

THE EFFECTS OF SEED TREATMENTS ON
DAMPING-OFF OF SPINACH AND BEET
SEEDLINGS IN NATURALLY AND
ARTIFICIALLY INFESTED MUCK SOILS

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ABSTRACT

THE EFFECTS OF SEED TREATMENTS ON DAMPING-OFF OF SPINACH AND BEET SEEDLINGS IN NATURALLY AND ARTIFICIALLY INFESTED MUCK SOILS

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Nine seed treatment chemicals, p-dimethyl amino benzene diazo sulfonate (Dexon), 1-benzoyl-1-2-p-nitroso phenyl hydrazine (Chemagro B-15080), copper 2-pyridinethione 1-oxide (Omadine-copper), captan (Captan 75), methylmercury dicyandiamide (Panogen 15), manganous dithiocarbamate (Tennam 10), yellow cuprous oxide, a chlorinated heterocyclic sulfur compound (Diamond Alkali 6N49), and trans 1,2, bis n-propyl sulfonyl ethylene (Chemagro B-1843), from commercial and experimental stocks were evaluated for control of damping-off of spinach and beet seedlings. The degree of specificity of the chemicals toward the pathogenic fungi, the extent of chemical control of disease in various soil infestations, and the interaction between soil fungi, seed treatments and the host plants were determined in a series of greenhouse experiments. Seeds were planted in pots or flats of naturally infested muck soil and in soil artificially infested with Rhizoctonia solani, Pythium irregulare, Fusarium sp., and the various combinations of these fungi. Evaluation of seed treatment chemicals was based on the

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final seedling stand and the amount of post-emergence damping-off.

The most severe pre- and post-emergence damping-off occurred in soils artificially infested with Pythium, Rhizoctonia and their various combinations. The Fusarium isolate used was only mildly pathogenic and appeared to have reduced severity of damping-off by Rhizoctonia in one experiment.

Nearly all seed treatments significantly increased stands of spinach and beets in pathogen-infested soils. Those which gave most consistent overall protection for spinach and beets, considering all soil infestations as a whole, were Dexon, Chemagro B-15080, Chemagro B-1843, Captan 75, and Tennam 10. None of the seed treatments protected spinach or beet seedlings effectively in soil infested with Rhizoctonia alone. Pythium-specific materials were most effective in these trials.

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INTRODUCTION

Damping-off is a severe disease of field and vegetable crops under conditions which favor its development. The disease is caused by invasion of the seedling by one or several pathogenic fungi which disorganize, weaken, or rot the tissues. Damping-off may be either pre-emergence or post-emergence depending on the stage of seedling development at which the plant succumbs to fungal invasion. Pre-emergence damping-off includes seed rotting or killing of the young seedling by fungi before it emerges from the soil.

The development of damping-off is influenced by various interactions of the environment on both host and pathogen. Generally the conditions most favorable for the pathogen that are at the same time unfavorable for the host will lead to maximum disease development. Delayed growth of the soft seedling tissue, for example, lengthens the susceptible period through which the seedling passes before its tissues become lignified or "hardened-off" sufficiently to resist invasion. At the same time the fungus may be able to grow vigorously and the probability of infection is increased.

Damping-off occurs in the field as well as in the greenhouse and affects both large and small-scale growers. Greenhouse operators have problems with damping-off in flats, pots, coldframes, and seedbeds. In spite of

expensive soil steaming processes, contamination of greenhouse flats and pots may occur as a consequence of careless handling or improper procedures (31).

Chemical seed treatment offers a simple and moderately effective solution to damping-off problems, either as a supplementary treatment or as the sole treatment where the damping-off complex is not severe. Seed treatment offers two types of protection for the growing seedling A) protection from invasion by fungi which are borne externally on the seed and B) at least partial protection of the emerging seedling while it is in it's most vulnerable state (58).

The studies reported here are concerned with the damping-off of spinach and beet crops in the greenhouse. The effectiveness of some promising chemical seed protectants was investigated particularly with regard to disease control and to the specificity of the chemicals. An equally important objective has been that of studying the characteristics and interactions of fungal soil microflora in naturally and artificially infested muck soil by observation of the behavior of selected seed treatments in those soils. Attempts were made to approximate a natural pathogenic field soil by artificially infesting steamed muck with controlled mixtures of soil fungi (Rhizoctonia, Pythium, and Fusarium) in order that an analysis of the damping-off phenomena could be made.

REVIEW OF LITERATURE

Damping-off of Vegetable Crops

Damping-off diseases are particularly severe in vegetable, conifer, and ornamental plants. The disease or group of diseases is a potential threat in most crop growing areas and few plant species are resistant when environmental conditions and stage of development of the host plant favor attack by damping-off fungi (9, 50).

One of the earliest observations on damping-off or root rot was made by De Candolle in 1815 who demonstrated the relation of Rhizoctonia to root rot (39). In 1874 Hesse, and others in Germany, described Pythium debaryanum as a plant parasite and described its effect on plants.

Apparently the damping-off phenomenon was well known in this country prior to 1891, but it was considered to be caused by excessive dampness in the soil (31, 34). In 1891, Humphrey (34) reported the occurrence of damping-off of cucumbers in Massachusetts and described the appearance of mycelium in the rotted tissues. He identified the mycelium as that of Pythium debaryanum and concluded that this was the same disease described by Hesse in Germany. The first detailed accounts of this fungus-disease relationship and that of the "sterile fungus" Rhizoctonia were given by Atkinson (2, 3). The parasitic nature of Rhizoctonia and its relationship to plant diseases was subsequently reviewed by Duggar and Stewart (21) and by

Peltier (50) in Experiment Station bulletins emphasizing the seriousness of root diseases caused by this fungus on a wide variety of crops. More recent reviews of damping-off and its control with particular regard to vegetable crops has been given by Alexander (1), Kadow and Anderson (39) and Ellis (23).

Host Range

Rhizoctonia, Pythium, and Fusarium, the most common damping-off fungi, are widely distributed and have an extensive host range, as evidenced by reports of damping-off disease in the literature (9, 39). Peltier (50) listed the distribution and host range of Rhizoctonia on various crops in the United States, Canada, South America, the West Indies, Europe, India and Australia. Plants belonging to the families Amaranthaceae, Caryophyllaceae, Cruciferae, Leguminosae, Solanaceae and Compositae were especially susceptible to Rhizoctonia species. Some strains of Rhizoctonia have a very restricted host range while others are less specialized (56).

Economic Importance

The cost of vegetable production is increased considerably by the damping-off hazard. The grower must pay for preventive measures such as soil steaming, chemical soil treatment, fumigation, seed treatment, and special cultural practices or he must be prepared to absorb cost

of reseeding if disease becomes severe (10, 13, 33, 39). Damping-off is often responsible for as much as 90% kill of seedlings and in especially susceptible varieties, seedling losses of 25--75 percent occur yearly (39). Secondary losses in yield or quality may occur as a result of mild infection of seedlings after they have begun to mature. Although such plants may survive, the injury to the meristematic or conductive tissue causes poor growth and yield.

Damping-off in seedling flats is important because it represents loss of labor and valuable seed as well as waste of greenhouse space and disrupted planting schedules. Healthy-appearing plants weakened by disease may succumb to the shock of transplanting. As an example of the seriousness of this disease, one large producer of tomato seedlings in New York discarded nearly a half million plants grown in 500 seedling flats as a result of damping-off (31). Cook (14) estimated that 20--30 percent less spinach seed was required in field seedings when the seed was treated for protection against damping-off. According to Natti (49) spinach is subject to a further loss because of damping-off disease. If seed is sown at the normal rate and large gaps result from damping-off disease, the remaining spinach plants grow too large and coarse for a quality product. If spinach is sown at a heavier rate to compensate for seedling disease loss and disease does not become severe, the plants must be thinned

or the lamina of the leaves will be decreased considerably in area without a corresponding decrease in the size of the midribs. Not only is this type an undesirable product but the crowded plants develop stem rot more readily.

It has also been pointed out that aged seed or seed of low vitality is benefited especially by seed treatment. The protection afforded by seed treatment enables the weaker seedlings to survive (58, 60, 61, 62).

Causal Organisms

According to Kadow and Anderson (39) most workers agree that Rhizoctonia, Pythium, Fusarium, Botrytis and Phytophthora are the most important fungi involved in damping-off of vegetable crops. The species most commonly involved are Pythium ultimum, Pythium debaryanum, Rhizoctonia solani (1, 5, 32) and Fusarium oxysporum (5). Other fungi, capable of causing damping-off under more restricted conditions are Phoma betae (16) and certain species of Sclerotinia, Sclerotium, Glomerella and Thielavia (39).

Damping-off may also be caused by fungi which are more specific in their host range. This type is represented by Aphanomyces laevis, and Aphanomyces cochliodes on beet (7, 24) and Ascochyta pinoidella and Mycosphaerella pinodes, root parasites of peas (38).

Symptoms

The most conspicuous symptom of damping-off is that of the toppling of the seedling after emergence from the soil. Post-emergence damping-off occurs as a result of fungal invasion of the hypocotyl at or just below the ground line. The tissues become rotted and soft at that point and the seedling collapses. The post-emergence phase of damping-off by Pythium is characterized generally by a water soaked and discolored appearance of the hypocotyl, particularly below the soil line. Plants which do not topple but have diseased roots or hypocotyl are stunted, abnormally dark green, and the cotyledon leaves roll downward (1). Invasion of the hypocotyl by Rhizoctonia produces a dry, shrivelled lesion instead of the water soaked one caused by Pythium (1). Tissues are frequently browned and sunken and roots may be covered with hyphae (50). Rhizoctonia may cause a soft rot which progresses rapidly without any browning or lesion formation, or invasion may cause the formation of reddish brown lesions which gradually deepen until they reach the vascular tissue. If the lesion is confined to the superficial tissues, the plant may recover but penetration to the vascular tissues, especially when the lesion girdles the stem, usually results in the death of the plant (3).

Fusarium causes a different type of seedling disease than Rhizoctonia or Pythium. Instead of attacking

the hypocotyl at the soil line, Fusarium penetrates the plant through the root (27), and often invades the plant systemically producing severe wilt symptoms and often death (35). The early stages of infection of spinach are recognized by the pale color of the leaves and the tendency of the leaf margins to roll inward. Leaf symptoms become progressively more severe and permanent wilting and death follow within three or four weeks. The roots of a diseased plant appear blackened and the vascular tissue is often discolored (15, 35). Older infected plants may survive until maturity or may die at any intermediate period. Infected plants may or may not be stunted, but the leaves yellow and wilt starting with the older outer leaves and spreading progressively to the younger ones (15). Although Fusarium is considered to be primarily a vascular pathogen, a strain of Fusarium oxysporum, causing a seedling blight of asparagus, was found to act primarily in the cortical region of the root (26). Penetration was through the root tip or stomata on the hypocotyl and the fungus grew cellularly and intercellularly through the cortex. In contrast to vascular strains, symptoms were associated with collapse of sections of the primary root and the roots of older seedlings had reddish-brown elliptical lesions.

The effects of pre-emergence damping-off can be observed in pots, seedling flats, cold frames or in the field where gaps in the rows show that seedlings have

not emerged. Infestation may radiate from the initial infection point outward in all directions, killing seedlings as it progresses (39).

Closely related diseases known as "wire stem", "stem rot" or "bottom rot" result from late seedling stage infection by the damping-off fungi. These seedlings, though they may not topple over, are slow in development and generally are stunted and of little commercial value (39).

Environment

Severity of damping-off, particularly of the pre-emergence type, is determined by several factors, among which soil temperature, moisture, and pH are usually the most important.

Temperature-- There is evidence that pre-emergence damping-off may be correlated with the effect of temperature on the growth of the host (4, 20, 37) as indicated by temperature studies with wheat and corn. Studies by Leach (45) have shown that the effect of temperature on the growth rate of both the host and the pathogen bears a definite relationship to disease severity. Using spinach, sugar beets, watermelon and peas in combination with Rhizoctonia, Pythium, and Phoma he found little correlation between temperature effect on the severity of infection and the growth rate of either the host or the pathogen. However, by determining the coefficient of velocity of

emergence of the host plant by the method of Kotowski (40) and the rate of growth of the organism in culture at various temperatures, Leach predicted the range of disease severity for a particular combination of host and pathogen. The percentage of seedlings emerging from infested soil at different temperatures agreed closely with the ratio between the coefficient of velocity of emergence and the growth rate of the organism at the same temperatures (42, 45). Spinach, which germinated equally well between 4 and 25° C became most severely diseased in Pythium-infested soil between 12 and 20° C. In Rhizoctonia-infested soil pre-emergence damping-off was least at 12° C or below, moderate at 16° C and severe at 20° C or above. Sugar beets suffered most severe pre-emergence damping-off in Pythium-infested soil between 12 and 20° C and in Rhizoctonia-infested soil between 16 and 30° C. In all combinations of host and pathogen pre-emergence infection was most severe at temperatures that were relatively less favorable to the host than to the pathogen as measured by the ratio of their growth rates (45).

Experiments with damping-off of Ladino clover and Lespedeza caused by Rhizoctonia solani, Pythium debaryanum and Fusarium roseum indicated that pre-emergence damping-off was greatest at temperatures which were relatively unfavorable to the host and that post-emergence damping-off with the exception of that caused by Fusarium, was greatest at temperatures favoring the growth of the host (27).

Studies on the influence of temperature and other factors on the damping-off of Red pine have shown that although Red pine germinated well between 18 and 33° C , about half of the seedlings were killed in Pythium-infested soil at the higher temperature and 90 percent were killed at temperatures near 12° C. At these low temperatures Rhizoctonia caused only a small loss, but damage increased to a maximum of about 58 percent at 24--30° C and then declined at 33° C (53).

Kadow and Anderson (39) in summarizing the results of various workers listed Pythium as being destructive over a wide range of temperatures, but usually most serious between 24 and 30° C (75--85° F). Rhizoctonia was listed as most destructive at temperatures between 16 and 25° C (61--77°F). In field and greenhouse experiments with many kinds of vegetables, Pythium was found to be the cause of damping-off at temperatures as low as 7° C (45° F) and as high as 33° C (90° F) and in serious form between 13 and 30° C (55--85° F).

Soil Moisture-- Beach (4) pointed out that damping-off can be severe when the soil holds just enough moisture for good plant growth, or about 50 percent water-holding capacity. Increasing soil moisture above this value leads to progressively poorer stands since this condition is less favorable to the host and more favorable to certain fungi.

Pythium irregulare caused damping-off at a soil moisture of 20 percent and the percentage of disease showed a continuous increase with increase in soil moisture up to saturation (53). Rhizoctonia damping-off gradually increased from 47 percent at 13 percent soil moisture to a maximum (65 percent) at 68 percent soil moisture and then decreased to a minimum at saturation.

Pythium is sensitive to drying especially at high temperatures but Rhizoctonia is able to survive in dry soil (4, 52). In controlled greenhouse experiments, growth of Rhizoctonia through the soil was greatest at 30 percent soil moisture and least at 80 percent (6).

The Interrelationship of Temperature and Moisture

In experiments with tomato seedlings under controlled moisture and temperature conditions, it was demonstrated that Rhizoctonia and Pythium have a wide range of parasitism. Disease development was retarded by high soil temperature and low soil moisture. Pythium injury increased as soil temperature was lowered to 18° C even though soil moisture was maintained at 35 percent. As soil moisture was increased at the low temperatures, damping-off became more severe. Rhizoctonia solani was most severe around 24° C at all soil moisture values tested (1).

Roth and Riker (53, 54) found that temperature was more important in determining severity of total

damping-off of Red Pine while soil moisture determined which fungus would predominate. Except for early pre-emergence and some post-emergence damage caused by Pythium at low temperatures, warm weather favored total damping-off irrespective of the fungus acting. When temperature was low, post-emergence damping-off was at a minimum.

Soil Acidity-- The fungi most concerned in damping-off are adapted to a pH range wider than that of most cultivated plants, or from pH 4 to pH 9. If the pH of the soil is optimum for a particular crop, it is better able to resist or escape attack, probably because the seeds germinate faster and more uniformly. In experiments with spinach, beets and tomatoes, damping-off tended to be two to three times greater below pH 6.5 than throughout the range above this level (4).

Variations in soil acidity between pH 5.5 and 7.0 had little influence on total damping-off of Red Pine. A pH of 5.5--6.0 was favorable to both Rhizoctonia and Pythium. The more acid soils favored Rhizoctonia while Pythium damping-off occurred in soil more nearly neutral. Increase in damping-off by both fungi at levels above pH 7.0 appeared to be associated with decline in host development (54).

Soil Solute Concentration-- An excess of solutes in the soil, caused by overfertilization may be a direct cause of physiogenic damping-off or in less severe form

may simply delay germination and predispose the seedling to fungal attack. It has been shown that common damping-off fungi grow well in concentrations of soil solutes which seriously inhibit growth of seed plants. As solute concentration increases, the growth rates of both fungus and host plant decrease, but the host plant is more severely affected and disease incidence becomes greater (5).

Control

Seed treatment for seed and soil borne disease control has been reviewed by a number of investigators (1, 8, 23). Leukel (47) surveyed the use of various organic and inorganic fungicides for seed treatment, and the materials and methods for seed treatment of cereals, forage crops, cotton, sugar beets and vegetables. Reviews with particular emphasis on vegetable crops have been written by Taylor and Rupert (57) and by Walker (59).

Prior to 1937 most of the chemicals used for treatment of spinach seed were inorganic metal salts such as copper sulfate, red and yellow oxides of copper, and zinc oxide (11, 12, 13, 14, 39, 51). Later the development of the organic fungicides led to the recommendation of thiram, dichlone and the organic mercuries along with the copper and zinc oxides for spinach seed treatment (25). Dichlone was reported to be less effective than the other materials for treatment of beet and spinach seeds (57).

In New Zealand, 40 chemicals, including copper compounds, quinones, organic mercury compounds, dithiocarbamates and other organic materials, were compared as spinach seed protectants. Yellow cuprous oxide, dichlone, organic mercuries, thiram, ferbam, copper-8-quinolinolate, nitrosopyrazole and captan were found to be most effective (36). Dichlone was more effective than chloranil as a quinone seed treatment and mercury combined with methoxy ethyl, ethyl phosphate, and dinaphthyl methanedisulphonate was more effective than other formulations. Of the dithiocarbamates, thiram in concentrations of not less than 50 percent was effective but cupram, ziram, and zineb were not effective for protecting spinach seeds.

Tests in the United States have shown that yellow cuprous oxide, Arasan, Phygon, Orthocide 75, Panogen (18, 48, 49), zinc 2,4,5 trichlorophenate and Vancide 51 (48) were effective as spinach seed treatments. It was noted that treatment with Panogen (1 percent by weight of seed) caused some seed injury (49).

The early recommendations for treatment of beet seed were cuprous oxide, copper sulfate seed soak, and hydroxymercurichlorophenol (Semesan) (39). Red oxide of copper was found to be a satisfactory seed treatment for spinach and sugar beets where damping-off was caused by Pythium ultimum. In the case of accompanying Rhizoctonia and Phoma infestation however, seed treatment with organic mercury compounds was more effective (41).

Field tests in 1943 indicated the usefulness of several inorganic and organic fungicides as vegetable seed protectants (25). Those which were effective as beet seed treatments were Arasan, Ceresan, Semesan and 2,4,5 trichlorophenol. Cooperative tests in 1944, in both greenhouse and field, indicated that Arasan, Ceresan and yellow cuprous oxide gave beneficial results in 60 percent of the areas in which tests were conducted (43, 44). In flat tests all three compounds were equally effective against Pythium but yellow cuprous oxide was less effective than the others in controlling Rhizoctonia. It was concluded from these tests that Detroit Dark Red beets are less susceptible to Pythium attack than other strains of garden or sugar beets (43).

In order to eliminate some of the objectionable features of dust treatments, a number of soluble or wettable treatments have been investigated (46). With beet seed, a .15 percent solution of ethyl mercury phosphate applied at 4 percent of seed weight was as effective against Pythium damping-off as Ceresan M or Phygon. The use of this material as a seed dip also controlled seed-borne Phoma betae.

Dichlone and thiram were highly suitable as beet seed treatments and both were superior to yellow cuprous oxide or chloranil, while captan, Vancide 51, and Panogen were moderately effective treatments in Michigan (18).

The effectiveness of captan and Vancide 51 was confirmed in other studies (48, 49). Arasan, captan, dichlone, chloranil, Semesan, Vancide 51 and zinc 2,4,5 trichlorophenate proved to be highly effective as beet seed treatments in Louisiana. Chloranil and thiram were somewhat less effective than the other materials (48). Dichlone, captan, and Vancide 51 were recommended as the best materials for beet seed treatment in New York (49). Dichlone consistently appeared best and thiram was less effective than the above materials.

Greenhouse experiments with various seed protectants on beets have shown that Dexon (p-dimethyl amino benzene diazo sodium sulfonate), captan, and Tennam (manganous dithiocarbamate) gave good overall protection in naturally and artificially infested muck soils (17).

MATERIALS AND METHODS

Ruby King pepper, Black Beauty eggplant, Giant Thick-leaved Nobel spinach and Detroit Dark-Red beets were selected for greenhouse experimental work /a . Seed treatment chemicals were obtained from companies participating in the support of a graduate assistantship, employing the author, for seed and soil treatment research at Michigan State University.

Seed Treatment

Seed treatments were applied as slurries or liquid treatments (Table 1). The seeds were tumbled in an Erlenmeyer flask to insure uniform distribution of the chemical over the seeds. Extra chemical (10% by weight) was added to approximately compensate for loss of material on the walls of the flasks. After drying, the seeds were stored in the laboratory for subsequent plantings.

Soil Infestation

Inoculum for infesting soil was grown on petri plates of Potato Dextrose agar (Fusarium sp. and Rhizoctonia solani Kühn), Nutrient Dextrose agar (Rhizoctonia solani Kühn), and Corn Meal agar (Pythium irregulare Buisman) until the mycelium covered the plate. The cultures were then chopped separately in a small-cup Waring Blendor with a small amount of distilled water for

/a Seeds were furnished by Ferry-Morse Seed Co.
Detroit, Michigan

Table 1. Seed-treatment chemicals applied to Giant Thick-leaved Nobel spinach or Detroit Dark Red beet seeds.

<u>Material & Mfr.</u>	<u>Active Ingredient</u>
Dexon (Chemagro)	20% p-dimethyl amino benzene diazo sulfonate.
B-15080 (Chemagro)	20% 1-benzoyl-1-2 p-nitrosophenyl hydrazine.
Omadine-Copper (Olin-Mathieson)	50% copper 2-pyridinethione 1-oxide.
Captan 75 (Stauffer)	75% captan.
Panogen 15 (liq.) (Panogen) <u>/a</u>	2.2% methylmercury dicyandiamide.
Tennam 10 (Tennessee Corp.)	90% manganous dithiocarbamate.
Cuprous oxide (Rohm & Haas)	100% yellow cuprous oxide.
DAC 6N49 (Diamond Alkali)	50% a chlorinated heterocyclic sulfur compound.
B-1843 (Chemagro)	20% trans 1,2 bis (n-propyl sulfonyl ethylene).
AC-14307 (American Cyan.)	50% trichloro methyl chlorobenzene thiosulfonate.
TPA-1 (Tennessee Corp.)	2% phytoactin-antibiotic.
Actidione M (Upjohn Co.)	1% cycloheximide.
Arasan 75 (duPont Co.)	75% thiram.
Omadine-Zn-disulfide (Olin-Mathieson)	50% Zn-disulfide 2-pyridinethione 1-oxide.
B-856 (Chemagro)	50% 1,3-dichloro-5,5 diphenyl hydantoin.

/a Panogen 15 applied to seeds at the rate of 4 oz./100 lb. of seed. All others at 8 oz./100 lb.

30--60 seconds at slow speed. The resulting fragments were about 1 mm in diameter. Screened muck soil pastuerized in flats was infested by distributing inoculum (1 plate suspended in 200 ml of water for each flat) in trenches made by a 10 row marker. The soil was allowed to stand for 3 to 5 days for colonization. The colonized soils were combined variously and mixed uniformly in a clean polyethylene-lined mixing bin. The various mixtures were placed in either steamed pots or flats and were planted immediately.

Planting

Seeds were planted in 4-replicate 25-seed units either in 4 inch pots or in 14 inch rows in flats (10 rows per flat). An effort was made to provide the same amount of soil in each planting unit and to plant the seeds at a uniform depth and spacing. Watering was done carefully to avoid splashing and cross contamination between flats or pots.

Data

After emergence, post-emergence damping-off was recorded daily or at 2-day intervals until the seedlings reached a resistant stage of maturity; generally within 14 days. Surviving seedlings were also recorded at this time. Pre-emergence data is graphically shown by difference between total emergence and germination of treated seeds in steamed soil. Analysis of variance was carried out on both

total emergence and survivor data. Wherever multiple-unit experiments were conducted, using both seed and soil treatment variables, a split-plot analysis was applied on the survivor or survivor and total emergence data and a Duncan Multiple Range (22) comparison was carried out wherever appropriate.

EXPERIMENTAL RESULTS

Selection of Experimental Materials

Preliminary experiments with treated eggplant and pepper seeds in flats of unsteamed mineral soil aided in the selection of the 15 materials listed in Table 1. Nineteen materials were tested in separate 4-replicate experiments using these crops. Both experiments were repeated and the data was analyzed statistically. Eight of the effective materials were selected for further use. The remaining seven materials were selected because of their known specific activity on certain soil fungi.

Although pepper and eggplant were satisfactory hosts for damping-off studies, a period of 12 to 16 days was required for emergence when they were grown at temperatures near 60° F. For this reason, spinach and beet were selected as test plants in subsequent experiments. Both germinate readily in 3 to 4 days at fairly low temperatures and are very susceptible to damping-off in infested soil if the seeds are unprotected.

Seed Treatment Effects in Artificially Infested Soils

Preliminary Experiments

Since seed treatment chemicals differed in effectiveness at certain stages of seedling development in unsteamed soil, a series of experiments was set up to determine the behavior of each seed treatment in a given

type of artificially infested muck soil. Soils were artificially infested with Rhizoctonia solani Kühn, Pythium irregulare Buisman, Fusarium sp. or combinations of these fungi. Spinach seeds treated with each of 15 chemicals were planted in the variously infested soils at intervals over a seven month period. Seeds from the same treated lots were used in each experiment of the series. Stand averages of the various seed treatments from each of the experiments appear in Table 2 and Fig. 1.

Rhizoctonia Soil (Expt. 1) -- In Rhizoctonia-infested muck soil, Chemagro B-1843 was the only seed treatment significantly better (5% level) than the untreated control. Cuprous oxide was significantly poorer at the same level of significance. Post-emergence damping-off was nearly the same in all treatments (ranging from 11.0--20.0%) including the control (Fig. 1).

Pythium Soil (Expt. 2) -- In Pythium-infested soil, 10 seed treatments produced stands significantly (1% level) higher than those of untreated seed. Both pre-emergence (2.0--14.0%) and post-emergence (3.8--6.8%) damping-off losses were small for Chemagro Dexon, Chemagro B-15080 and Chemagro B-1843. For the other seven effective materials, the average pre- and post-emergence damping-off was 15.2% and 34.5% respectively. Pre-emergence damping-off was very high (56.0--100.0%) for American Cyanamid 14307, Tennessee TPA-1, Diamond Alkali 6N49, Actidione M and the untreated control.

Table 2. Percent stand of Giant Thick-leaved Nobel Spinach seed treated with various seed protectants and planted in various soil infestations.

Seed Treatment	Experiment #									
	1 R /a	2 P	3 F	4 RF	5 PF	6 RP	7 RPF	8 U	9 S	Ave.
1. Chemagro Dexon	43.2	79.2	74.0	56.0	48.0	31.2	23.2	81.2	76.8	57.0
2. Chemagro B-15080	46.0	72.0	63.2	65.2	52.0	22.0	22.0	74.0	72.0	54.3
3. Omadine-copper	51.2	22.0	69.2	68.0	39.2	12.0	25.2	54.0	78.0	46.6
4. Captan 75	59.2	54.0	74.0	70.0	24.0	22.0	19.2	67.2	86.0	52.9
5. Panogen 15	58.0	31.2	72.0	71.2	27.2	26.0	34.0	64.0	82.0	51.8
6. Tennam 10	53.2	46.0	68.0	82.0	21.2	30.0	24.0	64.0	68.0	50.7
7. Amer.Cyan.14307	42.0	0.0	64.0	67.2	1.2	1.2	17.2	63.2	75.2	36.8
8. Tenn.Corp. TPA-1	46.0	0.0	63.2	54.0	0.0	1.2	12.0	6.0	64.0	27.4
9. Arasan 75	51.2	43.2	75.2	73.2	18.0	37.2	33.2	62.0	75.2	52.1
10. Cuprous oxide	28.0	27.0	73.2	72.0	15.2	14.0	27.2	65.2	74.0	44.0
11. Chemagro B-1843	68.0	76.0	79.2	72.0	32.0	31.2	28.0	64.0	76.0	58.5
12. Diamond Alk.6N49	44.0	4.0	74.0	73.2	7.2	3.2	23.2	53.2	74.0	39.6
13. Actidione M	45.2	0.0	75.2	63.2	0.0	3.2	13.2	9.2	75.2	31.6
15. Omad.Zn-disulf.	47.2	32.0	64.0	60.0	17.2	19.2	23.2	53.2	78.0	43.8
16. Chemagro B-856	34.0	11.2	75.2	59.2	17.2	3.2	27.2	49.2	77.2	39.2
C. Control	48.0	1.2	79.2	70.0	0.0	3.2	9.2	5.2	72.0	32.0
Ave.	47.7	31.2	70.3	67.2	20.0	16.3	22.6	52.2	75.4	
L.S.D. 5%	17.5	15.3	--	13.8	20.5	14.1	--	9.8	--	
1%	23.2	20.3	--	--	27.4	18.8	--	13.1	--	

/a Soil Infestations

R = Rhizoctonia P = Pythium F = Fusarium

U = unsteamed.

S = steamed.

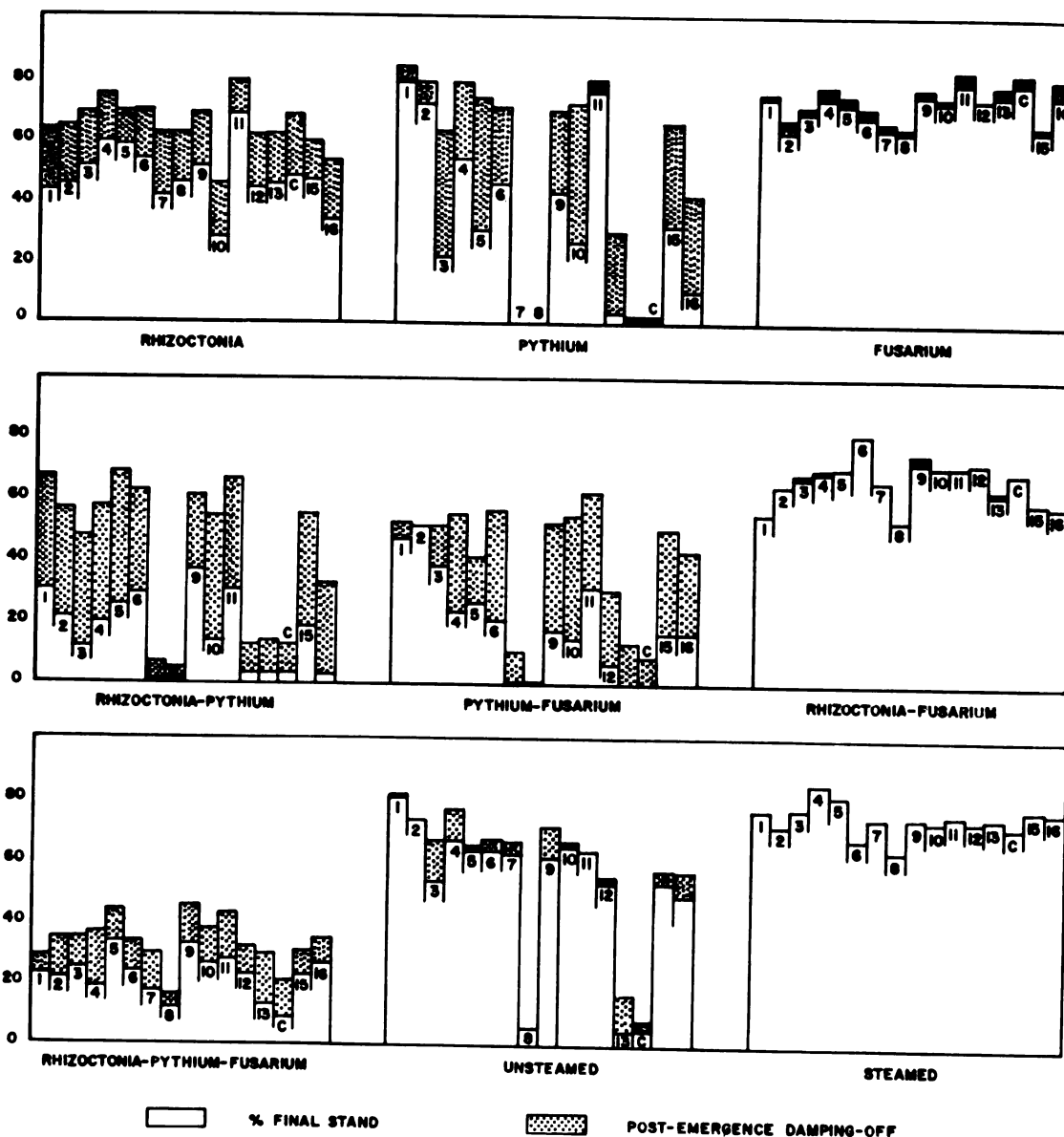


Figure 1. Percent stand and total emergence of Giant Thick-leaved Nobel spinach seed treated with various seed protectants and planted in variously infested muck soils. See Table 2 for individual treatments.

Fusarium and Rhizoctonia-Fusarium Soils (Expts. 3 and 4) --

Seed treatments produced no significant increase in stands over those of untreated seed. Fusarium pre-and post-emergence damping-off ranged from 1.0--5.8% and 3.0--22.0% respectively. In RF soil, stands were generally high (54.0--75.0%) and post-emergence damping-off was negligible. Some pre-emergence damping-off (3.0--21.2%) was evident in this soil.

Pythium-Fusarium Soil (Expt. 5) -- In contrast to Experiments

3 and 4, the stands from treated seeds in PF infested soil were low (0--51.8%) and both pre- and post-emergence damping-off were high. Five seed treatments gave significant increases in stands over those of untreated seed. Post-emergence damping-off was low (5.3% and 0% respectively) for Chemagro Dexon and Chemagro B-15080, moderate (12.8 and 15.0%) for Omadine-copper and Panogen 15, and higher (31.2%) for Chemagro B-1843 as compared to 27.0--40.0% for Captan 75, Tennam 10, Arasan 75, Cuprous oxide, Omadine-Zn-disulfide and Chemagro B-856, all of which were not effective seed treatments in this experiment. Pre-emergence damping-off caused losses of 22.8--44.0% for the effective treatments as compared to 55.0--80.0% for the poorer treatments (American Cyanamid 14307, Tennessee TPA-1, Diamond Alkali 6N49, Actidione M) and the untreated control. In the latter group, the combined losses of pre- and post-emergence damping-off reduced stands to zero. The results of this experiment were similar to those for Pythium-infested soil.

Rhizoctonia-Pythium Soil (Expt. 6) -- Stand averages ranged from 1.2--37.0% in soil infested with RP in combination. Six of the seed treatments produced stands significantly greater than those from untreated seeds. For the effective materials, pre- and post-emergence damping-off averaged 21.6 and 34.8% respectively. Pre-emergence damping-off was high (ave. 75.2%) for American Cyanamid 14307, Tennessee TPA-1, Diamond Alkali 6N49, Actidione M and the control. The additional post-emergence loss (ave. 8.0%) practically eliminated stands for these treatments.

Rhizoctonia-Pythium-Fusarium Soil (Expt. 7) -- There were no significant differences between seed treatments in RPF infested soil. Stands were generally low for all treatments, ranging from 34.1% for the best treatment to 9.4% for the untreated control. Differences between treatments were slight and pre- and post-emergence damping-off averaged 53.2% and 11.0%. Either pre-emergence damping-off or a loss in viability caused a general stand reduction for all treatments. The latter is suspected, since this experiment was conducted much later than the others in the series.

Unsteamed Soil (Expt. 8) -- Soil for this experiment was unsteamed field muck. Untreated seed produced the lowest stand and 13 treatments gave stands significantly greater than the untreated control. Both pre- and post-emergence damping-off losses were small for Dexon, Chemagro B-15080, Panogen 15, Cuprous oxide, and Chemagro B-1843. Pre-emergence losses were high (69.0--80.0%) for Tennessee TPA-1, Actidione M and the control.

Steamed Soil (Expt. 8a)-- Although the differences between seed treatments in steamed soil were not statistically significant, stands from the treated seeds were uniform and somewhat better than those of untreated seeds. The control stand average was 72.0% and 12 of the 15 seed treatments had stands averaging from 74.0 to 86.0%.

Spinach Seed Treatment

Analysis of the preceding experiments indicated the desirability of comparing the various soil treatments at one time in flats or pots in order to minimize some variability caused by environment. Both spinach and beets were used in this way in subsequent trials. Nine of the more effective and distinctive seed treatments were selected for these experiments (Table 3). Seed treatments were replicated in each type of inoculum with steamed and unsteamed soil as controls for the soil treatments.

Experiment 9 -- Flats were infested as previously described and planted immediately after mixing the inoculum components. Average total emergence, final stand and post-emergence damping-off are shown graphically in Fig. 2. Pre-emergence damping-off values may be approximated by extending the bars to 72.0 (the percent germination in steamed soil). Pre-emergence damping-off was relatively high and post-emergence damping-off was low in this experiment. Analysis revealed no significant interaction

Figure 2. Percent stands and total emergence of Giant Thick-leaved Nobel spinach seed treated with various seed protectants and planted in variously infested muck soils (Experiment 9).

<u>Seed Treatments</u>	<u>Soil Infestations</u>
1. Dexon.	R-- <u>Rhizoctonia solani</u> .
2. Chemagro B-15080.	P-- <u>Pythium irregulare</u> .
3. Omadine-copper.	F-- <u>Fusarium</u> sp.
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- "
8. Diamond Alkali 6N49.	U-- Unsteamed.
9. Chemagro B-1843.	S-- Steamed.
10. Control.	

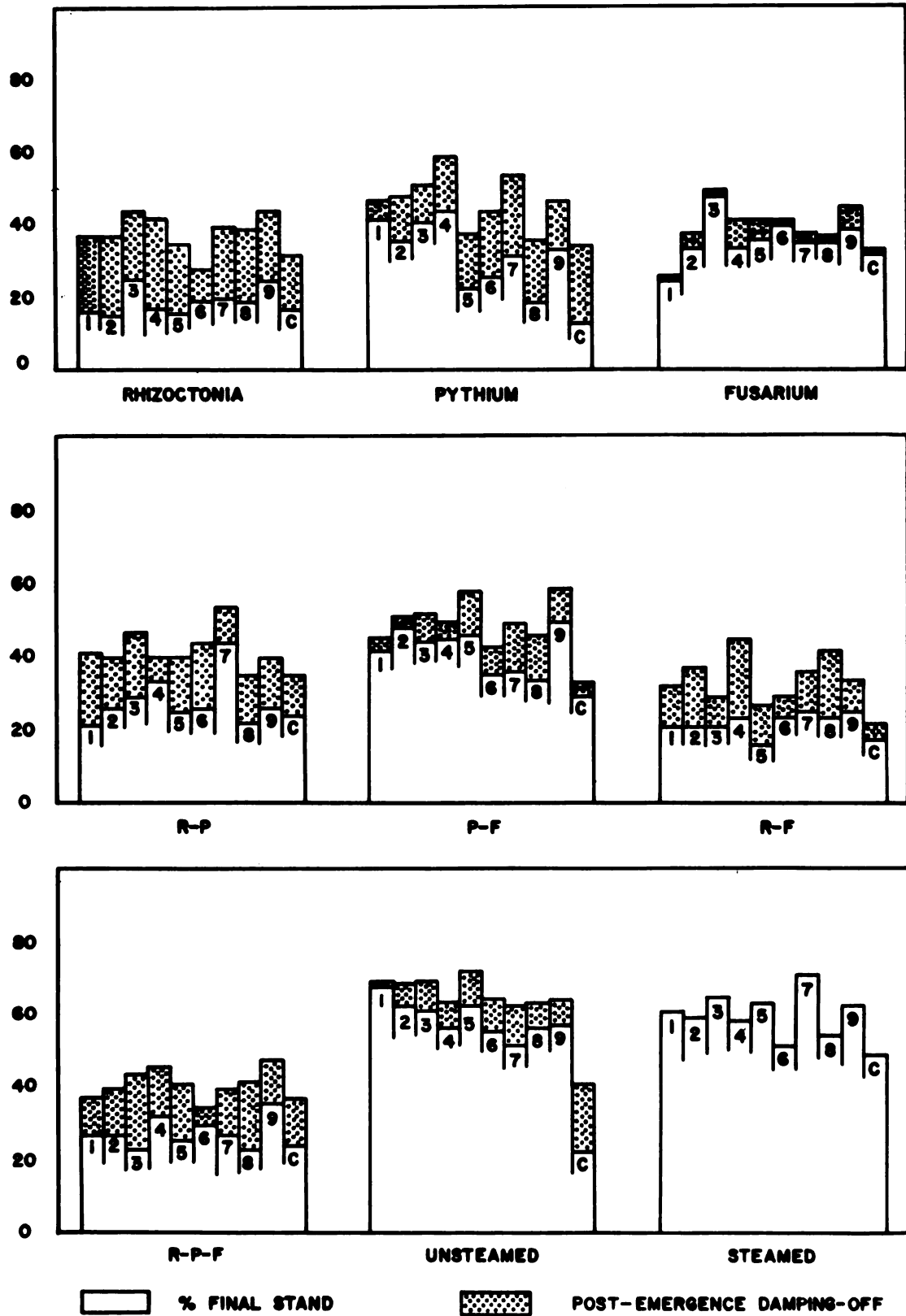


Figure 2.

Table 3. A Duncan Multiple Range comparison of stand differences due to soil infestation and seed treatment of Giant Thick-leaved Nobel spinach.

		Seed Treatment										
		3	9	4	7	2	1	5	6	8	10	<u>Means</u>
Soil Infestation	S	66.0	63.2	59.2	72.0	60.0	62.0	64.0	52.0	55.2	63.2	60.4
	U	62.0	58.0	57.2	52.0	63.2	68.0	63.2	56.0	57.2	58.0	56.0
	PF	44.0	50.0	45.2	36.0	48.0	42.0	46.0	35.2	34.0	50.0	42.0
	F	48.0	39.2	34.0	35.2	34.0	25.2	36.0	40.0	35.2	39.2	36.0
	P	41.2	34.0	44.0	32.0	36.0	42.0	23.2	26.0	19.2	34.0	31.2
	RP	29.2	26.0	33.2	44.0	26.0	21.2	25.2	26.0	22.0	26.0	27.6
	RPF	23.2	36.0	32.0	27.2	27.2	27.2	26.0	30.0	23.2	36.0	27.6
	RF	21.2	25.2	23.2	25.2	21.2	21.2	20.0	23.2	23.2	25.2	21.6
	R	25.2	25.2	17.2	20.0	15.2	16.0	16.0	19.2	19.2	25.2	18.8
	Means	40.0	39.6	38.4	38.0	36.8	36.0	35.2	34.0	32.0	25.6	

Means bracketed by the same line are not significantly different from each other.

<u>Seed Treatments</u>	<u>Soil Infestations</u>
1. Dexon.	R-- <u>Rhizoctonia solani</u> .
2. Chemagro B-15080.	P-- <u>Pythium irregulare</u> .
3. Omadine-copper.	F-- <u>Fusarium sp.</u>
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- " "
8. Diamond Alkali 6N49.	U-- Unsteamed.
9. Chemagro B-1843.	S-- Steamed.
10. Control.	

differences between seed and soil treatments or between seed treatments with all inoculum types taken as a whole. All seed treatments, except Diamond Alkali 6N49, significantly increased germination. Germination was also significantly affected by the various soil treatments (Table 3). In this experiment, stands of treated seed in unsteamed soil were not significantly different from those in steamed soil. Stand averages in Pythium, Fusarium and PF soils were essentially the same and stand reduction was greatest in soils containing Rhizoctonia (R, RF, RP, and RPF).

Experiment 10 -- Seeds treated with the nine fungicides were planted in pots of freshly infested soil. The inoculum was adjusted to give the same initial concentration for each component whether used in combination or alone. In previous trials, the initial concentration of each component was one-half or one-third that of the component when it was used alone. There was a definite increase in post-emergence damping-off in RP and RPF soils (Fig. 3). A Duncan Multiple Range analysis of stand averages, shows three significantly different groups (Table 4). The first group (stand ave. 51.2--78.0%) is composed for the most part of seed treatments which were effective primarily against Pythium. The second group (23.2--51.2%) was represented largely by seed treatments of intermediate effectiveness in P and PF soils. The last group (stand ave. 0--21%) encompassed nearly all of the seed treatments

Figure 3. Percent stand and total emergence of Giant Thick-Leaved Nobel spinach seed treated with various seed protectants and planted in variously infested muck soils (Experiment 10).

<u>Seed Treatment</u>	<u>Soil Infestation</u>
1. Dexon.	R-- <u>Rhizoctonia solani</u> .
2. Chemagro B-15080.	P-- <u>Pythium irregulare</u> .
3. Omadine-copper.	F-- <u>Fusarium</u> sp.
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- "
8. Diamond Alkali 6N49.	U-- Unsteamed.
9. Chemagro B-1843.	S-- Steamed.
10. Control.	

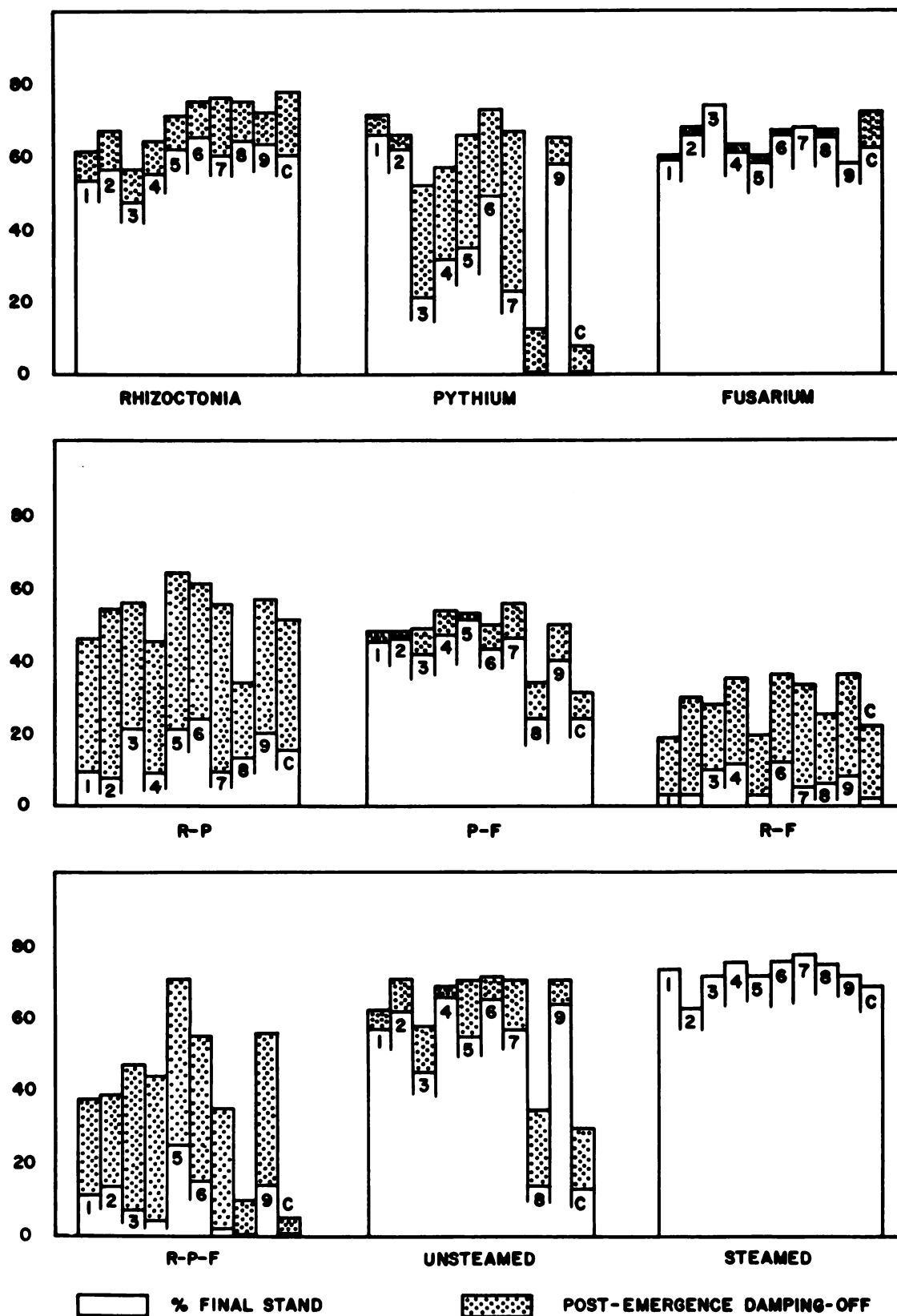


Figure 3.

Table 4. A Duncan Multiple Range comparison of percent stands of Giant Thick-leaved Nobel spinach produced by treated seed in variously infested muck soils.

<u>Seed Treatments</u>	<u>Soil Infestations</u>
1. Dexon.	R-- <u>Rhizoctonia solani</u> .
2. Chemagro B-15080.	P-- <u>Pythium irregulare</u> .
3. Omadine-copper.	F-- <u>Fusarium</u> sp.
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- "
8. Diamond Alkali 6N49.	U-- Unsteamed.
9. Chemagro B-1843.	S-- Steamed.
10. Control.	

Table 4. Experiment 10.

<u>Treatment</u>	<u>Means</u>	
S7	78.0	
S4-S6	76.0	
S8	75.2	
S1-F3	74.0	
S3-S5	72.0	
S9-F7	68.0	
U4-U6-F2-P1-F6	66.0	
R6-F8-S10	65.2	
R8-U9	64.0	
S2-R9	63.2	
U2-R5-P2-F10	62.0	
F4	61.2	
R7-R10	60.0	
F1	59.2	
P9-F5-F9	58.0	
U1-U7	57.2	
R2	56.0	
R4-U5	55.2	
R1	53.2	
PF5	51.2	
P6	49.2	
R3-PF4	47.2	
PF2-PF7	46.0	
PF1-U3	45.2	
PF6	43.2	
PF3	42.0	
PF9	40.0	
P5	35.2	
P4	32.0	
RPF5	25.2	
RP6-PF8-PF10	24.0	
P7	23.2	
RP5-P3-RP3	21.2	
RP9	20.0	
RPF6-RP10	15.2	
RPF9-U8	14.0	
RP8-RPF2-U10	13.2	
RF6	12.0	
RPF1-RF4	11.2	
RF3	10.0	
RP7-RP1-RP4	9.2	
RP2-RF9	8.0	
RPF3	7.2	
RF8	6.0	
RF7	5.2	
RPF4	4.0	
RF1-RF2-RF5	3.2	
RPF7-RF10	2.0	
P10-RPF10-RPF8-P8	0.0	

Means bracketed by the same line are not significantly different from each other.

Table 4A. A Duncan Multiple Range comparison of stand differences due to soil infestation and seed treatment of Giant Thick-leaved Nobel spinach.

		Seed Treatment										
		6	9	5	2	1	4	7	3	8	10	Means
Soil Infestation	S	76.0	68.0	72.0	63.2	74.0	76.0	78.0	72.0	75.2	65.2	71.6
	F	66.0	58.0	58.0	66.0	59.2	61.2	68.0	74.0	65.2	62.0	63.0
	R	63.2	63.2	62.0	56.0	53.2	55.2	60.0	47.2	64.0	60.0	58.4
	U	66.0	64.0	55.2	62.0	57.2	66.0	57.2	45.2	14.0	13.2	49.6
	PF	43.2	40.0	51.2	46.0	45.2	47.2	46.0	40.0	24.0	24.0	40.8
	P	49.2	58.0	35.2	62.0	66.0	32.0	23.2	21.2	0.0	0.0	34.4
	RP	24.0	20.0	21.2	8.0	9.2	9.2	9.2	21.2	13.2	15.2	14.8
	RPF	15.2	14.0	25.2	13.2	11.2	4.0	2.0	7.2	0.0	0.0	9.2
	RF	12.0	8.0	3.2	3.2	3.2	11.2	5.2	10.2	6.0	2.0	6.4
Means		46.0	43.6	42.4	42.0	41.6	40.0	38.8	37.6	29.2	26.8	

Means bracketed by the same line are not significantly different from each other.

<u>Seed Treatments</u>	<u>Soil Infestations</u>
1. Dexon.	R-- <u>Rhizoctonia solani</u> .
2. Chemagro B-15080.	P-- <u>Pythium irregulare</u> .
3. Omadine-copper.	F-- <u>Fusarium</u> sp.
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- "
8. Diamond Alkali 6N49.	U-- Unsteamed.
9. Chemagro B-1843.	S-- Steamed.
10. Control.	

in RF, RP, and RPF soils. Rhizoctonia was apparently less virulent in this experiment than in previous ones. The cause for this was not specifically determined. Compared to the previous experiment, stands in PF soil remained about the same. Post-emergence damping-off was increased considerably in RP and RPF soils and both pre- and post-emergence damping-off were higher in RF infested soils (Fig. 3). Stand differences due to seed treatment alone remained unchanged but differences due to soil treatment were more pronounced (Table 4A).

Experiment 11 -- Seed treated with the same materials was again planted in freshly infested muck soil in flats. The inoculum was adjusted as described above. After planting the flats were placed on shaded platforms outside the greenhouse. Pre-emergence damping-off was very low in this experiment and post-emergence damping-off was higher than in previous experiments (Fig. 4 and 4A). The distribution of stand averages for the seed and soil treatment combinations is given in Tables 5 and 5A. In this experiment, Fusarium-infested soil was non-virulent and allowed for stands comparable to steamed soil. Two seed treatments, Dexon and Chemagro B-15080, in U, P, and PF soils gave stands comparable to those in steamed soil. Other materials, especially B-1843, which are known to be effective against Pythium constitute most of the second significance group (stand ave. 35.2--65.2%).

Figure 4. Percent stands and total emergence of Giant Thick-Leaved Nobel spinach seed treated with various seed protectants and planted in variously infested muck soils (Experiment 11).

<u>Seed Treatments</u>	<u>Soil Infestations</u>
1. Dexon.	R-- <u>Rhizoctonia solani</u> .
2. Chemagro B-15080.	P-- <u>Pythium irregulare</u> .
3. Omadine-copper.	F-- <u>Fusarium</u> sp.
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- "
8. Diamond Alkali 6N49.	U-- Unsteamed.
9. Chemagro B-1843.	S-- Steamed.
10. Control.	

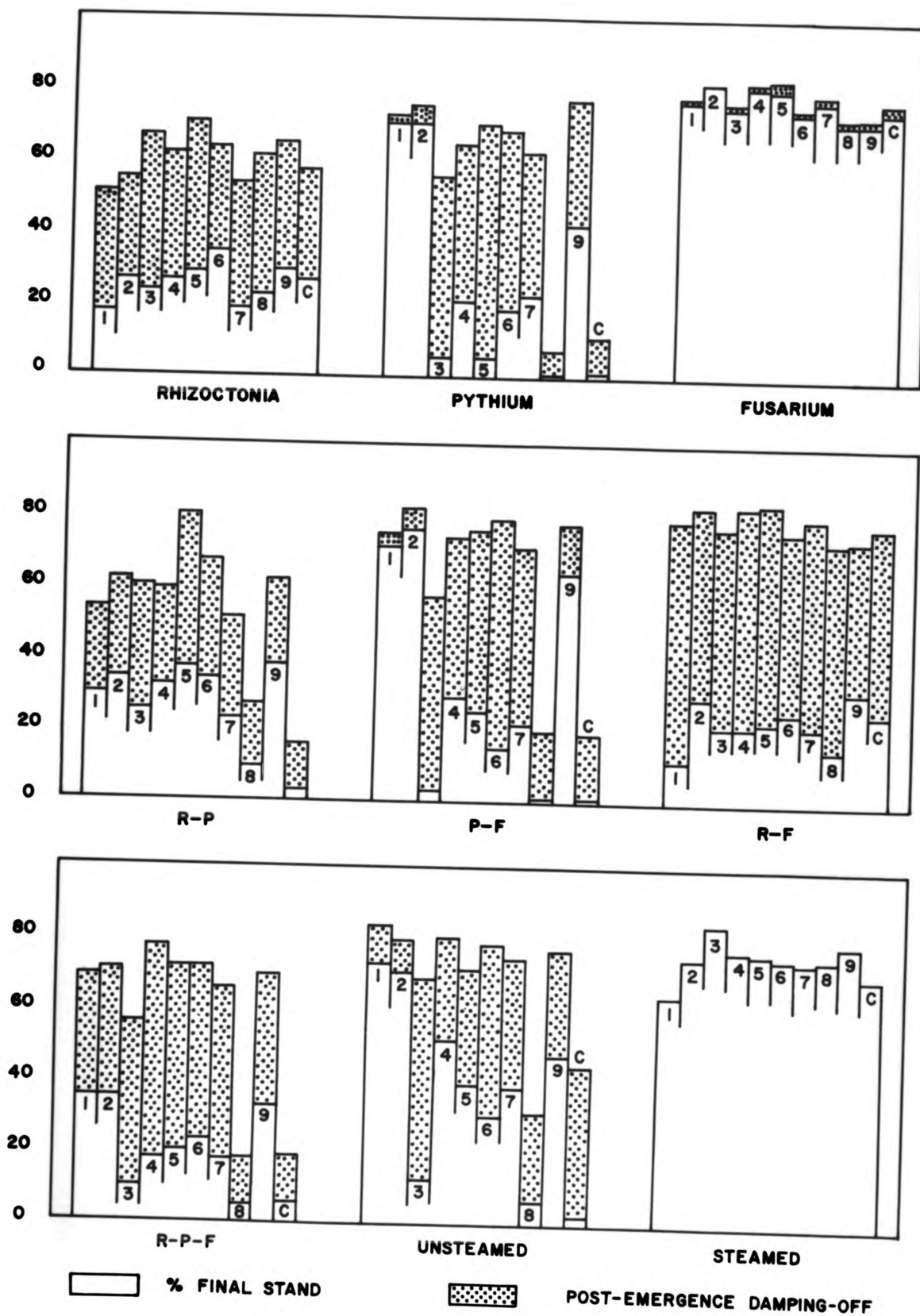


Figure 4.

Figure 4A. Percent stand and total emergence of Giant Thick-Leaved Nobel spinach seed treated with various seed protectants and planted in variously infested muck soils (Experiment 11).

Soil Infestations

R-- Rhizoctonia solani.

P-- Pythium irregulare.

F-- Fusarium sp.

RF- Combination of above.

PF- "

RP- "

RPF- "

U-- Unsteamed.

S-- Steamed.

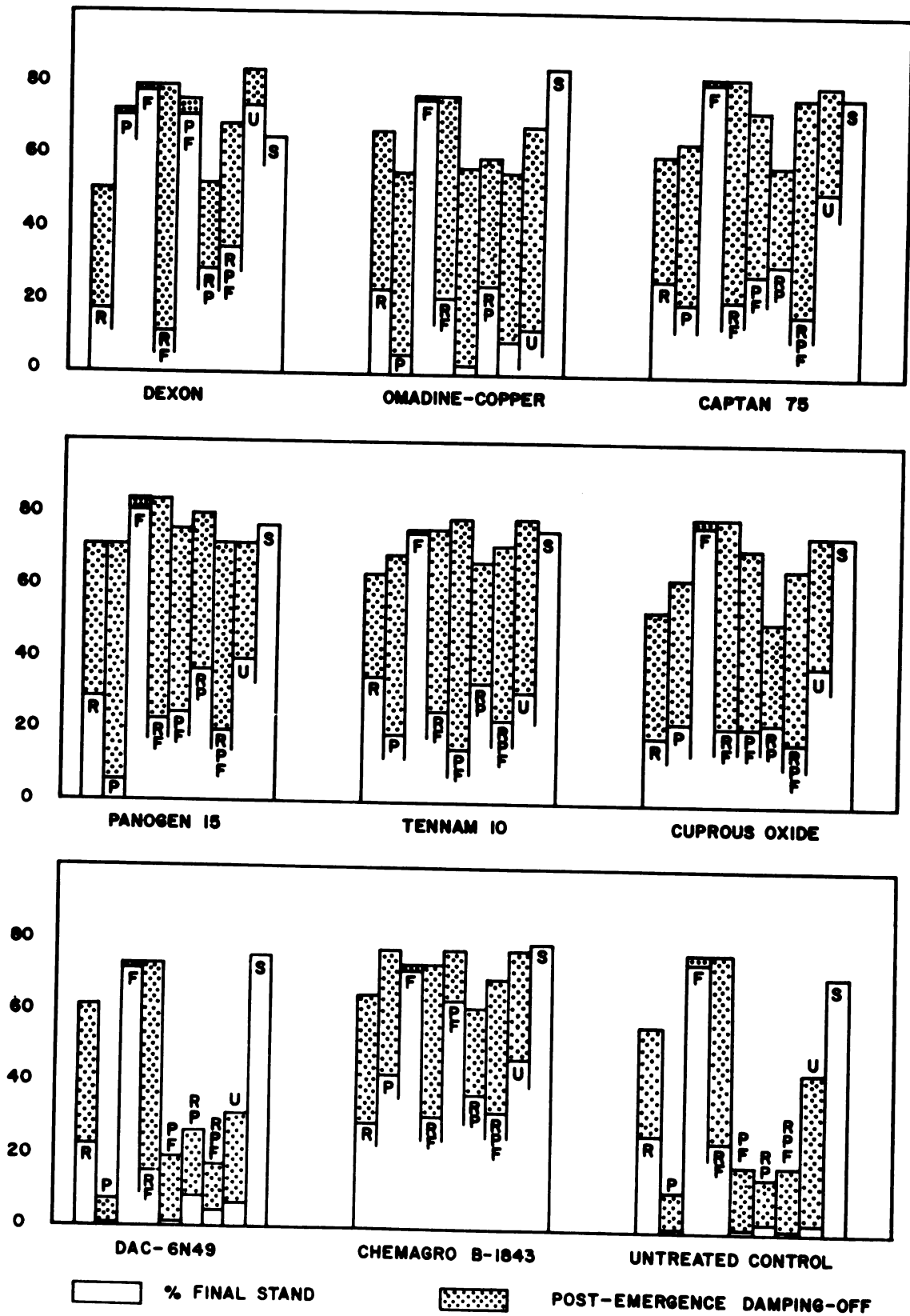


Figure 4A.

Table 5. A Duncan Multiple Range comparison of percent stands of Giant Thick-Leaved Nobel spinach produced by treated seed in variously infested muck soils.

<u>Seed Treatments</u>	<u>Soil Infestations</u>
1. Dexon.	R-- <u>Rhizoctonia solani</u> .
2. Chemagro B-15080.	P-- <u>Pythium irregulare</u> .
3. Omadine-copper.	F-- <u>Fusarium</u> sp.
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- "
8. Diamond Alkali 6N49.	U-- Unsteamed.
9. Chemagro E-1243.	S-- Steamed.
10. Control.	

Table 5. Experiment 11

<u>Treatment</u>	<u>Means</u>
S3	86.2
F2	83.2
F4	82.0
F5	81.2
S9	80.0
S4-F1-F7	78.0
S5	77.2
PF2-F3-S2-S6-S8	76.0
F10-F6-S7	75.2
U1	74.0
F8-F9	72.0
PF1-U2-S10-P1-P2	71.2
S1	65.2
PF9	64.0
U4	52.0
U9	48.0
P9	43.2
U5	40.0
U7	39.2
RP9	38.0
RP5	37.2
RPF1-R6-RPF2	35.2
RP2-RP6	34.0
RPF9	33.2
RP4-RF9	32.0
U6	31.2
R9-RF2	30.0
PF4-R5-RP1	29.2
R2-R4-R10	27.2
RF6-RF10	26.0
RP3-RF5	25.2
R3	24.0
RPF6-P7-RF5-R8-RP7	23.2
RF3-RF4-RF7-PF7	22.0
P4	21.2
RPF5	20.0
P6-R7	19.2
RPF4-R1-RPF7	18.0
RF8	16.0
PF6	15.2
U3	13.2
RF1	12.0
RPF3	10.0
RP8	9.2
U8	7.2
P3-P5	6.0
RPF8	5.2
RP10-U10-PF3	3.2
P10-RPF10	2.0
P8-PF8-PF10	1.2

Means bracketed by the same line are not significantly different from each other.

Table 5A. A Duncan Multiple Range comparison of stand differences due to soil infestation and seed treatment of Giant Thick-leaved Nobel spinach.

		Seed Treatment										
		2	1	9	4	5	6	7	3	8	10	<u>Means</u>
Soil Infestation	S	76.0	65.2	80.0	78.0	77.2	76.0	75.2	85.2	76.0	71.2	76.0
	F	83.2	78.0	72.0	82.0	81.2	75.2	78.0	76.0	72.0	75.2	77.2
	U	71.2	74.0	48.0	52.0	40.0	31.2	39.2	13.2	7.2	3.2	38.0
	PF	76.0	71.2	24.0	29.2	25.2	15.2	22.0	3.2	1.2	1.2	30.4
	P	71.2	71.2	43.2	21.2	6.0	19.2	23.2	6.0	1.2	2.0	26.4
	RP	34.0	29.2	38.0	32.0	37.2	34.0	23.2	25.2	9.2	3.2	26.4
	R	27.2	18.0	30.0	27.2	29.2	35.2	19.2	24.0	23.2	27.2	26.0
	RF	30.0	12.0	32.0	22.0	23.2	26.0	22.0	22.0	20.0	26.0	23.2
	RPF	35.2	35.2	33.2	18.0	20.0	23.2	18.0	10.0	5.2	2.0	20.0
Means		56.0	48.4	48.8	40.0	37.2	37.2	35.6	29.2	23.2	23.2	

Means bracketed by the same line are not significantly different from each other.

Seed Treatments

1. Dexon.
2. Chemagro B-15080.
3. Omadine-copper.
4. Captan 75.
5. Panogen 15.
6. Tennam 10.
7. Cuprous oxide.
8. Diamond Alkali 6N49.
9. Chemagro B-1843.
10. Control.

Soil Infestations

- R-- Rhizoctonia solani.
P-- Pythium irregulare.
F-- Fusarium sp.
RF- Combination of above.
PF- "
PP- "
RPF- "
U-- Unstermed.
S-- Stermed.

The third group (stand ave. 18--35.2%) is the largest and is represented principally by treatments #2, 4, 5, 6, and 7 (B-15080, Captan 75, Panogen 15, Tennam 10 and CuO) in R, RP, RF and RPF soils. Tennam 10, CuO, and Captan 75 appeared most frequently in this group. Untreated controls in U, P, PF, RP, and RPF soils had stands ranging from 1.2 to 3.2% while in R and RF soils the stands were 27.2% and 26.0% respectively. Rhizoctonia was less pathogenic than Pythium, in general, and none of the treatments gave obvious control. In general, pre-emergence damping-off in the flat experiment was not as severe as in either the uniform inoculum concentration or adjusted inoculum concentration pot experiments, but the post-emergence damping-off was greater (Fig. 4). Differentiation between seed treatments was greater than in the pot experiments, but differences due to soil treatment were not as distinct (Table 5A).

Beet Seed Treatment

Experiment 12 -- Nine seed treatments of beets were compared in variously infested soils at the same time, using the standard amounts of inocula (1 plate/flat for single infestation, 1/2 plate of each fungus per flat for double infestation, and 1/3 plate of each fungus per flat for triple infestation). There was a broad distribution of stand averages ranging from 1.0--48.0 seedlings per 25 seedballs for the various combination interactions (Table 6 and 6A).

Table 6. A Duncan Multiple Range comparison of stands of Detroit Dark Red beets produced by treated seed in variously infested muck soils (Experiment 12).

<u>Seed Treatments</u>	<u>Soil Infestations</u>
1. Dexon.	R-- <u>Rhizoctonia solani</u>
2. Chemagro B-15080.	P-- <u>Pythium irregulare</u> .
3. Omadine-copper.	F-- <u>Fusarium</u> sp.
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- "
8. Diamond Alkali 6N49.	U-- Unsteamed.
9. Chemagro B-1843.	S-- Steamed.
10. Control.	

Table 6. Experiment 12.

<u>Treatment</u>	<u>Means</u>
S6	48.0
S9	46.5
S3	46.0
S1	45.7
F5	45.2
RPF8	44.5
F7	44.2
S4-S7	44.0
S2	43.5
S8	43.2
RPF4	42.2
F6-RF4	42.0
F3-F9-PF2	41.5
RPF9	41.2
RF10-PF8-S5-S10	41.0
F1-RPF6	40.7
PF4	40.2
F8-RF6	40.0
F4-RF3-U1-U9	39.2
PF9	39.0
RF1	38.7
RF2	38.5
RF9	38.2
F10	38.0
P4	37.7
P9-U8	37.5
RPF1	37.2
RPF2-PF5	37.0
RP4	36.7
RF8-F2-PF1	36.5
U4	36.2
P1	36.0
RF5-PF6	35.5
RPF5-RP6	35.2
RPF7	35.0
RF7	34.7
P2	33.2
P6	32.7
RP1	31.7
RP5	31.5
PF7	30.7
U6	29.7
RP9	29.0
U2-RP2	28.5
RP8	26.7
RPF3	26.0
P8	23.2
P5	20.7
R1	18.2
R3	18.0
R5	17.5
R6-R8	16.7
RP7	16.5
R4	16.0
U7	15.5
R7	15.2
P7	13.5
R9	12.2
PF3	11.0
U5	10.7
R2-R10	9.7
RPF10	9.0
U3-RP3	5.0
RP10	4.2
PF10	3.0
U10	2.2
P3	2.0
P10	1.0

Means bracketed by the same line are not significantly different from each other.

Table 6A. A Duncan Multiple Range comparison of stand differences due to soil infestation and seed treatment of Detroit Dark Red Beets.

		Seed Treatment										
		4	9	1	6	8	2	5	7	3	10	<u>Means</u>
Soil Infestation	S	44.0	46.5	45.7	48.0	43.2	43.5	41.0	44.0	46.0	41.0	44.3
	F	39.2	41.5	40.7	42.0	40.0	36.5	45.2	44.2	41.5	38.0	40.9
	RF	42.0	38.2	38.7	40.0	36.5	38.5	35.5	34.7	39.2	41.0	38.5
	RPF	42.2	41.2	37.2	40.7	44.5	37.0	35.2	35.0	26.0	9.0	34.8
	PF	40.2	39.0	36.5	35.5	41.0	41.5	37.0	30.7	11.0	3.0	31.6
	RP	36.7	29.0	31.7	35.2	26.7	28.5	31.5	16.5	5.0	4.2	24.5
	U	36.2	39.2	39.2	29.7	37.5	28.5	10.7	15.5	5.0	2.2	24.4
	P	37.7	37.5	36.0	32.7	23.2	32.2	20.7	13.5	2.0	1.0	23.7
	R	16.0	12.2	18.2	16.7	16.7	9.7	17.5	15.2	18.0	9.7	15.0
Means		37.2	36.1	36.0	35.6	34.4	33.0	30.5	27.7	21.5	16.6	

Means bracketed by the same line are not significantly different from each other.

<u>Seed Treatments</u>	<u>Soil Infestations</u>
1. Dexon.	R-- <u>Rhizoctonia solani</u> .
2. Chemagro B-15080.	P-- <u>Pythium irregulare</u> .
3. Omadine-copper.	F-- <u>Fusarium</u> sp.
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- "
8. Diamond Alkali 6N49	U-- Unsteamed.
9. Chemagro B-1843	S-- Steamed.
10. Control.	

Non-treated seed averaged between 1.0 and 9.7 surviving seedlings per 25 seedballs in R, P, PF, RP, RPF and U soils and 38.0 or more in steamed soil and soil infested with F or RF. A large group of seed treatment-soil treatment interactions fell in the 37.0--48.0 range of survivors and did not differ significantly from each other. Most of these good stands are represented by the less virulent soil infestations and by the effective seed treatments used in the virulent soils (P, PF, RPF and Unsteamed). Chemagro Dexon, Captan 75, Diamond Alkali 6N49 and Chemagro B-1843, all of which are known to be effective against Pythium, provided good disease control. The combination of Rhizoctonia and Fusarium was less pathogenic to beets in this trial than was Rhizoctonia alone. Stands were nearly the same, regardless of seed treatment, as in the mildly pathogenic Fusarium soil.

The next significance range (26.7--37.0) is composed largely of inoculum types in which Pythium control by seed treatment was decisive in determining the stand. The soil treatments represented in this group are P, PF, RP, RPF and Unsteamed soil. From the relatively high stand averages of the Pythium-effective materials in unsteamed muck, it seems reasonable to assume that Pythium is a virulent component in this soil. The Fusarium inoculum used in this experiment appeared to be relatively non-virulent and the seed treatments in Rhizoctonia-infested soil allowed a stand average significantly below most stands

in the P, PF, RP, RPF, U group. The evidence, therefore, suggests that Pythium control was the factor determining stands in this group. The third group, ranging in stand from 9.0--26.0 is represented mainly by seed treatments in Rhizoctonia-infested soil and those treatments which were less effective in Pythium-infested soil.

Experiment 13 -- Because of the interesting "protective" effect of the Rhizoctonia-Fusarium combination observed in the beet experiment above, it was decided to replant in the same soil for confirmation. Parallel results were obtained even though there were no statistically significant differences due to the interaction of seed treatment and soil infestation. There were significant differences between soil treatment averages (Table 7). S, F, and RF infested soils were again represented in the higher stand averages. Although Rhizoctonia soil was significantly different from other types, it produced less severe damping-off and more nearly resembled the RF soil than at the time of the first planting (Fig. 5). Soils infested at least in part with Pythium (RP, PF, P, RPF, U) were most severe and were nearly alike in respect to stand averages. As in the previous experiment, the seed treatments with best overall effectiveness were those which were effective against Pythium. Differences due to seed treatment, soil treatment, and their interactions were obscured by replanting in the same infested soil (Table 6A and 7).

Table 7. Surviving seedlings of Detroit Dark Red Beets in variously infested muck soils after treatment with seed protectants.

		Seed Treatment										
		1	2	9	4	6	8	5	7	3	10	<u>Means.</u>
Soil Infestation	S	43.0	37.7	40.2	45.0	42.0	42.5	37.7	42.2	42.5	35.7	41.0
	F	38.5	39.0	41.0	40.7	41.2	40.2	40.2	40.2	40.5	38.5	40.1
	RF	39.0	38.0	37.2	39.2	43.2	36.5	38.0	35.5	40.7	38.0	38.6
	R	32.5	39.5	20.7	28.2	30.2	27.0	37.0	28.7	24.2	22.7	29.1
	RP	37.5	37.7	30.2	30.7	18.2	30.2	18.2	12.0	8.5	3.0	22.7
	PF	36.2	32.5	35.5	27.5	27.2	26.7	15.7	18.0	2.7	3.2	22.3
	U	28.5	32.2	26.2	24.0	23.2	24.5	19.0	19.2	9.0	5.5	21.2
	RPF	32.5	24.0	31.2	25.7	26.2	16.5	12.7	12.2	2.2	1.2	18.6
	P	40.0	33.0	39.2	19.0	13.7	13.7	5.0	7.7	.7	1.7	17.4
Means		36.4	34.6	33.5	31.2	29.6	28.2	24.9	24.0	19.0	16.6	

Means bracketed by the same line are not significantly different from each other.

<u>Seed Treatments</u>	<u>Soil Infestations</u>
1. Dexon.	R-- <u>Rhizoctonia solani.</u>
2. Chemagro B-15080.	P-- <u>Pythium irregulare.</u>
3. Omadine-copper.	F-- <u>Fusarium sp.</u>
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- "
8. Diamond Alkali 6N49.	U-- Unsteamed.
9. Chemagro B-1843.	S-- Steamed.
10. Control.	

Figure 5. Stand and total emergence of Detroit Dark Red beets treated with various seed protectants and planted in variously infested muck soils (Experiment 13).

<u>Seed Treatments</u>	<u>Soil Infestations</u>
1. Dexon.	R-- <u>Rhizoctonia solani</u> .
2. Chemagro B-15080.	P-- <u>Pythium irregulare</u> .
3. Omadine-copper.	F-- <u>Fusarium</u> sp.
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- "
8. Diamond Alkali 6N49.	U-- Unsteamed.
9. Chemagro B-1843.	S-- Steamed.
10. Control.	

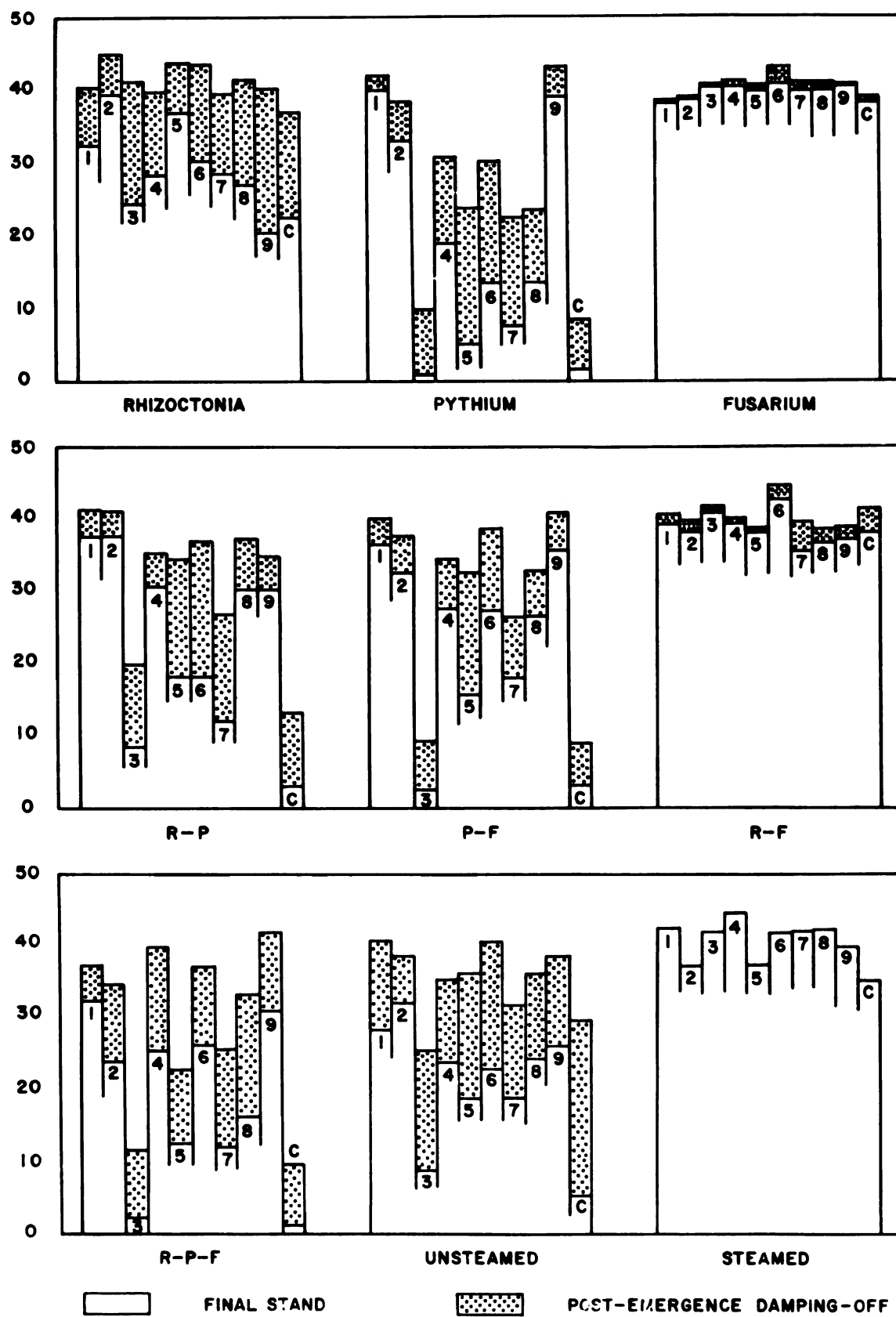


Figure 5.

Experiment 14 -- The beet experiment was repeated in freshly infested muck soil. Inoculum was adjusted, as in the spinach experiments, to provide the same concentration of each component in all flats. The highest average stands were obtained in steamed soil and Fusarium-infested soil (Table 8 and 8A). Certain Pythium-effective treatments produced stands in unsteamed soil comparable to those in steamed soil. The Fusarium proved to be more virulent in this experiment than before and significant differences in stand averages between seed treatments and between Fusarium-infested and steamed soil were obtained.

The first range of significance (40.8--48.8) included most of the seed treatments used in steamed soil. The second range (32.0--39.0) represents the seed treatments in Fusarium-infested soil. Chemagro B-15080, Diamond Alkali 6N49, and Panogen 15, which were moderately effective in Pythium-infested soils, allowed good stands in the range 22.0--29.8. The next significance group (12.0--18.5) was represented by Dexon, Tennam 10, and Chemagro B-1843 in PF soil and CuO in unsteamed soil. The significance group (1.75--9.75) included most of the seed treatments used in R or P soils and treatments # 2, 3, 4, 5, 7, and 8 (Chemagro B-15080, Omadine-copper, Captan 75, Panogen 15, CuO, and Diamond Alkali 6N49) in PF soil.

The lowest range (0--1.75) was represented mostly by the remaining seed treatments in R and P soils as well as nearly all materials used in RF, RP, and RPF soils.

Table 8. A Duncan Multiple Range comparison of stands of Detroit Dark Red beets produced by **treated** seed in variously infested muck soils (Experiment 14).

<u>Seed Treatments</u>	<u>Soil Infestations</u>
1. Dexon.	R-- <u>Rhizoctonia solani</u> .
2. Chemagro B-15080.	P-- <u>Pythium irregulare</u> .
3. Omadine-copper.	F-- <u>Fusarium</u> sp.
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- "
8. Diamond Alkali 6N49.	U-- Unsteamed.
9. Chemagro B-1843.	S-- Steamed.
10. Control.	

Table 8. Experiment 14.

<u>Treatment</u>	<u>Means</u>
S5	48.8
S6-S9	43.5
S2	42.2
S1	42.0
S3	41.0
S8-U4	40.8
S10	39.0
S4-F4	38.8
S7	38.0
F9	37.5
U9	36.5
F3	36.0
F7	35.5
U1	34.8
F2	33.8
F5-F6	32.2
U6	32.0
F8	30.3
U2	29.8
F10	27.5
F1	27.3
U5-U8	22.0
PF1	18.5
PF6	15.5
U7-PF9	12.0
PF5	9.75
PF4	9.25
PF2	8.75
PF7	8.50
PF8	6.25
R5-RF5-P1-P2	6.00
P6	4.75
U3-RPF5	4.50
R3	3.00
RP5	2.75
R6-P9	2.50
RF6-R4-R7	2.25
P4-R10	2.00
PF3-RPF6	1.75
R8-R9	1.50
RF4-RPF9-R1-P5-P7	1.25
P3-RF9-RF10-R2-RP2-U10	1.00
RP7-PF10-RF2-RPF2-RPF4-P10	.75
RF1-RF3-RP3-RP4-P8-RPF7-RP9	.50
RF7-RP1-RP8-RPF1-RPF3-RPF10	.25
RP6-RP10-RPF8-RF8	.00

Means bracketed by the same line are not significantly different from each other.

Table 8A. Surviving seedlings of Detroit Dark Red Beets in variously infested muck soils after treatment with seed protectants.

		Seed Treatment										<u>Means</u>
		9	4	6	5	1	2	8	7	3	10	
Soil Infestation	S	43.5	38.8	43.5	48.8	42.0	42.2	40.8	38.0	41.0	39.0	41.7
	F	37.5	38.8	32.2	32.2	27.3	33.8	30.3	35.5	36.0	27.5	33.1
	U	36.5	40.8	32.0	22.0	34.8	29.8	22.0	12.0	4.5	1.0	23.5
	PF	12.0	9.3	15.5	9.8	18.5	8.8	6.3	8.5	1.8	.8	9.1
	P	2.5	2.0	4.8	1.3	6.0	6.0	.5	1.3	1.0	.8	2.6
	R	1.5	2.3	2.5	6.0	1.3	1.0	1.5	2.3	3.0	2.0	2.3
	RF	1.0	1.3	2.3	6.0	.5	.8	0.0	.3	.5	1.0	1.4
	RPF	1.3	.8	1.8	4.5	.3	.8	0.0	.5	.3	.3	1.3
	RP	.5	.5	0.0	2.8	.3	1.0	.3	.8	.5	0.0	.7
Means		15.2	14.9	14.9	14.8	14.6	13.8	11.3	11.0	9.8	8.0	

Means bracketed by the same line are not significantly different from each other.

<u>Seed Treatments</u>	<u>Soil Infestations</u>
1. Dexon.	R-- <u>Rhizoctonia solani</u> .
2. Chemagro B-15080.	P-- <u>Pythium irregulare</u> .
3. Omadine-copper.	F-- <u>Fusarium sp.</u>
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- "
8. Diamond Alkali 6N49.	U-- Unsteamed.
9. Chemagro B-1843.	S-- Steamed.
10. Control.	

Figure 6. Stands and total emergence of Detroit Dark Red beets treated with various seed protectants and planted in variously infested muck soils (Experiment 14).

<u>Seed Treatments</u>	<u>Soil Infestations</u>
1. Dexon.	R-- <u>Rhizoctonia solani</u> .
2. Chemagro B-15080.	P-- <u>Pythium irregulare</u> .
3. Omadine-copper.	F-- <u>Fusarium</u> sp.
4. Captan 75.	RF- Combination of above.
5. Panogen 15.	PF- "
6. Tennam 10.	RP- "
7. Cuprous oxide.	RPF- "
8. Diamond Alkali 6N49.	U-- Unsteamed.
9. Chemagro B-1843.	S-- Steamed.
10. Control.	

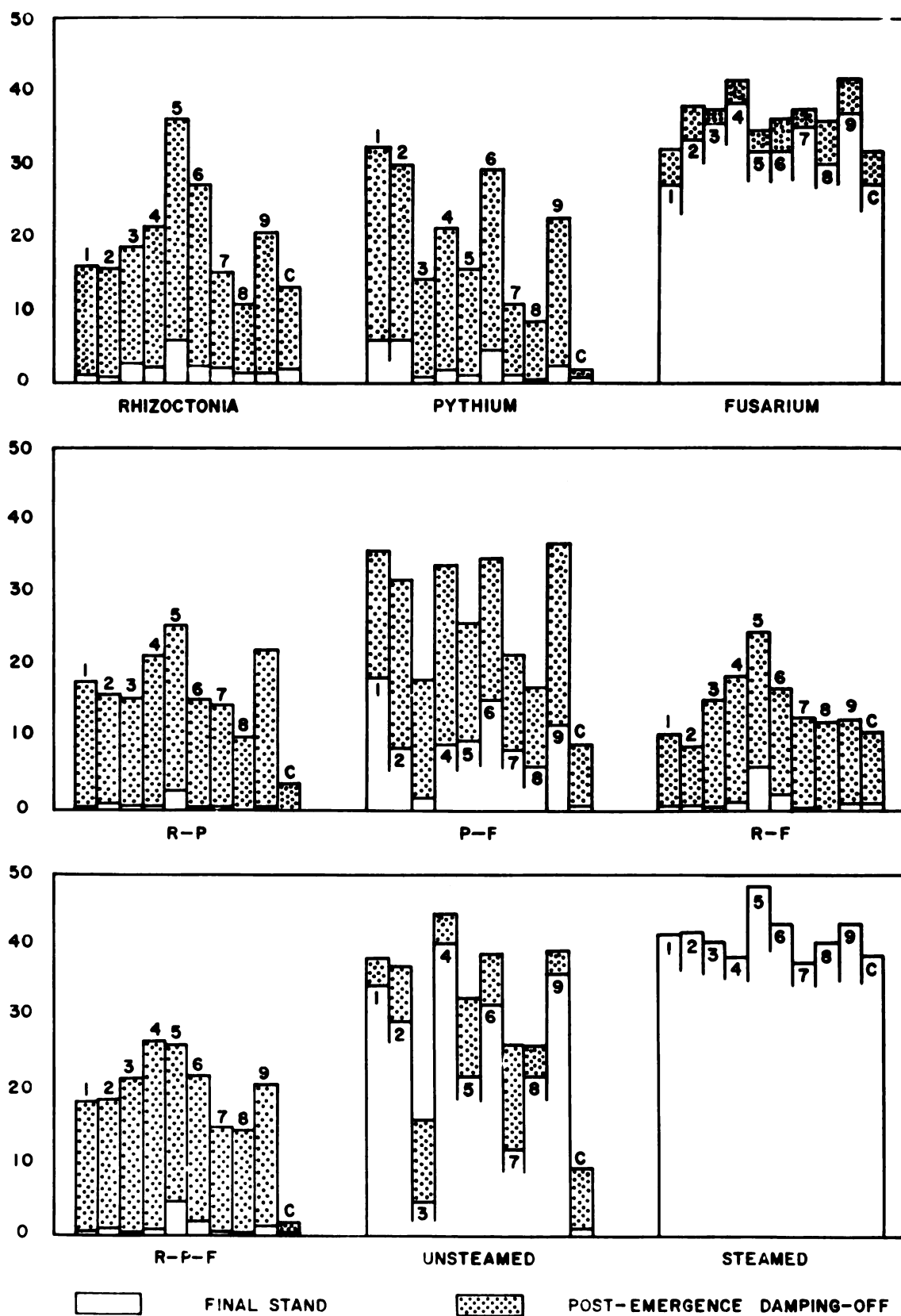


Figure 6.

Stand averages in the artificially infested soils were very low due to the severe pre- and post-emergence damping-off (Fig. 6). In this trial there was no apparent antagonism between Rhizoctonia and Fusarium. The stands in the RF soils were in the lowest range, in contrast to previous experience.

In general, both pre- and post-emergence damping-off was more severe than in the uniform inoculum concentration experiment. However, since the pre- and post-emergence damping-off losses were also high for Rhizoctonia and Pythium alone, it is difficult to attribute the increased losses in the combination infestations to increased inoculum concentration (Fig. 6). Seed treatment, soil treatment and interaction differences were more distinct than those of the previous beet experiments (Table 8A).

DISCUSSION

All of the seed treatments, except Diamond Alkali 6N49, significantly increased stands of spinach in the pathogen-infested soils. In two of the experiments, none of the seed treatments differed significantly from each other with the exception of Diamond Alkali 6N49 which was not effective. In the third experiment of the series, three seed treatment groups were apparent and differed significantly from each other: Dexon, Chemagro B-15080, and B-1843 were in the most effective group; Captan 75, Panogen 15, Tennam 10, and Cuprous oxide were intermediate; and Omadine-copper was least effective.

Except for one experiment where damping-off was less severe, all beet seed treatments gave a definite increase in stands. Dexon, Chemagro B-15080 and B-1843, Captan 75, and Tennam 10 provided best overall protection in all soil infestations. Panogen 15, Diamond Alkali 6N49 and Cuprous oxide were also effective seed protectants in some of the infested soils.

None of the seed treatments protected spinach or beet seed effectively in Rhizoctonia-infested soil. There were no interaction differences in Fusarium-infested soil, except in Experiment 14 where Captan 75, Chemagro B-1843 and Omadine-copper provided protection against damping-off of beets. There were also no significant differences due to the interaction of seed and soil treatments in

Rhizoctonia-Fusarium infested soil. Dexon, Chemagro B-15080 and B-1843, Captan 75, Panogen 15 and Cuprous oxide significantly increased stands of both spinach and beets in P, PF, RP, RPF and Unsteamed soils, all of which contained pathogenic Pythium. Tennam 10 provided protection for beets but not for spinach in PF infested soil. Diamond Alkali 6N49 also increased beet stands significantly in one experiment with RP and RPF soils. Omadine-copper was only moderately effective as a spinach and beet seed treatment in these soils.

Comparison of spinach stands (average of all treatments in a given soil infestation) showed that RF, RP, and RPF soils were not significantly different from each other (Tables 3, 4A, and 5A). The P and PF soils were also essentially alike in all three experiments. Overall stand averages (average of all seed treatments) in Rhizoctonia-infested soil were comparable to those in P, RP, RF, and RPF soils in two of the experiments but the stand average was distinctly different from that group in Experiment 10. Damping-off by Rhizoctonia in the latter experiment was much less severe and stands were comparable to those in Fusarium-infested soil.

No consistent relationships between the various soil infestations could be determined in the case of beet seed treatments, except for Rhizoctonia which was consistently different from other infestation types. In two of the three beet experiments, P, RP and U soils had essentially

the same stand averages for all treatments. Stands in F soil were similar to steamed soil as in the spinach experiments, and F was comparable to RF-infested soil. This latter is in contrast to spinach where no protective effect of Fusarium against Rhizoctonia infestation was seen.

Stand averages in unsteamed soil were comparable to those in P, RP, PF, and RPF with treated beets and to R, PF, P, and RP with treated spinach seeds. Although difficult to demonstrate conclusively from this type of comparison, it appears that Pythium was the most pathogenic component among the damping-off organisms in the unsteamed muck soil used in these trials.

Increasing the inoculum concentration, as in several of the combination infestation experiments, increased the amount of pre- and post-emergence damping-off to some extent. The principle result of this increase, however, was the sharpening of differences due to soil treatment, seed treatment or their interactions. Soil treatment differences were more pronounced (Table 4A) in the spinach pot experiment; seed treatment differences were more distinct in the spinach flat experiment; and differences due to seed and soil treatment interactions were significant in both experiments as compared to the standard inoculum concentration experiment (Expt. 9) where no significant interaction differences occurred. Replanting of treated beet seed in artificially infested soils (standard inoculum concentration) which had been used for a previous test

tended to obscure differences due to soil treatment, seed treatment, and their interactions.

The sharpest distinctions between seed treatment, soil treatment, and seed and soil treatment interactions were obtained with beets as test plants in soil in which the inoculum concentration of each pathogen was 1 plate per flat, whether used alone or in combination with other organisms. Under these conditions beets were apparently more satisfactory than spinach as test plants.

An interesting observation was made in the preliminary experiments with treated spinach seed and in subsequent experiments with treated beet seed in Fusarium and Rhizoctonia-Fusarium infested soils. Stand averages in the F and RF soils were essentially the same and were similar to those in steamed soil. The absence of significant differences between seed treatments in Fusarium-infested soil was probably due to the low virulence of the Fusarium species used in these experiments. This species, which was isolated from a damped-off Michelite bean seedling, appeared to be quite virulent in laboratory and greenhouse tests with spinach in mineral soil. In the experiments with muck soil, this Fusarium species failed to show a high level of virulence. Sanford (55) has shown that Rhizoctonia was less virulent in steamed soil than in naturally infested soil. This was thought to be the result of conditions which favored marked vegetative growth of the pathogen and thus had a tendency to depress its virulence. Perhaps this

phenomenon may also occur with Fusarium species since they are generally regarded as vigorous saprophytes.

It is not known, why the stand averages in the RF infested soil were so high. Since Rhizoctonia was highly virulent, one might expect stand averages in RF soil to be intermediate between R and F or nearer to R alone, if a simple physical competition for space were responsible. A possible explanation may lie in the initial inoculum concentration of each of the components in the combination infested soil. Where the "protective" effect was observed only 1/2 or 1/3 as much of each component was seeded into the steamed muck soil for combination infestations as in later trials when an attempt was made to achieve more uniformity. This view is supported by the increased damping-off in the combination infested flats when the inoculum concentration was increased, and by the absence of the "protective" effect with both spinach and beets.

Another possibility is that there may have been some antagonistic or competitive reaction between R and F which reduced the virulence of Rhizoctonia. However, no antagonism was apparent when the cultures were grown together in vitro. The situation encountered here may be similar to that described by Ho (30) in which two pathogens, Pythium debaryanum and Gibberella saubenetii, in artificially infested soil exhibited an additive effect on disease severity with corn while a combination of a pathogen and

a non-pathogenic fungus, Gibberella saubenetti and Aspergillus niger (or Penicillium oxalicum) resulted in an inhibitive effect and the disease was less severe than with the pathogen alone.

In retrospect, the study of the interactions between soil fungi in various combinations would have been more meaningful had a more virulent strain of Fusarium been used. Any further study should involve the use of a pathogenic Fusarium and perhaps other pathogenic or saprophytic fungi (Verticillium, Phoma, Botrytis, Trichoderma, Penicillium or others). Evidence indicates that not only the fungus microflora but the actinomycete and bacterial populations in the soil have a definite influence on the pathogenicity or survival of organisms in the soil (28, 29, 63).

The use of other materials with known specific activity such as PCNB (pentachloronitrobenzene), would have aided in evaluation of interactions between soil fungi. By manipulating environmental conditions and using specific seed treatments, it might be possible to correlate stands or rate of damping-off with a particular fungus pathogen or complex. For example, in this study a comparison of stands and damping-off in unsteamed muck soil and soils containing Pythium seemed to indicate that Pythium caused the most damage in unsteamed soil and that damping-off was most effectively controlled by chemicals which were highly

effective against Pythium. A rapid evaluation by this process would afford a basis for selecting fungicides or other measures that might be effective for disease control. The ideal goal, of course, would be to have some basis for selecting specific fungicides which would be effective in controlling a pathogen without radically disturbing the microfloral balance and soil fertility.

LITERATURE CITED

1. Alexander, L.J., H.C. Young and C.M. Kiger. 1931. Causes and control of damping-off of tomato seedlings. Ohio Agr. Exp. Sta. Bull 496.
2. Atkinson, G.F. 1895. Damping-off. N.Y. (Cornell) Agr. Exp. Sta. Bull. 94: 231-272.
3. Atkinson, G.F. 1896. Diseases of cotton. U.S.D.A. Office Exp. Stas. Bull. 33: 279-316.
4. Beach, W.S. 1946. Pathogenic and physiogenic damping-off. Soil Sci. 61: 37-46.
5. Beach, W.S. 1949. The effects of excess solutes, temperature and moisture upon damping-off. Penn. Agr. Exp. Sta. Bull. 509.
6. Blair, I.D. 1943. Behavior of the fungus Rhizoctonia solani Kühn in the soil. Ann. Appl. Biol. 30: 118-127.
7. Buchholtz, W.F. 1944. The sequence of infection of a seedling stand of sugar beets by Pythium debaryanum and Aphanomyces cochlioides. Phytopathology 34: 490-496.
8. Clayton, E.E. 1928. Increasing stands from vegetable seeds by seed treatment. N.Y. (Geneva) Agr. Exp. Sta. Bull. 554.
9. Clayton, E.E. 1931. Vegetable seed treatment with special reference to the use of hot water and organic mercurials. N.Y. (Geneva) Agr. Exp. Sta. Tech. Bull. 183.
10. Clayton, E.E. 1931. Vegetable seed treatment. N.Y. (Geneva) Agr. Exp. Sta. Bull. 597.
11. Cook, H.T., and J.A. Callenbach. 1935. A comparison of zinc seed treatments for control of seed and seedling rots of spinach. Proc. Va. Acad. Sci. 1934-1935: 36.
12. Cook, H.T., and J.A. Callenbach. 1935. Spinach seed treatments in Virginia. Phytopathology 25: 12.
13. Cook, H.T., and J.A. Callenbach. 1935. Spinach seed treatment. Va. Truck Exp. Sta. Bull 87: 1213-1233.

14. Cook, H.T. 1937. Spinach and cabbage seed treatment. Va. Truck Exp. Sta. Bull. 96: 1491-1510.
15. Cook, H.T., T.J. Nugent, G.K. Parris and R.P. Porter. 1947. Fusarium wilt of spinach and the development of a wilt resistant variety. Va. Truck Exp. Sta. Bull. 110: 1810-1820.
16. Coons, G.H., and D. Stewart. 1927. Prevention of seedling disease of sugar beets. Phytopathology 17: 259-296.
17. Davis, R.A., and D.J. deZeeuw. 1959. Interactions of damping-off fungi on red beets. Phytopathology 49: 537.
18. deZeeuw, D.J. 1954. Fungicide treatment of table beet and spinach for the prevention of damping-off. Mich. Agr. Exp. Sta. Quarterly Bull. 37: 105-118.
19. deZeeuw, D.J., and R.A. Davis. 1957. Comparative effectiveness of 4 classes of seed treatment materials on peas, beans, and cucumbers. Phytopathology 47: 7.
20. Dickson, J.G. 1923. Influence of soil temperature and moisture on the development of the seedling blight of wheat and corn caused by Gibberella saubinetii. Jour. Agr. Res. 23: 837-870.
21. Duggar, B.M., and F.C. Stewart. 1901. The sterile fungus Rhizoctonia as a cause of plant disease in America. N.Y. (Cornell) Agr. Exp. Sta. Bull. 186: 50-76.
22. Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11: 1--42.
23. Ellis, D.E., and R.S. Cox. 1951. The etiology and control of lettuce damping-off. N. Carolina Agr. Exp. Sta. Tech. Bull. 94.
24. Esmarch, F. 1942. Der Wurzelbrand der Ruben. (Root rot of beets). Kranke Pflanze 19 (1/8) 19-23. Biol. Abs. 17: 19180.
25. Gould, C.J. 1943. Vegetable seed treatment tests in western Washington. Plant Disease Reprtr 27: 594-601.
26. Graham, K.M. 1955. Seedling blight, a Fusarial disease of asparagus. Can. Jour. Bot. 33: 374-399.

27. Graham, J.H., V.G. Sprague and R.R. Robinson. 1957. Damping-off of Ladino clover and Lespedeza as affected by soil moisture and temperature. *Phytopathology* 47: 182-185.
28. Gregory, K.F., O.N. Allen, A.J. Riker and W.H. Peterson. 1952. Antibiotics and antagonistic micro-organisms as control agents against damping-off of alfalfa. *Phytopathology* 42: 613-622.
29. Griffeths, E., and M.A. Siddiqi. 1958. Microbial antagonism of Fusarium culmorum. *Nature* 182: 956.
30. Ho, Wen-Chun. 1944. Soil inhabiting fungi attacking the roots of maize. *Iowa Agr. Exp. Sta. Res. Bull.* 332: 403-446.
31. Horsfall, J.G. 1930. Combating damping-off of tomatoes by seed treatment. N.Y. (Geneva) *Agr. Exp. Sta. Bull.* 586.
32. Horsfall, J.G. 1932. Dusting tomato seed with copper sulfate monohydrate for combating damping-off. N.Y. (Geneva) *Agr. Exp. Sta. Tech. Bull.* 198.
33. Horsfall, J.G. 1932. Red oxide of copper as a dust fungicide for combating damping-off by seed treatment. N.Y. (Geneva) *Agr. Exp. Sta. Bull.* 615.
34. Humphrey, J.E. 1891. Damping-off. *Mass. Agr. Exp. Sta. (Amhurst) 8th. Ann. Rept.* : 220-221.
35. Hungerford, C.W. 1923. A *Fusarium* wilt of spinach. *Phytopathology* 13: 205-209.
36. Jacks, H. 1954. Seed disinfection. X. Effect of seed protectants on emergence of spinach. *New Zealand Jour. Sci. and Tech. Ser. A* 36: 129-133.
37. Jones, L.R., J. Johnson and J.G. Dickson. 1926. The Wisconsin studies upon the relation of soil temperature to plant disease. *Wis. Agr. Exp. Sta. Res. Bull.* 71.
38. Jones, L.K. 1927. Studies of the nature and control of blight, leaf and pod spot, and footrot of peas caused by species of Ascochyta. N.Y. (Geneva) *Agr. Exp. Sta. Bull.* 547.
39. Kadow, K.J., and H.W. Anderson. 1937. Damping-off control: an evaluation of seed and soil treatments. *Ill. Agr. Exp. Sta. Bull.* 439: 290-348.

40. Kotowski, F. 1926. Temperature relations to germination of vegetable seed. Amer. Soc. Hort. Sci. Proc. 23: 176-184.
41. Leach, L.D. 1940. Influence of the pathogen, environment, and host response on the efficacy of seed treatment with sugar beets and some vegetable crops. Phytopathology 30: 788.
42. Leach, L.D. 1943. Ratio of velocity of seedling germination to fungus growth rate as a measure of pre-emergence damping off. Phytopathology 33: 7.
43. Leach, L.D. 1944. Beet seed treatments. Plant Disease Reprtr. Supp. 145: 31-35.
44. Leach, L.D. 1946. Garden beet seed treatments. Plant Disease Reprtr. Supp. 161: 6-10.
45. Leach, L.D. 1947. Growth rates of host and pathogen as factors determining the severity of pre-emergence damping-off. Jour. Agr. Res. 75: 161-179.
46. Leach, L.D. 1949. Use of soluble and wettable fungicides for seed treatment. Phytopathology 39: 12.
47. Leukel, R.W. 1948. Recent developments in seed treatment. Bot. Rev. 14: 235-239.
48. Martin, W.J., and J.G. Atkins. 1954. Results of vegetable seed treatments in Louisiana. Plant Disease Reprtr. 38: 348-349.
49. Natti, J.J., and W.T. Schroeder. 1955. Protectant seed treatments for vegetable processing crops. N.Y. (Cornell) Agr. Exp. Sta. Bull. 771.
50. Peltier, G.L., 1916. Parasitic Rhizoctonias in America. Ill. Agr. Exp. Sta. Bull. 189: 279-390.
51. Pirone, P.P., A.G. Newhall, W.W. Stuart, J.G. Horsfall, and A.L. Harrison. 1933. Copper seed treatments for the control of damping-off of spinach. N.Y. (Cornell) Agr. Exp. Sta. Bull. 566.
52. Roth, L.F., and A.J. Riker. 1943. Life history and distribution of Pythium and Rhizoctonia in relation to damping-off of red pine seedlings. Jour. Agr. Res. 67: 129-148.
53. Roth, L.F., and A.J. Riker. 1943. Influence of temperature, moisture, and soil reaction on the damping-off of red pine seedlings by Pythium and Rhizoctonia. Jour. Agr. Res. 67: 273-293.

54. Roth, L.F., and A.J. Riker. 1943. Seasonal development in the nursery of damping-off of red pine seedlings caused by Pythium and Rhizoctonia. Jour. Agr. Res. 67: 417-431.
55. Sanford, G.B. 1941. Studies on Rhizoctonia solani Kühn. V. Virulence in steam-sterilized and natural soil. Can. Jour. Res. (C) 19: 1-8.
56. Storey, I.F. 1941. A comparative study of strains of Rhizoctonia solani Kühn with special reference to their parasitism. Ann. Appl. Biol. 28: 219-228.
57. Taylor, C.F., and J.A. Rupert. 1946. A study of vegetable seed protectants. Phytopathology 36: 726-749.
58. Tisdale, W.B., A.N. Brooks and G.R. Townsend. 1945. Dust treatments for vegetable seed. Fla. Agr. Exp. Sta. Bull. 413.
59. Walker, J.C. 1948. Vegetable seed treatment. Bot. Rev. 14: 588-601.
60. Wallen, V.R., and A.J. Skolko. 1953. Treatment of vegetable seed of low germination. Plant Disease Reptr. 37: 66-68.
61. Wallen, V.R. 1953. Treatment of vegetable seed for improved emergence. Plant Disease Reptr. 37: 620-622.
62. Wallen, V.R., M. Angela Wallace and W. Bell. 1955. Response of aged vegetable seed to seed treatment. Plant Disease Reptr. 39: 115-117.
63. Wilson, K.S., and C.L. Porter. 1958. The pathogenicity of Verticillium albo-atrum as affected by muck soil antagonists. Applied Microbiol. 6: 155-159.

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