasts (Unidentified)	1	—	—	1	—	—	—	—	2	8	—	—

Table 22. Organisms isolated from cuttings grown in sterilized and unsterilized sand beds.

Treatment	Fungal Isolates
Sterilized sand	*Alternaria* spp. *Alternaria tenuis* *Ascochyta imperfecta* *Colletotrichum* spp. *Colletotrichum graminicolum* *Cladosporium* spp. *Chaetomium* *Fusidium* spp. *Fusarium* spp. *Fusarium roseum* *Fusarium moniliforme* *Fusarium solani* *Gliocladium* spp. *Metarrhizium* spp. *Monilia* spp.
Unsterilized sand	*Alternaria* spp. *Alternaria tenuis* *Cladosporium* spp. *Chaetomium* spp. *Fusarium* spp. *Fusarium solani* *Fusarium moniliforme* *Fusarium roseum* *Fusidium* spp. *Phoma* spp. *Pevronellaea* spp.
Rhizoctonia solani	*Alternaria* spp. *Fusidium* spp. *Rhizoctonia solani*
Ascochyta imperfecta	*Alternaria* spp. *Ascochyta imperfecta* *Fusarium* spp. *Fusarium roseum* *Fusidium* spp.
Colletotrichum graminicolum	*Alternaria* spp. *Colletotrichum graminicolum* *Fusidium* spp.

Table 22. (continued)

Treatment	Fungal Isolates
Alternaria tenuis	*Alternaria tenuis* *Alternaria* spp. *Fusidium* spp.
Fusidium sp.	*Alternaria* spp. *Fusidium* spp. *Fusarium* spp.
Pythium debaryanum	*Alternaria* spp. *Fusidium* spp. *Pythium debaryanum*
Fusarium roseum	*Alternaria* spp. *Colletotrichum* spp. *Fusarium roseum*
Fusarium solani	*Alternaria* spp. *Fusarium solani* *Fusidium* spp.

Table 23. Isolates recovered from diseased alfalfa seedlings

Treatment	Fungal Isolates
Sterilized sand	*Alternaria* sp. *Chaetomium* sp. *Fusidium* sp. *Fusarium* sp. *Fusarium moniliforme*
Unsterilized sand	*Alternaria* sp. *Didymaria* spp. *Fusarium* sp. *Fusarium solani* *Fusarium roseum* *Helminthosporium* *Pythium* spp. *Pythium irregulare* *Rhizoctonia solani*
Rhizoctonia solani	*Rhizoctonia solani*
Ascochyta imperfecta	*Ascochyta imperfecta* *Alternaria* sp.
Colletotrichum graminicolum	*Alternaria* spp. *Colletotrichum graminicolum*
Alternaria tenuis	*Alternaria* spp. *Alternaria tenuis* *Fusarium* spp.
Fusidium sp.	*Fusidium* sp. *Fusarium roseum*
Pythium debaryanum	*Pythium debaryanum*
Fusarium roseum	*Fusarium roseum* *Fusarium* spp. *Alternaria* spp.
Fusarium solani	*Alternaria* spp. *Fusarium solani*

Table 24. Organisms isolated from cuttings treated with chemicals

Treatment	Fungal Isolates
Captan 75	*Alternaria* sp. *Ascochyta imperfecta* *Fusarium* sp. *Fusarium moniliforme* *Fusarium roseum* *Fusidium* spp. *Rhizoctonia solani*
Captan-Dieldrin	*Ascochyta imperfecta* *Fusarium* sp.
Actidione-Captan	*Ascochyta imperfecta* *Fusarium* sp. *Fusarium roseum*
Panogen-Drench	*Alternaria* spp. *Ascochyta imperfecta* *Fusarium* spp. *Fusarium roseum* *Rhizoctonia solani*
Manzate	—
Dyrene	*Alternaria* spp. *Monilia* sp.
Phaltan	*Alternaria* spp. *Fusarium solani*
Spergon	*Alternaria* spp. *Fusarium roseum*
Thiram	*Fusarium* sp. *Fusarium solani* *Fusidium* sp.
Ferbam	*Fusarium solani*
Semesan	—
Crag Mylone	—
Vapam	—

Table 24. (continued)

Treatment	Fungal Isolates
Dithane D-14	Alternaria spp. Ascochyta imperfecta Colletotrichum spp. Fusarium spp. Fusarium roseum Rhizoctonia solani
D-113	Alternaria spp. Ascochyta imperfecta Colletotrichum sp. Fusarium spp. Fusidium spp. Rhizoctonia solani
PCNB	—
Dexon	—

(2) Cladosporium spp., (3) Penicillium spp., (4) Aspergillus spp., (yellow type), (5) Fusidium spp., (6) Helminthosporium spp., (7) Fusarium spp. (8) Chaetomium spp., (9) Trichoderma viride., (1) Botrytis spp., (11) Pullularia spp., (12) Thielavia spp., (13) Curvularia spp., and (14) Epicoccum spp. Other fungi occasionally isolated were: (1) Rhizoctonia solani (2) Pythium spp., (3) Paecilomyces spp., (4) Fusarium roseum (5) Aspergillus niger (6) Sordaria spp., (7) Verticillium spp., (8) Nigrospora spp., (9) Phoma spp., (10) Ascochyta spp., (11) Yeasts (12) Phycomycetes (unknown) (13) Cephalosporium spp., (14) Mucor spp., (15) Cylindrocephalum spp., and (16) Schizotrichella and (17) Gleocladium spp.

There seemed to be a build up of (1) Alternaria spp., (2) Penicillium spp., (3) Aspergillus spp., (4) Helminthosporium spp., (5) Cladosporium spp., and (6) Trichoderma viride with the time of exposure of the sand beds.

Few isolates of Rhizoctonia solani and Pythium spp. (organisms usually considered as soil- or sand-borne pathogens associated with damping-off of alfalfa cuttings in the greenhouse), were recovered from the sand beds. Their population did not seem to increase any during the experiment.

Generally the number of colonies dwindled as time went on. The Rhizoctonia and Pythium were discovered by this dilution technique. On the whole the results obtained by this method were less satisfactory than the one used in the previous experiment.

The buildup of the various organisms tested above seemed to be caused primarily by the air-conditioner which blew spore laden air into the chamber, and also possibly by their multiplication in the wet sand beds. Very few or no isolates were recovered from the covered sterilized sand beds.

This showed that the sterilization procedure was satisfactory, if not perfect. A greater number of colonies were usually found among the watered sand beds. This is clearly seen from the results of later isolations by which time most of the fungi infesting the dry sand beds had either died off or lost their viability while those infesting the wet sand beds had grown and multiplied or maintained their viability.

Plants in sterilized sand beds generally had more top growth than plants in unsterilized sand beds. They also had about twice as much root length as plants in unsterilized sand beds. Plants in unsterilized sand beds, however, had large and numerous nodules while plants in sterilized sand beds had no visible nodules on their roots. Besides plants in unsterilized sand beds were more green than plants in sterilized sand beds.

The results obtained in these experiments were exceedingly gratifying. A minimum of 95 percent of the plants survived and a minimum of 92.5 percent of healthy plants were obtained in all the treatments after five weeks. On the whole plants in the sterilized sand treatments had less infection than plants grown in unsterilized sand. Ninety-six and twenty-five one-hundred percent survived and 92.5 percent healthy plants were obtained from the sterilized sand watered with tap water as compared with 95.0 percent and 92.5 percent from the unsterilized sand watered the same way. Corresponding values for other treatments were 98.75 percent and 98.75 percent for sterilized sand watered with distilled water, 96.25 percent and 95 percent for unsterilized sand watered the same way. By similar comparison, the sand beds watered with distilled water gave slightly better results than the sand beds watered with tap water.

It seemed, therefore, that merely surface sterilizing the cuttings with 1/1000 mercury chloride and treating them with 1/1000 solution of indole butyric acid gave better results when planted in sterilized and unsterilized sand than merely treating the sand beds and plants with chemicals or even planting the unsterilized cuttings in sterilized sand.

The percent of healthy plants obtained by these treatments far surpassed any previous treatments in earlier experiments. The results herein obtained also showed that either: (1) organisms and pathogens are carried on the cuttings themselves prior to planting or; (2) the indole butyric acid induced the cuttings to root much quicker than any pathogens could invade the stem bases.

Seedlings in the sterilized sand beds were less green and had smaller number of nodules than seedlings grown in the unsterilized sand. The seeds in the sterilized sand beds watered with tap water germinated and survived 10 percent more than those in the sterilized sand beds watered with distilled water. There was very little difference, however, between the two water treatments when unsterilized sand was used. It seemed, therefore, that seedlings in the sterilized sand beds watered with distilled water were slightly weakened by this treatment and were consequently slightly more susceptible to the fungal organisms than the seedlings grown in sterilized sand watered with tap water.

Fungus isolates recovered from diseased stems (when cuttings were treated with mercury chloride) included: (1) Gliocladium spp. (2) Alternaria tenuis (3) Alternaria spp., (4) Fusarium spp. (5) Fusidium spp., (6) Cladosporium spp., (7) Peyronellaea spp., and (8) Chaetomium spp. A few sterile forms were also found.

From the number of colonies isolated from the sand beds and the limited infection on the stems, it seemed that these organisms were only weak parasites or saprophytes.

Rhizoctonia solani and Pythium spp., were recovered from the sand beds, but not from the diseased plants. Thus the strains were either weak pathogens or their population was small enough as not to cause any damage to the plants.

Organisms isolated from diseased seedlings grown in the unsterilized sand beds were mainly Rhizoctonia solani, Pythium irregulare, Pythium spp., Fusarium roseum and Fusarium spp.

Rhizoctonia and Pythium were not isolated from seedlings taken from the sterilized sand beds. Saprophytic forms such as Alternaria sp., Fusidium sp., Chaetomium sp., Didymaria sp., and Helminthosporium spp., were also found among diseased seedlings. Pythium spp., and Rhizoctonia spp., were found to be more destructive than the other species. They were isolated more than any of the other organisms from infected seedlings during the second week after sowing. Some of these organisms, except R. solani, and Pythium spp., were also found among diseased seedlings taken from the sterilized sand beds.

Organisms isolated from the diseased plants untreated with chemicals or fungi included for the most part: (1) Alternaria spp., (2) Fusidium spp., (3) Fusarium spp., (4) Fusarium roseum, (5) Fusarium solani, (6) Fusarium moniliforme, (7) Colletotrichum spp., (8) Colletotrichum graminicolum, and (9) Ascochyta imperfecta.

Pythium spp. and Rhizoctonia solani were only recovered from plants taken from sand beds which had been treated with the organisms. When

seedlings were grown in sterilized sand beds, however, they were found parasited readily by these two sand-borne parasites.

From this study it seemed that the best way to find pathogenic organisms in the sand was by the use of susceptible seedlings.

As compared with the experiment with cuttings, this method gave much quicker results. However, the population of the pathogens Rhizoctonia solani, Pythium irregulare and Pythium sp., was still too small to cause any great damage. The last conclusion is drawn from the results obtained from harvesting the seedlings.

The organisms isolated from diseased cuttings treated with various chemicals are presented in Table 24. Rhizoctonia solani has been found to infest greenhouse sterilized sand beds (22, 25, 32). In these experiments it was never found infesting the sterilized sand beds or infecting the cuttings and seedlings grown in such sand beds. Other pathogens described above were, however, invariably found associated with the chemically treated cuttings. The most common of these were: (1) Alternaria spp., (2) Fusidium spp., (3) Colletotrichum spp., and (4) Ascochyta imperfecta. Rhizoctonia solani and Pythium spp. were only isolated from the cuttings which had been inoculated, through sand or on the stems themselves.

Pathogenicity Tests

By this time various fungal organisms had been isolated from various portions of, or from entire diseased cuttings and seedlings. Stock cultures of the more common and apparently important species collected

from previous experiments were selected.

This experiment was then initiated to test the importance of these isolates as possible pathogens on alfalfa seedlings, cuttings, and mature stems. In this way the important isolates could be selected for further testing on cuttings.

The organisms selected were: (1) Rhizoctonia solani (A54R); (2) Pythium debaryanum; (3) Fusarium roseum; (4) Fusarium solani; (5) Ascochyta imperfecta; (6) Alternaria tenuis; (7) Colletotrichum graminicolum (8) Colletotrichum sp.; and (9) Fusidium sp.

Sterilized sand beds in loaf pans were inoculated with one half plate each of ten day old fungus cultures, grown on P.D.A. One-quarter plate of inoculum was used in the case of Rhizoctonia solani (A54R). The required amount of inoculum was blended with 100 cc. of distilled water for 60 seconds in a Waring blender. The same number of uninoculated sand beds were seeded with 100 seeds of alfalfa each. The sand beds were watered and placed in the growth chamber with plastic sides (Fig. 2) to incubate. After six days the inoculated sand beds were seeded with 100 seeds each and the seedlings in the "uninoculated" sand beds were infested with similar fungal inoculum. Two replications of the following treatments were made:

1A Rhizoctonia solani	Pre-emergence inoculation
1B Pythium debaryanum	Pre-emergence inoculation
1C Fusarium roseum	Pre-emergence inoculation

1D *Fusarium solani*	Pre-emergence inoculation
1E *Ascochyta imperfecta*	Pre-emergence inoculation
1F *Alternaria tenuis*	Pre-emergence inoculation
1G *Colletotrichum graminicolum*	Pre-emergence inoculation
1H *Colletotrichum* sp.	Pre-emergence inoculation
1I *Fusidium* sp.	Pre-emergence inoculation
1J Control	
2A *Rhizoctonia solani*	Post-emergence inoculation
2B *Pythium debaryanum*	Post-emergence inoculation
2C *Fusarium roseum*	Post-emergence inoculation
2D *Fusarium solani*	Post-emergence inoculation
2E *Ascochyta imperfecta*	Post-emergence inoculation
2F *Alternaria tenuis*	Post-emergence inoculation
2G *Colletotrichum graminicolum*	Post-emergence inoculation
2H *Colletotrichum* sp.	Post emergence inoculation
2I *Fusidium* sp.	Post-emergence inoculation
2J Control	

Survival and infection counts were made on the seedlings at four to five day intervals for 18 days after sowing the seeds.

The seedlings were then harvested and rated as described in the materials and methods. The average percent healthy seedlings obtained are presented graphically in Figures 9 and 10.

Isolations were also made from infected seedlings. The results are presented in Table 25.

The experiment was repeated twice by growing healthy cuttings in sterilized sand beds artificially inoculated with the fungal organisms.

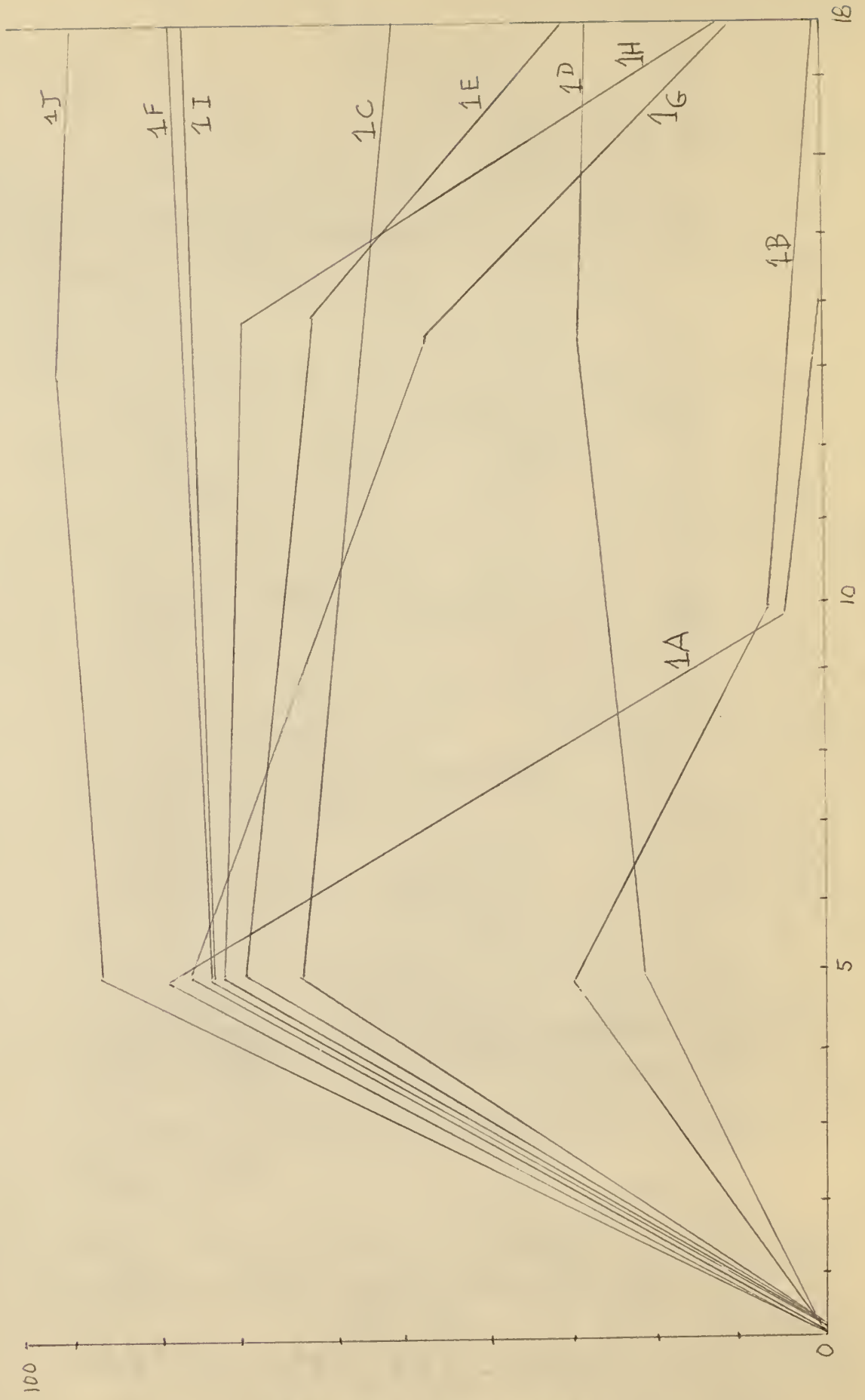
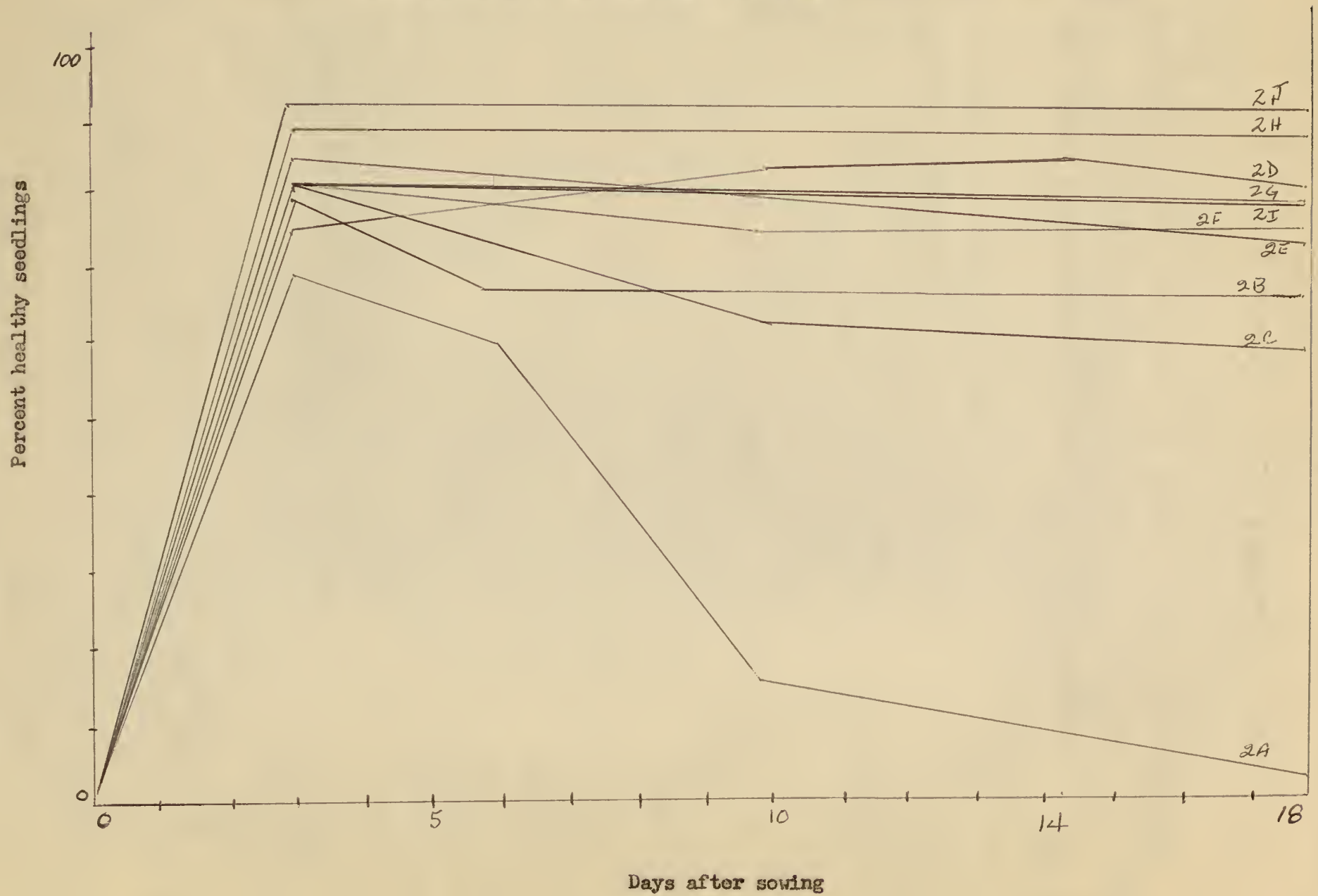


Figure 9. Graph showing percent of healthy seedlings obtained from pre-emergence inoculations with various fungal isolates.

Figure 10. Graph showing percent of healthy seedlings obtained from post-emergence inoculations with various fungal isolates



Footnote to Figure 9.

1A = *Rhizoctonia solani*	Pre-emergence inoculation
1B = *Pythium debaryanum*	Pre-emergence inoculation
1C = *Fusarium roseum*	Pre-emergence inoculation
1D = *Fusarium solani*	Pre-emergence inoculation
1E = *Ascochyta imperfecta*	Pre-emergence inoculation
1F = *Alternaria tenuis*	Pre-emergence inoculation
1G = *Colletotrichum graminicolum*	Pre-emergence inoculation
1H = *Colletotrichum* sp.	Pre-emergence inoculation
1I = *Fusidium* sp.	Pre-emergence inoculation
1J = Control	

Footnote to Figure 10.

2A = *Rhizoctonia solani*	Post-emergence inoculation
2B = *Pythium debaryanum*	Post-emergence inoculation
2C = *Fusarium roseum*	Post-emergence inoculation
2D = *Fusarium solani*	Post-emergence inoculation
2E = *Ascochyta imperfecta*	Post-emergence inoculation
2F = *Alternaria tenuis*	Post-emergence inoculation
2G = *Colletotrichum graminicolum*	Post-emergence inoculation
2H = *Colletotrichum* sp.	Post-emergence inoculation
2I = *Fusidium* sp.	Post-emergence inoculation
2J = Control	

Forty-two loaf pans were filled with sterilized sand. Six replications of the treatments summarized above were then made by cutting up the required amount of inoculum into little pieces and thoroughly mixing with the sand in the pans. The sand beds were then watered and placed in the air-conditioned growth chamber. They were kept moist all the time and allowed to incubate for a week.

After the one week incubation period, 20 cuttings from 3 to 4 week old stems of clone 50-1266 were made and planted in each sand bed. Symptoms caused by the different fungi on the cuttings is summarized in Table 25.

Twenty-one days after planting, the plants were harvested and rated according to degree of infection as outlined under materials and methods (Table 26). Isolations were then made from diseased plants and the results presented in Table 22. Photographs of the test plants before and after harvest are presented in Figures 11 and 12.

The fungal isolates used in the previous pathogenicity tests with seedlings and cuttings were again selected to: (1) test for the pathogenicity of these isolates on alfalfa plants; (2) test for their pathogenicity on cuttings made from infected stems.

Two plates each of ten-day old fungus inoculum on 2.0 percent P.D.A., were blended in a Waring blender with 100 cc of distilled water. Potted plants of clone 30-1108 with four week old top growths were selected and inoculated with the fungi by spraying the inoculum with an atomizer.

The plants were then placed in a moist chamber for three days. The moist chamber in which the plants were incubated was the same as that used

Table 25. Symptoms caused by the different sand-borne fungi on cuttings during the growth period.

Fungal Treatments	Symptoms
Rhizoctonia solani	Some plants showed signs of wiltingaabout two to three days after planting. 80 to 90° of them were damped-off in 7 to 14 days.
Pythium debaryanum	Plants were all stunted and pale green in color. Most of the damping-off occurred during the first week.
Fusarium roseum	Disease development was slow. Few plants had died, however, by the end of the second and third weeks.
Fusarium solani	Plants were normal and vigorous. No signs of infection were observed until plants had been harvested.
Ascochyta imperfecta	Disease development was slow. Few plants showed signs of decay about the second week. Damage estimated, however, after harvesting.
Ascochyta sp. (air)	Disease development was slow. Few plants showed signs of decay about the second week. Damage estimated, however, after harvesting.
Alternaria tenuis	No signs of infection.
Colletotrichum graminicolum	Disease development was slow. Few plants were decayed in two weeks. Actual damage estimated after harvesting.
Colletotrichum sp.	Disease development was slow. Actual damage estimated after harvesting.
Fusidium sp.	No signs of wilting.
Control	No signs of wilting.

Table 26. Classification of plants according to the degree of infection by the different supposed pathogenic sand-borne fungi.

Fungal Treatment	Classifications				
	Total av. % living	Total av. % healthy	Total av. % plants with minor infection	Total av. % plants with 50% stem infection	Total av. % dead or not rooted
Rhizoctonia solani	3.6	1.0	1.2	1.4	96.4
Pythium debaryanum	25.0		25.0	—	75.0
Fusarium roseum	82.5	40.0	27.5	15.0	17.5
Fusarium solani	70.0	60.0	10.0	—	30.0
Ascochyta imperfecta	81.4	46.4	14.4	15.6	22.5
Ascochyta sp. (air)	81.5	54	13.5	10.0	18.5
Alternaria tenuis	87.4	45.4	30.0	12.0	12.6
Colletotrichum graminicolum	85.6	38.6	31.1	16.0	14.4
Colletotrichum sp.	87.5	60.0	15.0	12.5	12.5
Fusidium sp.	92.0	52.0	25.0	15.0	8.0
Control	94.0	81.0	8.0	5.0	6.0

FIGURE 11

Cuttings in sand beds at time of harvesting to show effects of different sand-borne fungi on:

LEFT

1. Control (check)
2. Ascochyta sp. (air)
3. Rhizoctonia solani (A54R)
4. Colletotrichum graminicolum

RIGHT

5. Alternaria tenuis
6. Fusidium sp. (Cephalosporium)
7. Ascochyta imperfecta

FIGURE 11

FIGURE 12

Harvested, rooted cuttings showing the effects of the different sand-borne fungi on cuttings.

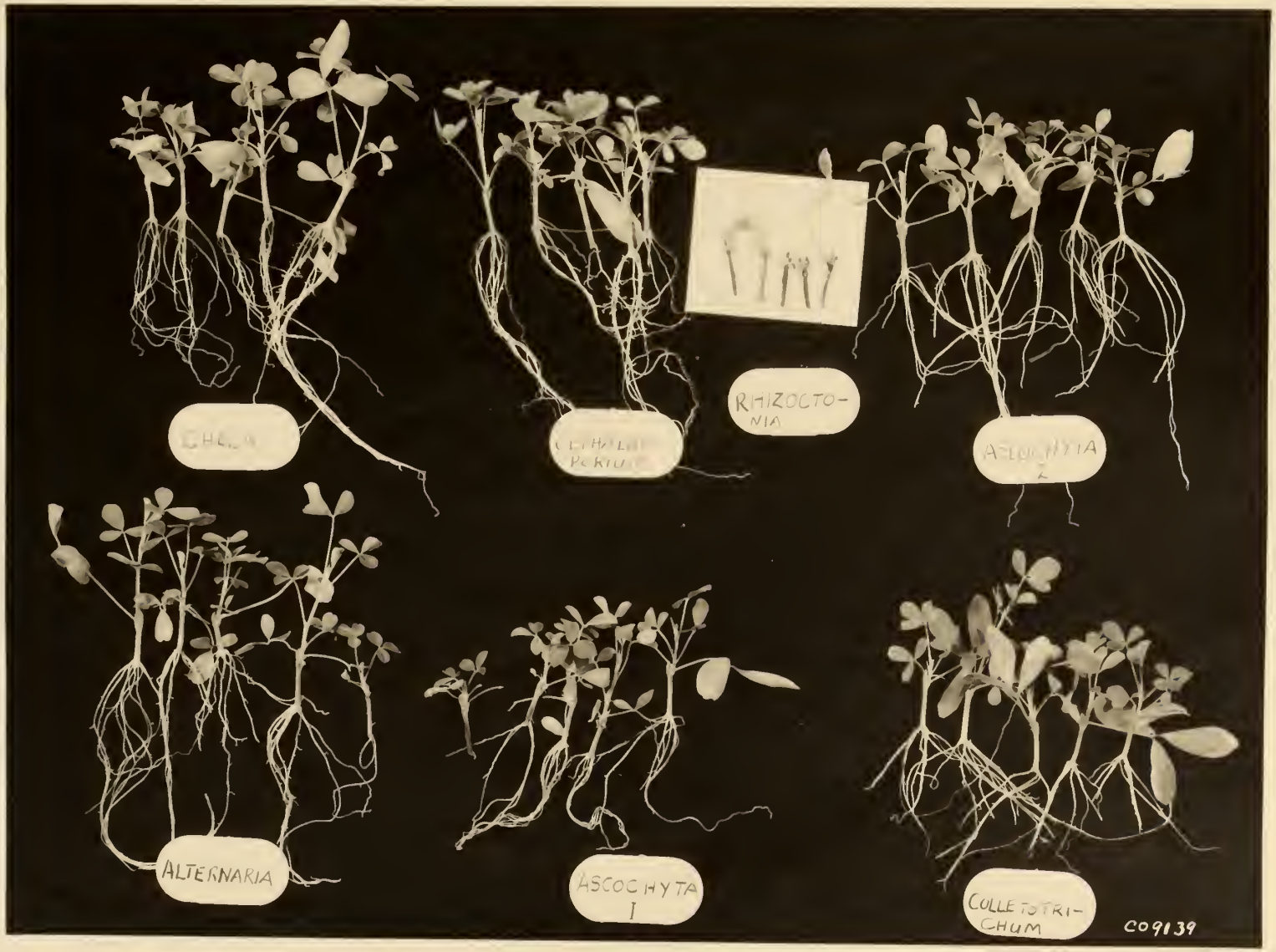
LEFT

1. Ascochyta sp. (air)
2. Rhizoctonia solani (A54R)
3. Fusidium sp. (Cephalosporium)
4. Control (Check)

RIGHT

5. Colletotrichum graminicolum
6. Ascochyta imperfecta
7. Alternaria tenuis

FIGURE 12


RHIZOCTO-
NIA
ALTERNARIA
ASCOCHYTA
I
COLLETOTRI-
CHUM
C09139

by Haskett et al. (25), and Peterson and Melchers (45). They were then taken out and kept in shade for five days.

Ten cuttings were then made from each potted plant and planted in sterilized sand beds in loaf pans. Three replications of each fungus treatment were then made and randomized. After four weeks growth, the plants were harvested and rated as before. The results are presented in Table 29. The effect of the different stem-borne fungi on the alfalfa plants and cuttings is presented in Table 27-29.

In the pre-emergence inoculation studies (Figure 9) 87 percent of the seeds had germinated by the fifth day after sowing. By the 18th day, 92 percent germination and healthy seedlings were obtained in the control. When the sand beds were treated with Rhizoctonia solani, 80 percent germination was obtained by the fifth day, 97 percent and 100 percent diseased seedlings were obtained on the tenth and 18th days respectively. Corresponding values were 30 percent, 94 percent and 98 percent when treated with Pythium debaryanum, 63 percent, 45 percent and 49 percent for Fusarium roseum, 20 percent, 75 percent and 72 percent for Fusarium solani, 65 percent 40 percent and 70 percent for Ascochyta imperfecta, 76 percent, 22 percent and 22 percent for both Alternaria tenuis and Fusidium sp., 76 percent, 38 percent and 92 percent for Colletotrichum graminicolum, 73 percent, 27 percent and 90 percent for Colletotrichum sp. Of the latter, 70 percent of the seedlings harvested were normal but almost every one of them had a few tiny spots which were at first considered physiological. However, the organism was recovered in all the isolations made from these tiny spots (see Results of Isolations).

Table 27. Symptoms caused by the different fungi on potted plants.

Treatment	Symptoms
Rhizoctonia solani	Numerous dark-brown oval and round sunken spots on stems, nodes and stipules.
Pythium debaryanum	No apparent symptoms on stems, but abundant mycelial strands all over plants. All flower buds and opened flowers were overgrown with mycelium and wilted off.
Fusarium roseum	Few, thread-like, brown streaks along the stems. Mycelial mat on leaves dried up without any infection.
Fusarium solani	No apparent symptoms
Ascochyta imperfecta	Very abundant black spots on both stems and leaves. Some merged into larger spots after standing in the shade, destroying most of the stems and leaves which wilted and died off before cuttings were made. Reference Grandfield et al. (29), Peterson and Melchers (45) Kernkamp and Hemerick (31).
Alternaria tenuis	No apparent symptoms. Plants were covered with black fungus material.
Colletotrichum graminicolum	No apparent symptoms on plants. Plants were merely covered with black fungus materials.
Colletotrichum sp.	No apparent symptoms. Plants were covered with black fungus material.
Fusidium sp.	No apparent symptoms.

Table 28. Symptoms caused by the different stem-borne fungi on cuttings during the growth period.

Fungal Treatments	Symptoms
Rhizoctonia solani	There was gradual damping-off of cuttings until by the end of the second week 20 to 30 percent damping-off had occurred in individual sand beds.
Pythium debaryanum	About 40.0 percent of the plants in individual pans had damped-off by the second week. A few others were still weak and pale dry.
Fusarium roseum	Most of the older leaves had wilted and died off by the first week. About 50 percent of them had damped-off completely by the third week. Symptoms observed here were very similar to those observed at the University alfalfa breeding greenhouse in the fall of 1960. The few plants that survived were pale, stunted and weak.
Fusarium solani	Apparently the plants were normal. Real damage estimated only after harvesting.
Ascochyta imperfecta	Few plants had already died off by the first and second weeks. These were probably the cuttings that had very heavy infection before they were planted. Older leaves died off and the newly developed leaves showed no apparent infection. Percent survival was far greater than expected on account of the severe infection of the stems.
Alternaria tenuis	Apparently the plants were normal. Real damage estimated only after harvesting.
Colletotrichum graminicolum	Symptoms developed very slowly. By the end of the third week a few plants were dead; many others were weak and stunted.
Colletotrichum sp.	Apparently the plants were normal. Real damage estimated only after harvesting.
Fusidium sp.	Apparently the plants were normal. Real damage estimated only after harvesting.
Control	No symptoms

Table 29. Classification of plants according to the degree of infection by the different supposed pathogenic stem-borne fungi.

Fungal Treatments	Total av. % living	Total av. % healthy	Total av. % plants with minor infection	Total av. % plants 50% stem infection	Total av. % dead or not rooted
Rhizoctonia solani	65.6	11.2	54.4	1.2	33.2
Pythium debaryanum	77.0	—	54.0	23.0	23.0
Fusarium roseum	75.0	7.5	42.5	25.0	25.0
Fusarium solani	99.0	21.2	74.8	3.0	1.0
Ascochyta imperfecta	90.0	—	73.4	16.6	10.0
Ascochyta sp. (air)	—	—	—	—	—
Alternaria tenuis	95.0	52.5	40.0	2.5	5.0
Colletotrichum graminicolum	94.0	1.0	60.0	33.0	6.0
Colletotrichum sp.	100.0	78.75	17.5	3.75	—
Fusidium sp.	98.75	31.25	66.25	1.25	1.25
Control	100.0	83.7	13.0	3.3	—

From this study, the most pathogenic isolates were: (1) Rhizoctonia solani; (2) Pythium debaryanum; (3) Fusarium solani; (4) Colletotrichum graminicolum; (5) Colletotrichum sp.; (6) Ascochyta imperfecta and (7) Fusarium roseum. Alternaria tenuis and Fusidium sp., were thus very weak pathogens.

From the post-emergence studies (Figure 10), the most pathogenic isolates were (1) Rhizoctonia solani with 98 percent diseased seedlings, (2) Fusarium roseum with 40 percent diseased seedlings, (3) Pythium debaryanum with 35 percent diseased seedlings, (4) Ascochyta imperfecta with 26 percent diseased seedlings, (5) Alternaria tenuis with 25 percent diseased seedlings. Twenty-two percent diseased seedlings were obtained from both Colletotrichum graminicolum and Fusidium sp. treatments, 20 percent from Fusarium solani treatment, 13 percent from Colletotrichum sp., treatment, and 10 percent from the control.

Thus Rhizoctonia solani caused both pre-emergence and post-emergence damping-off of seedlings by about equal rates, Pythium debaryanum caused severe pre-emergence damping-off and little post-emergence damping-off. Fusarium solani, Fusarium roseum, Colletotrichum graminicolum, Colletotrichum sp., and Ascochyta imperfecta caused relatively severe pre-emergence damping-off but little post-emergence damping-off, when the seedlings were about three days old.

The effect of the different sand-borne fungi on alfalfa cuttings is presented in Tables 25 and 26, and in Figures 11 and 12. Ninety-seven and eight tenths percent plants were badly diseased (total average percent plants with 50 percent stem infection + total average percent plants

dead or not rooted) in three weeks by Rhizoctonia solani when sand-borne, 75 percent by Pythium debaryanum, 38.1 percent by Ascochyta imperfecta, 32.5 percent by Fusarium roseum, 30.4 percent by Colletotrichum graminicolum, and 30.0 percent by Fusarium solani, and 23 to 25 percent by Colletotrichum sp., Alternaria tenuis and Fusidium sp. The corresponding result for the control was 11.0 percent.

On the basis of these results, the virulent fungi were (1) Rhizoctonia solani and (2) Pythium debaryanum. Ascochyta imperfecta, Fusarium roseum Fusarium solani and Colletotrichum graminicolum were slightly pathogenic. The remaining fungi were also weakly pathogenic.

The effect of the different stem-borne fungi on alfalfa plants and cuttings is presented in Table 27 - 29.

The most virulent of the fungal isolates was Ascochyta imperfecta. Infection caused by this isolate was so severe that it was doubtful whether the cuttings would survive or root at all. A very high percentage of cuttings made from the infected stems, however, survived and rooted. On the one hand, the number of infection sites or diseased spots seemed to increase on the stems below soil level without inhibiting the rooting ability of the plants. This increase in infection sites without actually inhibiting rooting or the survival of the plants caused most of them to be rated as "minor infection" (73.4 percent). On the other hand, a few cuttings that were badly infected before planting succumbed to the black stem disease and either partially survived (50 percent stem infection) or completely died off within the four week growth period. An average of 73.4 percent of the cuttings made from heavily Ascochyta imperfecta infected stems could be used as transplants (with minor infection), and

only 26.3 percent were either partially or completely dead and could not be used. These observations seemed to support as well as contradict the observations made by Haskett et al. (25) at this station, even though we may be comparing the pathogenicity of two different isolates of A. imperfecta.

When the stems were infected with Rhizoctonia solani, and cuttings were made and planted, 34.4 percent of the cuttings were severely diseased and could not be used as transplants. Corresponding figures were 50.0 percent for Fusarium roseum, 46.0 percent for Pythium debaryanum, and 39.0 percent for Colletotrichum graminicolum. The results obtained for Alternaria temuis, Fusarium solani, Colletotrichum sp. and Fusidium sp. were 2.5 - 7.5 percent, as compared with 3.3 for the control.

From these last results the virulent stem-borne fungi were Fusarium roseum, Pythium debaryanum, Colletotrichum graminicolum, Rhizoctonia solani and Ascochyta imperfecta.

On the basis of the results obtained from these pathogenicity tests, Rhizoctonia solani and Pythium debaryanum were considered the most important of the fungi tested on alfalfa cuttings. Fusarium roseum, Ascochyta Colletotrichum graminicolum, and Ascochyta sp. were considered possible pathogens. Alternaria temuis, Fusidium sp., Fusarium solani and Colletotrichum sp., were considered weak pathogens or saprophytes on alfalfa cuttings.

Effect of Chemicals on Nodule Bacteria

This study was undertaken to: first find whether or not the chemicals Captan 75 and Panogen-Drench (two satisfactory chemicals against

damping-off organisms), and Dithane D-14 and D-113 (two chemicals that were phytotoxic to alfalfa cuttings) have any effects on sand-borne nodule bacteria; and secondly, to find out the effects of sand-borne Rhizoctonia solani on nodulation of alfalfa cuttings.

For this purpose 1/10 plate of 3-day old Rhizoctonia solani (A54R) grown on 2.0 percent P.D.A. was cut into little pieces and mixed with sterilized sand in each of 20 loaf pans. Another 20 sand beds were kept as controls. They were watered and placed in the air-conditioned growth chamber. After seven days, four replications of the following treatments were made and randomized.

Treatment	Chemical Conc.	Amount/pan
1. Captan 75	0.4 gm/liter distilled water	100 cc
2. Captan 75 + R. solani	0.4 gm/liter distilled water	100 cc
3. Panogen-Drench	1 cc/1 1/3 liters distilled water	100 cc
4. Panogen + R. solani	1 cc/ 1 1/3 liters distilled water	100 cc
5. Dithane D-14	2 cc/liter distilled water	100 cc
6. Dithane + R. solani	2 cc/liter distilled water	100 cc
7. D-113	0.6 cc/liter distilled water	100 cc
8. D-113 + R. solani	0.6 cc/liter distilled water	100 cc
9. R. solani only	—	—
10. Control (No treatments)	—	—

After the seven-day incubation period, 20 cuttings were made from three week old stems of clone 50-1266 and planted in each sand bed. The chemicals were applied then and after every two weeks.

One week after the second chemical application, one-half teaspoonful of Nitragin (alfalfa), a nodule bacterial preparation, was suspended in water in a watering can and sprinkled on all the 40 sand beds.

One week after the fourth chemical application, the plants were dug up and examined for fungal infection, root formation and nodule formation.

For nodulation studies five healthy plants were taken from each treatment (unless otherwise indicated). The total number of roots per plant and the total number of nodules, visible to the naked eye, were counted and averaged (Tables 30 and 31).

In the Rhizoctonia solani treated sand beds, there was an average 10.85 roots and 3.6 nodules per plant when treated with Captan, 11.5 roots and 16.7 nodules per plant when treated with Panogen, 5.25 roots and 11.7 nodules per plant when treated with Dithane, 13.75 roots and 33 nodules per plant in the check (R. solani only).

In the absence of R. solani in the sand beds there were 10.65 roots and 2.4 nodules per plant when treated with Captan, 11.0 roots and 11.75 nodules per plant when treated with Panogen, 5.35 roots and 7.2 nodules per plant when treated with Dithane, 15.2 roots and 20.9 nodules per plant in the Control.

In the Captan 75 treatments, there were 0.32 nodules per root in the presence of Rhizoctonia solani (A54R) as compared with 0.24 nodules per root in the absence of R. solani (A54R). Corresponding results were: 1.46 and 1.1 for Panogen-Drench; 2.37 and 1.4 for Dithane D-14. When R. solani was untreated with any chemical, there were 2.4 nodules per root as compared with 1.4 nodules per root in the Control (no chemical and no R. solani).

Table 30. Effect of chemicals on nodulation.

Replications	Captan		Captan + R		Panogen		Panogen + R		R (Only)	
	Roots	Nodules	Roots	Nodules	Roots	Nodules	Roots	Nodules	Roots	Nodules
1 Total	61	4	58	4	65	26	61	55	23	44
Av. number of roots per plant	12.2		11.6		13		12.2		11.5	
Av. number of nodules per plant		.8		.8		5.2		11		22
Av. number of nodules per root		.065		.07		.4		.9		1.9
2 Total	56	3	73	15	54	65	54	52	16	44
Av. number of roots per plant	11.2		14.6		10.8		10.8		16	
Av. number of nodules per plant		.6		3		13		10.4		44
Av. number of nodules per root		.05		.2		1.2		.96		2.75
3 Total	49	8	34	0	46	55	61	81	—	—
Av. number of roots per plant	9.8		6.8		9.2		12.2			
Av. number of nodules per plant		1.6		0		11		16.2		
Av. number of nodules per root		.16		0		1.2		1.3		
4 Total	47	33	52	53	55	89	54	147		
Av. number of roots per plant	9.4		10.4		11		10.8			
Av. number of nodules per plant		6.6		10.6		17.8		29.2		
Av. number of nodules per root		.7		1		1.6		2.7		

Table 30. (continued)

Replications	D-113		D-113 + R		Dithane		Dithane + R		Control	
	Roots	Nodules	Roots	Nodules	Roots	Nodules	Roots	Nodules	Roots	Nodules
1 Total	—	—	—	—	17	15	10	20	83	37
Av. number of roots per plant	—	—	—	—	5.7		5		16.6	
Av. number of nodules per plant	—	—	—	—		5		10		7.2
Av. number of nodules per root	—	—	—	—		.9		2		.4
2 Total	—	—	—	—	16	8	15	55	68	118
Av. number of roots per plant	—	—	—	—	5.3		5		13.6	
Av. number of nodules per root	—	—	—	—		2.6		18.3		23.6
Av. number of nodules per root	—	—	—	—		.5		3.7		1.7
3 Total	—	—	—	—	21	55	27	54	78	112
Av. number of roots per plant	—	—	—	—	4.2		5.4		15.6	
Av. number of nodules per plant	—	—	—	—		11		10.6		22.4
Av. number of nodules per root	—	—	—	—		2.6		2		1.4
4 Total	—	—	—	—	25	41	28	40	75	152
Av. number of roots per plant	—	—	—	—	6.2		5.8		15	
Av. number of nodules per plant	—	—	—	—		10.2		8		30.4
Av. number of nodules per root	—	—	—	—		1.6		1.4		2

Table 31. Summary of effects of chemicals on nodulation.

Treatments	Av. number roots/plant	Av. number nodules/plant	Av. number nodules/root
Captan	10.65	2.4	0.24
Captan + R	10.85	3.6	0.32
Panogen	11.0	11.75	1.1
Panogen + R	11.5	16.7	1.46
Dithane	5.35	7.2	1.4
Dithane + R	5.25	11.7	2.37
D-113	0	0	0
D-113 + R	0	0	0
R- Only	13.75	33.0	2.4
Control (No treatment)	15.2	20.9	1.4

In the presence of R. solani, nodulation was increased by 33 percent in the Captan treatment, 33 percent in the Panogen treatment, 74 percent in the Dithane treatment, and about 74.0 percent in the Control.(No treatment), on the basis of nodules per root.

The only chemical that reduced nodulation was Captan 75. The rest did not seem to have any effect on nodule bacteria. On the whole, the presence of Rhizoctonia solani increased nodulation by 33-74 percent. It is possible both organisms have synergistic effect on one another. The exact nature of their relationship is not known, however.

DISCUSSION

Damping-off of alfalfa cuttings has been a problem among alfalfa breeders. At the time this study was begun there were five reports that pertained directly to the asexual propagation of alfalfa in the greenhouse (18, 22, 25, 32, 68). Essentially, early methods advocated for the asexual propagation of alfalfa were those of sanitation and culture (18, 68). Later chemical methods were advanced which included the use of Daptan, Panogen-Drench, and dry Phygon (25, 32). Fusarium sp., Rhizoctonia sp., Rhizoctonia solani, and Ascochyta imperfecta had been suggested as the cause of damping-off of alfalfa cuttings in the greenhouse (22, 25, 32). Grandfield et al. (22) considered Rhizoctonia sp., Fusarium sp., and Ascochyta imperfecta the main organisms recovered from diseased cuttings taken from unsterilized sand beds. Kernkamp et al. (32) on the other hand encountered R. solani in greenhouse benches, rooting media and diseased cuttings. They considered R. solani the main organism that parasitized alfalfa cuttings in the greenhouse; it was either stem-borne or was splashed from the greenhouse benches on to the rooting medium. Other workers have encountered Rhizoctonia spp. in greenhouse nurseries.

In the present study, organisms found infesting exposed sterilized sand beds included about 16 genera of fungi. The most common of these were: (1) Alternaria spp.; (2) Fusarium spp.; (3) F. roseum; (4) F. solani; (5) Fusidium spp.; (6) Botrytis spp.; (7) Helminthosporium spp.; (8) Cladosporium spp.; (9) Penicillium spp.; and (10) Aspergillus spp. Species which were commonly found associated with diseased alfalfa cuttings taken from such sand beds were: (1) Alternaria spp., (2) A. tenuis,

(3) Fusarium spp., (4) F. roseum, (5) F. solani, (6) F. moniliforme, (7) Ascochyta imperfecta, (8) Colletotrichum spp., (9) C. graminicolum, and (10) Fusidium spp. Organisms found associated with diseased seedlings taken from the sterilized sand beds were: (1) Alternaria spp.; (2) Fusarium spp.; (3) Fusidium spp.; and (4) Chaetomium spp.

About 33 genera of fungi were found infesting the unsterilized sand beds. These included (1) Rhizoctonia solani, (2) Pythium spp., (3) Pythium irregulare, (4) Schizotrichella spp., (5) Phoma spp., (6) Gliocladium spp., (7) Chaetophoma spp., (8) Thielavia spp., and (9) Verticillium spp. However, only (1) Alternaria spp., (2) Fusarium spp., (3)Fusidium spp., (4) Cladosporium spp., (5) Chaetomium spp., (6) Phoma spp., and (7) Peyronellaea spp., and (8) a few unknown sterile fungi, while (1) Rhizoctonia solani, (2) Pythium spp., (3) Pythium irregulare (4) Fusarium roseum, and (5) Fusarium solani were the important fungi recovered from diseased seedlings. Under the conditions of these experiments, no Rhizoctonia spp., or Pythium spp., were found parasitizing the cuttings thus differing from the report by Grandfield et al. (22) and Kernkamp et al. (32). From the amount of infection obtained from the cuttings, the organisms found associated with them were definitely weak pathogens or saprophytes, while the Rhizoctonia spp. and Pythium spp. were either non-pathogenic to the alfalfa cuttings, or their populations in the sand were too small to cause any damage to the cuttings. A quick and efficient way to isolate them from sand was thus by growing susceptible alfalfa seedlings which would be parasitized in a few days.

Pathogenicity tests conducted by Grandfield et al. (22) showed that Rhizoctonia sp. was pathogenic to alfalfa cuttings. By planting cuttings

in sand beds infested with Rhizoctonia sp. they obtained 89% rooted cuttings and 75 percent diseased ones in three weeks, and 75 percent rooted and 37 percent diseased cuttings when the cuttings were infected with Rhizoctonia sp. before planting them in sand beds. Kernkamp et al. (32) reported that 100 percent of cuttings were damped-off by R. solani in a few days. They also found that at 60-65°F cuttings were killed in 23 days; at 70-75°F some roots were formed but the cuttings were killed in 16 days; while at 80-85°F no roots were formed but the cuttings were killed in three days. Elmer (19) reported similar effects of soil temperature on survival and pathogenicity of Rhizoctonia spp. in Kansas. In the present study, it was found that at the rates of 3/10 plate or less of Rhizoctonia solani (A54R) per sq. ft. surface area of sand, 20-23 percent of the cuttings rooted but about 97 percent were dead or diseased in three to five weeks. (Tables 3, 5, 9; Figures 3-8). At the rates of 3/4 plate or more of the inoculum per sq. ft., 89-100 percent of the plants died and it was almost impossible to control the organism by any chemical means (Table 14). When the cuttings were infected with the organism before plant, 31-60 percent of the plants had minor infection while 40-70 percent were badly infected or dead after three to four weeks. These results confirmed those of Grandfield et al. (22) and Kernkamp et al. (32). It was also found that at 75°F and above (in the growth chamber with plastic sides), the cuttings were damped-off much quicker than at 66-68°F (in the air-conditioned growth chamber). Rhizoctonia solani was also found to be a virulent pre- and post-emergence damping-off organism of alfalfa seedlings.

Grandfield et al. (22) obtained 98-100 percent dead or diseased plants in two trials with only 0-11 percent being rooted when the cuttings were

infected with Ascochyta imperfecta. When sand beds were infested with the organism, they obtained 97 percent diseased cuttings with only 15 percent rooting. They concluded that A. imperfecta was more pathogenic and would probably be more difficult to control among cuttings than Rhizoctonia sp. In the present study, when cuttings were made from stems infected with A. imperfecta and planted, 16.6 percent of the plants with 50 percent stem infection and 10.0 percent dead plants were obtained in four weeks. The remaining 73.4 percent of the plants were rooted and had about the same amount of infection as they had before they were planted. They were, however, considered as plants with minor infection. The older leaves died off while the young leaves that developed later were disease-free and normal.

When healthy cuttings were planted in sand beds artificially infested with Ascochyta imperfecta, 12.5 - 24 percent of the plants with 50 percent stem infection and 3.4 - 30 percent dead plants were obtained. From this study, therefore, it seemed that A. imperfecta was weakly pathogenic on alfalfa cuttings. It did not prevent rooting or cause any appreciable damage to the cuttings. It did, however, cause about 50 percent pre-emergence damping-off of seedlings and was destroyed on the seeds by the sterilization procedures, as reported by Cormack (12).

It is possible that the strains of A. imperfecta used in the two experiments were different. However, severe infection of mature stems was obtained by both workers. A reasonable explanation for the wide differences in the results obtained in this study and that of Grandfield et al. (22) seems to be the water content of the sand medium in which the cuttings were planted. It is the belief of this author that if the sand beds are kept constantly moist, as was done in these trials, A. imperfecta would have little effect on the cuttings on which it was borne, or on the

cuttings planted in the sand beds in which it was infesting.

Fusarium sp. was reported by Grandfield et al. (22) to be non-pathogenic to alfalfa cuttings. In these trials, contrary to what Grandfield et al. (22) found, plants with 27.5 percent to 86.7 percent minor infection, 13.5 percent with 50 percent stem infection and 0 - 85 percent dead plants were obtained when cuttings were made from infected stems and rooted. It was also found to cause severe pre-emergence damping-off of seedlings. Fusarium solani, on the other hand, was non-pathogenic on cuttings but was virulent as a pre-emergence damping-off organism of seedlings.

Pythium spp. have been reported pathogenic to alfalfa seedlings and mature plants (5, 6, 24, 26, 27, 39). Some workers (26) have indicated that it is non-pathogenic to old seedlings (3-14 days or older). In this study similar results were obtained. When cuttings were infected with P. debaryanum and planted, 46 percent of them were damped-off in three to four weeks; when sand-borne, it damped-off 75 percent of them in three to four weeks. This fungus was also found to be a virulent pre-emergence damping-off organism of seedlings, but non-pathogenic when seedlings were 3 to 5 days old.

Colletotrichum graminicolum was also found associated with diseased cuttings. When cuttings were infested with this organism and planted, 0-65 percent of the plants with 50 percent stem infection, and 0-12.5 percent dead plants were obtained. But when sand-borne, it was non-pathogenic. It was also found to be a virulent pre-emergence damping-off organism of seedlings.

In the present study 300 ppm (active ingredient) of Captan 75,

3ppm (active ingredient) of Panogen-Drench applied at the rate of 1/3 liters per sq. ft. every one - two weeks, were found satisfactory against Rhizoctonia and Pythium damping-off among cuttings. Haskett et al. (25) suggested single dosages of 1000 cc at these concentrations per sq. ft. When the latter procedure was tried, the cuttings were stunted and rooting was reduced. In addition the chemicals produced phytotoxic effects on the cuttings and failed to prevent damping-off by R. solani, applied at the rate of three plates of inoculum per sq. ft. of sand.

While Captan and Panogen seemed to give a practical control of R. solani, their actions were considered to be strongly fungistatic rather than fungicidal. They were also found to be slightly phytotoxic to cuttings in some cases. Captan in particular was found to reduce nodulation, while the presence of R. solani in the sand seemed to enhance it. The phenomenon suggested synergistium, but the actual mechanism could not be explained. The above mentioned chemicals were also effective in controlling most of the fungi tested. As a result of these findings, it was suggested that they be used only as preventive measures against damping-off by Rhizoctonia and Pythium, species in particular, when they are known to infest the soil. Commercial preparations of PCNB (Penta chloro nitrobenzene) and Phaltan were also found effective against Rhizoctonia and Pythium species, as well as most of the other fungi tested. Similar reports made by other workers (23, 28, 35) were thus confirmed.

One method was found superior to both chemical treatments and steam sterilization of the sand. This was by sterilizing the cuttings first with 1/1000 bichloride of mercury and later treating them with 1/1000 solution of indole butyric acid for 1/4 - 1 minute before planting. This way, 92 -

95 percent of the 7,500 cuttings per acre of a single cross field as suggested by Elling (18), could be satisfactorily grown in the greenhouse.

CONCLUSIONS

Various organisms were tested on cuttings, mature stems and seedlings. Rhizoctonia solani was found the most pathogenic of all the organisms on cuttings and seedlings. Pythium debaryanum, Fusarium roseum and Colletotrichum graminicolum were found pathogenic on cuttings and seedlings. Fusarium solani was found pathogenic on seedlings, while Ascochyta imperfecta was found pathogenic on mature stems, and on cuttings when sand-borne.

Captan 75 (300 ppm) and Panogen-Drench 0.4 (3.0 ppm) applied at the rate of 1/3 liters per sq. ft. every one to two weeks satisfactorily prevented damping-off of alfalfa cuttings by Rhizoctonia solani and Pythium debaryanum. They were also effective against Fusaria species. Panogen-Drench was superior to Captan 75 against Fusaria spp. PCNB and Phaltan were the least phytotoxic of the chemicals used on alfalfa cuttings. They also showed great promise against Rhizoctonia solani, Pythium debaryanum, Colletotrichum graminicolum and Fusarium roseum. Very small quantities of R. solani and Pythium spp. were found in the unsterilized sand but they were not found parasitizing the cuttings grown in the sand beds. Ascochyta imperfecta was found extremely pathogenic on alfalfa stems but it neither inhibited the rooting ability of the cuttings made from stems on which it was borne nor did it cause any practical damage to the cuttings when it was borne on them.

In order to grow alfalfa cuttings satisfactorily in the greenhouse, therefore, the following measures should be used: (1) adequate sanitation (2) adequate and careful watering of plants (3) shading-off of cuttings from direct sunlight during the first 10-14 days (4) the use of nutritionally healthy, vigorously growing, two to three weeks old plants and (5) the use of Captan 75, Panogen-Drench, PCNB or Phaltan as a precautionary measure against damping-off by Pythium and Rhizoctonia. The only method however, that should be preferred to all the above ones would be to wash the stems thoroughly in water, sterilize the cuttings with 1/1000 mercury chloride solution for 1/2 - 1 minute and wash in water, treat with 1/1000 indole butyric acid solution (to counter the ill effects of the mercury) for 1/2 - 1 minute, before planting in sterilized or unsterilized sand.

The best procedure gave the most satisfactory results in all the trials and 92 - 95 percent of the cuttings rooted very satisfactorily without any signs of infection. It should be recommended because of its efficiency. It would save steam sterilization, the use of chemicals and their unwanted phytotoxic effects, as well as time and labor. It would also destroy organisms borne on the plants (or cuttings), particularly Ascochyta imperfecta, the cause of black stem, leaf spot of alfalfa and a common parasite of alfalfa plant materials.

Well aerated sand beds were also found very satisfactory, and the cuttings should be spaced 1/2 - 1 inch in row and about two inches between the rows to allow for aeration as well as prevent the rapid spread of pathogens among the cuttings.

ACKNOWLEDGMENT

I sincerely thank Dr. Paul C. Duffield who directed the course of this study during the 1959-60 academic year, and similarly Dr. Charles L. Kramer who directed and advised me during the second half of this study. In particular do I want to thank them for their time and patience and generosity.

I also wish to thank my two major advisers for helping me obtain the pictures presented in this manuscript, and for their invaluable help in the identification of the organisms described in this text. Dr. Duffield also provided me with the Rhizoctonia solani (A54R) used throughout this study. Dr. Kramer supplied the Ascochyta sp. (collected from the air) used in some of the trial tests and identified the organisms Colletotrichum graminicolum, Fusarium roseum, Fusarium solani, Fusarium moniliforme, and Pythium irregulare. He also identified the Schizotrichella sp., isolated from sand, and checked most of the isolates recovered from sand.

The person who extended the courtesy of allowing me the use of the fine growth chambers was Dr. O. H. Elmer of our department. Without these chambers most of these experiments could not have been so satisfactorily carried out in the greenhouse.

Valuable suggestions were also offered by Dr. W. H. Sill, Jr., Dr. O. H. Elmer and Dr. E. L. Sorensen of the Agronomy Department, U.S.D.A. Alfalfa Breeding section. In particular do I want to thank Dr. Sorensen for the supply and identification of all the plants used for this study.

I also acknowledge with special gratitude the aid and patience of Drs. C. L. Kramer, S. M. Pady, W. H. Sill, Jr., and H. L. Mitchell in

connection with the presentation of this manuscript.

The Chemagro Corporation, Kansas City, Mo., supplied me with the Dexon and PCNB (Pentachloro nitro benzene) used in later experiments. E. I. duPont deNemours and Company Incorporated, Houston, Texas, furnished me with "Manzate" used in the greenhouse and in one of my trial tests, together with "Arasan" 75 and "Ceresan" M. I am grateful to both companies for their generosity and quick response to my request.

In conclusion, I wish to acknowledge, with sincere appreciation, the various help offered by Miss Bonnie Wiley and Mrs. Nelson (staff of Drs. S. M. Pady and C. L. Kramer), and all persons who have contributed in any way to the success of this study but whom space and time do not permit me to single out individually.

LITERATURE CITED

1. Ark, Peter A., and W. S. Sibray.
Use of pancgen-drench to control damping-off in nursery flats. Plant Disease Reptr. 38:204-206. 1954.

2. Blair, I. D.
Studies on the growth in soil and parasitic action of certain Rhizoctonia solani isolates from wheat. Canad. Jour. Res. C. 20:174-185. 1942.

3. _____.
Behavior of Rhizoctonia solani Kuhn in the soil. Ann. Appl. Biol. 30:118-127. 1941.

4. Brown, W.
Experiments on the effect of chlorinated nitrobenzenes on the sprouting of potato tubers. Ann. Appl. Biol. 34:422-429. 1947.

5. Bucholtz, W. F. and C. H. Heredith.
Pythium debaryanum and other Pythium species caused alfalfa seedling damping-off (Abs.) Phytopath 28:4. 1938.

6. Bucholtz, W. F.
Influence of cultural factors on alfalfa seedling infection by Pythium debaryanum Hesse. Iowa Agr. Expt. Sta. Bul. 296. 1942.

7. Cherewick, W. J.
Rhizoctonia root rot of sweet clover. Phytopath. 31:673-674. 1941.

8. Chilton, S. J. P., L. Hensen, and H. W. Johnson.
Fungi reported on species of Medicago, Melilotus, and Trifoliorum. U.S.D.A. Misc. Publ. 499. 1943.

9. Chrisholm, R. D. and L. Koblitsky.
Absorption of methyl bromide by soil in a fumigation chamber. Jour. Econ. Entm. 36:549. 1943.

10. Cormack, M. W.
Cylindrocarpon ehrenbergi Wr., and other species, as root parasites of alfalfa and sweet clover in Alberta. Canad. Jour. Res. 15:403-424. 1937.

11. _____.
Fusarium spp. as root parasites of alfalfa and sweet clover in Alberta. Canad. Jour. Res. 15:493-510. 1937.

12. _____.
Studies on Ascochyta imperfecta, a seed- and soil-borne parasite on alfalfa. Phytopath. 35:838-855. 1945.

13. Crops Research Division, Agricultural Research Service, U.S.D.A.
Plant pests of importance to North American agriculture. Index of Plant Diseases in the United States. U.S.D.A. Agricultural Handbook No. 165. 259-261. 1960.

14. Crosier, W. F.
Detection and identification of seed-borne parasite. Proc. Assn. Off. Seed Analyst Nor. Amer. 27:82-92. 1933.

15. Daines, R. H.
Antagonistic action of Trichoderma on Actinomycetes scabies and Rhizoctonia solani. Amer. Potato Jour. 14:85-93. 1937.

16. Duggar, B. M.
Rhizoctonia crocorum (Pers.) DC and Rhizoctonia solani Kuehn Corticium vagum B and C with notes on other species. Ann. Mo. Bot. Gard. 2:403-458. 1915.

17. Duggar, B. M., and F. C. Stewart.
The sterile fungus Rhizoctonia as a cause of plant disease in America. Cornell Univ. Agr. Expt. Sta. Bul. 186:50-74. 1901.

18. Elling, Laddie Joe.
Vegetative propagation of alfalfa Medicago sativa. M. S. Thesis. University of Minnesota. Univ. Minn. Library Cat. No. 378.7M66, q.OE1562. 1948.

19. Elmer, O. H.
Effect of environment on the prevalence of soil-borne Rhizoctonia. Phytopath. 32:972-977. 1942.

20. Faucett, H. S.
Experiments in the control of Rhizoctonia damping-off of citrus seedlings. Hilgardia 10:1-16. 1936.

21. Garner, R. J.
Propagation by cuttings and layers. Recent work and its application with special reference to pome and stone fruits. Imperial Bureau of Horticulture and Plantation Crops Technical Communications. No. 14. 1944.

22. Grandfield, C. O., E. D. Hansing, and H. L. Hackerott.
Losses incurred in asexual propagation of alfalfa clones. Jour. Amer. Soc. Agron. 40:804-808. 1948.

23. Gram, E.
Klornitrobenzol-forbindelser som Middel mod kaalbrook, kartoffelskurv, klover-baegersvamp og' brune rodder' paa tomat. tidsskr. Planteavl. 49:119-143. (Citation from Rev. Appl. Mycol. 24: 483. 1945.)

24. Gregory, K. E., C. N. Allen, A. J. Riker, and W. H. Peterson.
Antibiotics and antagonistic microorganisms as control agents against damping-off of alfalfa. Phytopath. 42:613-622. 1952.

25. Haskett, W. C., E. D. Hansing, and E. L. Sorensen.
Chemical control of damping-off in alfalfa cuttings. (Abs.) Phytopath. 47:15. 1957.

26. Halpin, J. E., and E. W. Hanson.
Effect of age on seedlings of alfalfa, red clover, Ladion clover and sweet clover on susceptibility to Pythium. Phytopath. 48:481-485. 1958.

27. Halpin, J. E., E. W. Hanson, and J. G. Dickson.
Studies on the pathogenicity of seven species of Pythium on alfalfa, sweet clover, Ladino clover seedlings. Phytopath. 33:572-574. 1954.

28. Hooker, W. J.
Pentachloro-nitrobenzene soil treatment for potato scab and Rhizoctonia control. Plant. Dis. Reptr. 38:187-192. 1954.

29. Jones, L. K.
Studies on the nature and control of blight, leaf and pod spot and foot rot of peas caused by species of Ascochyta. N. Y. (Beneva) Agr. Expt. Sta. Bul. 574. 1927.

30. Jones, F. R.
Growth and decay of the transient (non-cambial) roots of alfalfa. Jour. Amer. Soc. Agron. 35:625-634. 1943.

31. Kernkamp, M. F. and G. A. Hemerick.
The relation of Ascochyta imperfecta to alfalfa seed production in Minnesota. Phytopath. 43:378-383. 1953.

32. Kernkamp, M. F., J. W. Gibler and L. J. Elling.
Damping-off of alfalfa cuttings caused by Rhizoctonia solani. Phytopath. 39:928. 1949.

33. Kilpatrick, R. A.
Fungi associated with red and white clover in New Hampshire. (Reprint from Plant Disease Reptr. 43:1111, October 15, 1959).

34. Koch, L. W.
Methyl bromide as a soil fumigant for disease, insect and weed control in tobacco and vegetable seed beds. Down to Earth 7:1-2. Dow Chem. Co. Midland, Mich. 1951.

35. Leach, L. D., R. H. Garber, and W. J. Tolmsoff.
Selective protection afforded by certain seed and soil fungicides. (Abs.) Phytopath. 50:643. 1960.

36. LeClerg, E. L.
Parasitism of Rhizoctonia solani on sugar beet. Jour. Agr. Res. U. S. 49:407-431. 1934.

37. _____.
Pathogenicity studies with isolates of Rhizoctonia solani obtained from potato and sugar-beet. Phytopath. 31:49-61. 1941.

38. _____.
Comparative studies of sugar-beet and potato isolates of Rhizoctonia solani. Phytopath. 31:274-284. 1941.

39. McDonald, W. C.
The distribution and pathogenicity of the fungi associated with crown and root rotting of alfalfa in Manitoba. Canad. Jour. Agr. Sci. 35:309-321.

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

41. Miller, H. J.
Control of damping-off fungi with halogen substituted nitrobenzens. Phytopath. 42:470. 1952.

42. Neal, D. C.
Rhizoctonia leaf spot of cotton. U. S. Dept. Agr., Plant Dis. Repts. 27:435. 1943.

43. Newhall, A. G. and B. Lear.
Soil fumigation for fungus control with methyl bromide. Phytopath. 38:38-43. 1948.

44. Peltier, G. L.
Parasitic Rhizoctonia in America. Ill. Agr. Expt. Sta. Bul. 189. 1961.

45. Peterson, M. L., and L. E. Melchers.
Studies on black stem of alfalfa caused by Ascochyta imperfecta. Phytopath. 32:590-597. 1942.

46. Richards, B. L.
Reaction of alfalfa varieties to stem blight. Phytopath. 28:824-828. (Also Utah Acad. Sci. Arts and Letters Proc. 14:33-38. 1936-37.) 1934.

47. Rensberg, Ruth and C. W. Hungerford.
Blackstem of alfalfa in Idaho. Phytopath. 26:1015-1020. 1936.

48. Rushdi, M. H. K., and W. F. Jeffers.
Effect of some soil factors on efficiency of fungicides in controlling Rhizoctonia solani. Phytopath. 46:88. 1956.

49. Sanford, G. B.
Studies on Rhizoctonia solani Kuhn III. Racial differences in pathogenicity. Canad. Jour. Res. 16:53-64. 1938.

50. _____.
Studies on Rhizoctonia solani (Kuhn) V. Virulence in steam sterilization and natural soil. Canad. Jour. Res. 19:1-8. 1941.

51. _____.
Pathogenicity tests on sugar-beets of random isolates of Rhizoctonia solani Kuhn from potato. Sci. Agr. 21:746-749. 1941.

52. _____.
Effect of various chemicals on the natural healing of freshly cut potato. Phytopath. 41:1077. 1951.

53. Smith, Oliver F.
Rhizoctonia root canker of alfalfa (Medicago sativa). Phytopath. 33:1081-1085. 1943.

54. _____.
Parasitism of Rhizoctonia solani from alfalfa. Phytopath. 35: 832-837. 1945.

55. Sprague, R.
Host range and life history studies of some leguminous Ascochytae. Phytopath. 19:917-932. 1929.

56. Stewart, L. C., G. French, and J. K. Wilson.
Troubles of alfalfa in New York. N. Y. (Geneva) Agr. Expt. Sta. Bull. 305. 416. 1908.

57. Tysdal, H. M., and H. S. Westover.
Alfalfa improvement. U. S. Dept. Agr. Yearbook. 1122-1153. 1937.

58. Tysdal, H. M., T. A. Kiesselbach, and H. S. Westover.
Alfalfa breeding. Nebraska Agr. Expt. Sta. Bul. 124. 1942.

59. Valleau, W. B. and E. M. Fergus.
Blackstem of alfalfa, sweet clover, and red clover. Phytopath. 19:507-509. 1929.

60. Waint, J. S.
The Rhizoctonia damping-off of conifers and its control by chemical treatment of the soil. Cornell Agr. Expt. Sta. Mem. 124:1-64. 1929.

61. Waint, J. S.
Effect of various soil supplements on the virulence and persistence of Rhizoctonia solani Kuhn. Sci. Agric. 27:533-544. 1947.

62. _____.
The control of diseases of lettuce by the use of antagonistic organisms. II. The control of Rhizoctonia solani. Kuhn. Ann, Appl. Biol. 38:203-216. 1950.

63. _____.
Persistence of Rhizoctonia solani Kuhn in soil. Canad. Jour. Bot. C. 30:652-654. 1952.

64. Wallen, V. R. and I. Hoffman.
Fungistatic activity of captan in pea seedlings after treatment of seeds or roots of seedlings. Phytopath. 49:680. 1959.

65. Weindling, R.
Various fungi recently found to be parasitic on Rhizoctonia solani Kuhn. Phytopath. 24:1141. 1934.

66. _____.
Studies on a lethal principle in the parasitic action of Trichoderma lignorum on Rhizoctonia solani and other soil fungi. Phytopath. 24:1153-1179. 1934.

67. Weimer, J. L.
A wilt disease of alfalfa caused by Fusarium oxysporum var. medicaginis. Jour. Agr. Res. 37:419-433. 1928.

68. White, W. J.
An improved method of rooting alfalfa cuttings. Sci. Agric. 26:194-198. 1946.

69. Wilson, G. W.
Colletotrichum graminicolum (Cesati). Phytopath. 4:106. 1914.

STUDIES ON DAMPING-OFF OF ALFALFA CUTTINGS IN THE GREENHOUSE

by

PAUL GYAMERA ADDOH

B. S., (Agric.) Purdue University, 1959

AN ABSTRACT OF A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Botany and Plant Pathology

KANSAS STATE UNIVERSITY
Manhattan, Kansas

1961

From the fall of 1959 until the end of spring 1961, studies were undertaken to determine the cause of damping-off of alfalfa cuttings in the greenhouse and possible measures for controlling the disease.

Captan 75 and Panogen Drench gave satisfactory control of Rhizoctonia solani and Pythium debaryanum. PCNB (Penta-chloro nitrobenzene) and Phaltan were also satisfactory but not as effective as Captan and Panogen. In addition all four of these chemicals were effective against most of the other organisms tested. Their action on R. solani was, however, only very strongly fungistatic rather than fungicidal. In heavily infested sand (three plates of inoculum per square foot), 20 percent of the cuttings rooted and 11.25 percent of them were without infection when treated with Captan 75. Out of 27.5 percent rooted cuttings in the Panogen treatment, only 7.5 percent were without infection.

At all the levels and conditions tested Rhizoctonia solani was the most pathogenic of the organisms. It caused from 89 to 100 percent damping-off of cuttings in 7 to 21 days. It was also extremely pathogenic to seedlings but it had little or no effect on mature stem portions of plants.

Pythium debaryanum caused severe damping-off of cuttings but had little effect on mature stem portions. It did, however, cause decay and abortion of flowers and was extremely effective in pre-emergence damping-off of seedlings but had no effect on seedlings three days and older.

Ascochyta imperfecta caused relatively high damping-off of cuttings when it was sand-borne but had little or no effect on the rooting ability and survival of cuttings made from infected stems (provided the stems were not very severely damaged before cuttings were made). It was also effective in causing pre-emergence damping-off of alfalfa seedlings.

Colletotrichum graminicolum was slightly pathogenic on alfalfa cuttings, and relatively virulent as a pre-emergence damping-off organism of seedlings. It had no effect on mature stems, however.

Fusarium roseum was pathogenic to cuttings made from infested stems but weakly pathogenic when sand-borne. It was a virulent pre-emergence damping-off organism of seedlings and had very little effect on mature stems.

Alternaria temuis, Fusidium sp. and Fusarium solani were weak or secondary pathogens of cuttings and mature stems. Fusarium solani was a virulent pre-emergence damping-off organism of seedlings while A. temuis and Fusidium sp. were non-pathogenic

In isolation studies, 33 genera of fungi were found inhabiting and infesting unsterilized sand in the greenhouse. The most common of these were: Cladosporium spp.; Alternaria spp.; Penicillium spp.; Aspergillus niger; Aspergillus spp.; Trichoderma viride; and Fusarium spp. Their populations in the sand beds seemed to increase with the time of exposure in the greenhouse.

Only very small amounts of Rhizoctonia solani and Pythium spp. were found in the unsterilized sand beds. However, neither of them were ever recovered from the sterilized sand or cuttings planted in sterilized and unsterilized sand. Because of their small populations in the unsterilized sand and because they failed to parasitize the cuttings in such beds, it was thought uneconomical to sterilize the sand beds before planting the cuttings.

Mature stems, four weeks old or older, and especially near blooming stage, were found to be more susceptible to all of the 18 chemicals used

in these studies than were younger alfalfa stems. Cuttings made from such mature stems rooted poorly or failed to root at all.

In order to grow alfalfa cuttings successfully in the greenhouse the following measures were found necessary: (1) the use of young and vigorously growing plants; (2) adequate sanitation; (3) careful and ample watering once a day; (4) shading of cuttings from direct light during the first 10 to 14 days; and (5) spacing of cuttings 1/2 inch to 1 inch in the rows and 2 inches between the rows, in well watered and moist sand beds to prevent the rapid spread of pathogens among cuttings.

The application of either of the chemicals, Panogen-Drench, Captan 75, Phaltan or PCNB can be made as a precautionary measure against damping-off organisms. However, the most practical method was found to be to surface sterilize the cuttings with 1/1000 solution of bichloride of Mercury followed by a treatment of 1/4 to 1 minute in a 1/1000 solution of indole butyric acid. These cuttings could then be planted in unsterilized sand where 92 - 95 percent of them would root and show no signs of infection. This procedure was highly preferred to chemical treatments and/or sand sterilization. It destroys organisms carried on the stems (especially Ascochyta imperfecta which causes the black stem of alfalfa and is commonly carried to the greenhouse on the stems). It saves much labor, chemicals and their unwanted phytotoxic effects as well as equipment used in sand sterilization.