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Etiology and Control of a Seedling Blight of Flax Caused by Rhizoctonia solani Kühn

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ETIOLOGY AND CONTROL OF A SEEDLING BLIGHT OF FLAX CAUSED BY RHIZOCTONIA SOLANI KÜHN

by

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ETIOLOGY AND CONTROL OF A SEEDLING BLIGHT OF FLAX CAUSED BY RHIZOCTONIA SOLANI KÜHN

This thesis is approved as a creditable, independent investigation by a candidate for the degree, Master of Science, and acceptable as meeting the thesis requirements for this degree; but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser

Head of the Major Department

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INTRODUCTION

A seedling disease has been observed throughout the flax-growing areas of South Dakota for a number of years. The importance of this disease has probably been overlooked, however, because of the sporadic and unpredictable serious outbreaks; but in recent years the disease has reached epiphytotic proportions and hundreds of acres have been destroyed and plowed under. In some fields, stands have been so severely thinned that weeds have become a serious problem and yields of flax have been markedly reduced.

The specific cause of these serious outbreaks of seedling blight in South Dakota was not known. Preliminary observations, however, suggested that it may have been due to Rhizoctonia solani Kühn, one of several pathogens known to cause similar losses in stands of flax.

In the present study, basic information essential to development of control measures for South Dakota has been sought. This paper deals with the identification of the causal fungi, study of the symptoms they induce, conditions which influence the expression of the disease, and investigation of methods of control.

LITERATURE REVIEW

Although the literature concerning Rhizoctonia solani and the disease caused by it on various plants from the time Kühn described the fungus in 1858 is extensive, literature specifically related to the disease on the flax plant is relatively scarce. Brentzel, 1923, (7) apparently was the first to describe this disease of flax as it appeared in the field. Since that time, other workers have also contributed specifically to the study of the seedling disease of flax. Of importance among them are Vanterpool (36, 37), Flor (9), Tervet (32), and Tsiang (33).

Seedling blight of flax is very similar in some respects to the disease caused by R. solani on other plants. For this reason, it seems necessary to give consideration to the pertinent literature pertaining to certain aspects of R. solani on other plants.

Rhizoctonia solani attacks many different plants, both cultivated and wild. Peltier (24), in 1916, compiled a list of 165 species and sub-species of plants which had been reported as being susceptible to R. solani in the United States up to that time. The list involves about 50 families of flowering plants, including dicotyledons, monocotyledons, gymnosperms, and Equisetum. Peltier states, "It is obvious that as long as investigations on this disease are continued, such a list cannot be regarded as complete or final." A review of the more recent literature indicates that many other plant species could be added to this list. It would seem, therefore, that most of the horticultural plants, vegetable and field crops, tree seedlings, and many weeds are susceptible to this fungus.

Numerous strains of R. solani have been acknowledged and several investigators have studied the parasitism of R. solani on a wide host range. Le Clerg (21) has adequately reviewed the literature on the physiologic specialization of R. solani prior to 1934, and Kernkamp et al. (17) have summarized the pertinent work up to 1952. As early as 1916, Peltier (24), in making a comprehensive study of a number of R. solani cultures and their pathogenic effects on a variety of plants, concluded that strains of R. solani obtained from a wide range of hosts of diverse geographical origin can attack the same species of plants and produce the same characteristic symptoms. His studies showed that the virulence of the isolates was variable and that no marked specialization to any particular host could be noted in any of the strains.

Houston (15) categorized R. solani isolates into certain cultural characteristic types which could be correlated with pathogenicity levels. Most workers, however, report that there is as much difference between cultures from the same host as between cultures from different hosts. Tsiang (33), in studying the disease on flax, concluded that isolates from flax varied considerably in pathogenicity levels and certain isolates from other crops were fully as capable of injuring flax seedlings. Furthermore, he found that pathogenicity of strains of R. solani to flax varieties was not specialized, because a strain highly virulent on one variety of flax was usually highly virulent on all varieties. Boosalis (6) reached similar conclusions in studying the parasitism of R. solani on soybeans; and Chen (8), in studying parasitism of R. solani isolates from potatoes, sugar beets, cotton, flax, and sweet clover, found that

they varied widely in their ability to parasitize grasses. Furthermore, he also found that some isolates had a wider host range than others, so that one isolate could attack only two or three hosts, while another could attack all of the hosts.

Ecological studies by Winter (41) showed that R. solani could grow from malt agar inoculum without being influenced by soil properties. Growth of the mycelium was entirely from the nutrient reserve of inoculum, as growth in pure sand moistened with tap water was equally successful as growth in soil. He showed that mycelial growth was related to the amount of inoculum, and concluded that R. solani could develop as a saprophyte only from plant tissues which already had been parasitized. Roth (27), in studying the disease on pine seedlings, observed that R. solani grew freely from diseased tissue into the soil and continued saprophytic development, apparently enhanced by the nutrient supply furnished by the dead seedlings. He also observed that R. solani grew a foot or two from an original infection point and spread both along and across the rows, killing irregular circular patches. Blair (4), on the other hand, demonstrated that R. solani was able to grow for relatively long distances through tubes of unsterilized soil, quite independently of the inoculum from which the growth was initiated.

The persistance of R. solani in the soil has been studied by many workers. In general, they agree that under dry conditions, the mycelium and sclerotia of the organism can persist indefinitely. Peltier (24) kept dried soil cultures of R. solani for three years and viability was not impaired. Gratz (13) found that soil containing R. solani maintained

in an air-dried condition for six months at greenhouse temperatures, caused as severe killing of cabbage seedlings as before drying.

Flor (9) found that the amount of seedling blight obtained in inoculated steamed and unsteamed field soil was diminished in successive monthly plantings of flax. This effect was most pronounced in unsteamed field soil where seedling blight had practically vanished in the third sowing.

The effects of temperature on the growth and pathogenicity of isolates of R. solani on various crops have been studied by several workers. Richards (25, 26) was probably one of the first to report on the effect of soil temperature on the incidence and virulence of R. solani. He noted that 18° C. was the optimum temperature for the infection of potatoes, peas, beans, and cotton, and that this temperature corresponded closely to the temperature found most favorable for the optimum growth of potatoes and peas. He considered this relationship coincidental, however, as the temperature requirement for optimum growth of beans and cotton was above the range of temperatures at which most injury of the plants resulted. He concluded that the optimum temperature range for parasitism of various hosts by R. solani is not influenced seriously by the species of hosts attacked and their optimum temperature requirements for growth, but is a condition determined primarily by a fixed physiological characteristic of the pathogen. Leach (20) was able to demonstrate a probable general relationship between temperature and pre-emergence killing of seedlings. He found that the incidence of pre-emergence killing at different temperatures was directly related to the ratio:

"Growth rate of pathogen
Velocity of seedling emergence"

Thus, if the velocity of emergence of a seedling is increased and other factors remain constant, disease severity would be reduced.

Other investigators (6, 18, 27, 30, 38) have noted considerably higher temperatures to be conducive to optimum disease development on cotton, alfalfa, soybeans, sugar beets, and pine. Vasudeva (38), working on the disease of cotton in India, studied the effect of temperature and humidity on disease development under field conditions by covering portions of plots with thatching. The disease development was less severe under the covered areas where the temperatures were lower and the humidity higher, than in the uncovered areas. Vanterpool (36) observed low average temperatures to favor the disease on flax, and Tsiang (33), in temperature tank studies, found that temperatures between 15° and 20° C. were optimum for the parasitism of R. solani isolates on flax. He concluded that soil temperatures for infection were not correlated with the optimum temperatures for growth of the isolates in culture.

Kernkamp et al. (17) have reviewed these temperature studies and have aptly summarized their significance: "These results serve to emphasize the interrelationship of host, strain of the pathogen, and temperature as they affect virulence or susceptibility, and point out that one cannot generalize too broadly regarding the influence of temperature on the pathogenicity of a fungus such as Rhizoctonia solani."

The literature contains many suggestions for control of seedling blight caused by R. solani. Excellent control has been ascribed to

certain chemicals, but their application is limited to greenhouse use or for establishing seedling stands for transplanting. As a result of four years of tests, Flor (10) concluded that little benefit can be expected from treating flax seed. This was confirmed by Greany (14) when uncracked seeds were treated. Moore (23), on the other hand, found increases in yield resulting from seed treatment. Tsiang (33) reported investigations involving five fungicides in greenhouse tests. Few benefits were derived; however, slight improvement of stands using New Improved Ceresan was noted.

Various chemicals and fungicides have been added to the soil in an attempt to control soil-borne organisms on several crop plants (1, 2, 3, 5, 12, 16, 31), but this technique has not been reported for control of R. solani on flax. Bird et al. (3) reported on the use of fungicides mixed with the covering soil at planting time for the control of R. solani on cotton seedlings. They indicated that this method of treatment was at least partially successful in improving stands of cotton in the field, but they found responses to chemicals were different in different types of soil. Strong (31) studied 25 fungicides for their value in controlling R. solani on pine seedlings in greenhouse tests and field experiments. When certain fungicides were mixed with the top five inches of soil, they showed considerable promise. In addition, certain fungicides used as a sprinkle treatment in applications at weekly intervals after seeding, resulted in good control of the seedling disease.

It has been suggested by many authors that soil microflora play

an important part in the development of root rots. Weindling (39) showed that Trichoderma lignorum inhibits the growth of R. solani and parasitizes it. Weindling and Fawcett (40) have shown that T. lignorum possibly could be used for control of damping-off of citrus seedlings because of its antagonistic action. Tsiang (33), in studying the effect of soil microflora on the development of seedling blight of flax, reported signs of antagonism toward R. solani isolates by other soil organisms. He observed that destruction was not as severe when isolates of R. solani were added to natural soil as when they were added to steamed soil. He also studied the parasitic action of T. lignorum on R. solani and reported experiments testing T. lignorum as a control for seedling blight of flax. He noted general increases in flax stands with this treatment, but responses were different with different races of R. solani.

Interesting side effects have been noted by various investigators from the use of soil fumigants for the control of soil-borne pathogens. Vaartaja (34) noted that tri-nitrotoluene exerts a fungistatic effect primarily due to the peculiarly encouraged growth of the antagonistic T. lignorum. Smith (29) observed the dominance of T. lignorum after partial sterilization of the soil by Chloropicrin.

The fact that soil-borne fungi may be of greater importance than seed-borne fungi in causing poor stands of flax has been emphasized recently by Millikan (22). This is particularly true where a high percentage of cracked or broken seeds occurs in the sample (19, 22, 28). There is general agreement in the literature that the application of

fungicides to cracked seed before sowing will improve stands in soil infested with seedling blight pathogens. Millikan (22) treated flax seed with several fungicide dusts and obtained significant improvement of stands over the untreated checks, apparently due to control of Pythium spp. in the soil. Schuster (28) found that species of Alternaria and Penicillium, which were ordinarily non-pathogenic to flax, could produce greater reduction in stands from cracked seed, whereas sound seed was not affected.

A few observations have been made regarding varietal resistance of flax to R. solani. Vanterpool (37) did not find a high degree of varietal resistance to R. solani but, of the varieties tested, Dakota appeared to be superior and Redwing was inferior. Tsiang (33), on the other hand, reported Redwing to have somewhat more resistance than any of the other commonly grown flax varieties which he tested.

MATERIALS AND METHODS

A number of field surveys were made during the spring of 1956 to ascertain the prevalence and severity of seedling blight of flax in northeastern South Dakota. Flax fields were examined at five-mile intervals in areas where fields were abundant and less frequently where fields were greater distances apart. Diseased flax seedlings were collected and placed in small plastic bags, labelled, and brought to the laboratory for isolation of the causal organisms.

In the laboratory, sections of root and hypocotyl were removed and washed in running water for approximately two hours. The segments were then blotted between paper towels and small pieces of the tissues were plated on a two per cent potato-dextrose agar in Petri dishes. The plates were left at room temperature, which ranged between 22° and 25° C. Hyphal tip transfers, made from the fungus originating from the diseased tissues, were used to establish pure cultures and to facilitate identification.

Greenhouse experiments reported were conducted during the period of January, 1956, to May, 1957, in automatic temperature-controlled greenhouses. Three and four-inch clay pots were used in these experiments. Steamed soil was prepared by autoclaving moist field soil at 15 pounds steam pressure for four hours. Field soil used in all greenhouse experiments was obtained from one of the Agronomy Farm land ranges on which flax stand losses of over 50 per cent due to seedling blight occurred in 1956. The soil was collected at random from the furrow slice after the land was plowed in the fall. It was stored in cloth sacks at 8° C.

Marine flax seed was used for all experiments except where indicated. The germination was 93 per cent. Eighteen per cent of the seed was cracked. Twenty-five seeds were planted in each pot of soil. Seedling counts were usually made twice. The first count was made following emergence when the flax was in the two-leaf stage, and the second count was made when the plants were in the six to eight-leaf stage.

When cracked and uncracked seed was required, the seed was separated under a stereoscopic microscope. Cracked seed contained hairline cracks, breaks, or splits in the seed coat. Uncracked seed had no visible injury on the seed coat. Broken seed was discarded.

Sixteen strains of flax were selected at random from the world collection of flax provided by the Cereal Crops Branch of the United States Department of Agriculture. Commercial varieties were obtained from the Agronomy Department. These varieties and strains were tested for their reaction to Rhizoctonia solani.

The inoculum used in steamed soil in greenhouse experiments was prepared by two methods. In one, cultures were increased on a sterile soil medium containing five per cent cornmeal in 300 milliliter Erlenmeyer flasks. When the substrate was completely colonized, usually in about two weeks, the inoculum was removed from the flasks and added to steamed soil. The inoculum was placed either over the planted seed or throughout the entire lot of steamed soil. In the second method, the pathogen was cultured on a sterilized liquid potato-dextrose medium in 300 milliliter Erlenmeyer flasks for five days and a weighed

portion of the wet mycelial mat was then removed and macerated in 100 milliliters of distilled water for one minute in a Waring Blendor. The suspension was then diluted as required and poured uniformly over the soil on which flax seeds were to be planted.

In determining the growth rate of various isolates of R. solani at different temperatures, a uniform source of potato-dextrose agar was used for the medium. Agar discs, three millimeters in diameter, of vigorously growing mycelium were transferred from five-day old cultures to Petri plates containing 20 milliliters of this medium. Three replications of each culture were placed at each of seven temperatures, ranging from 6° to 40° C. After 24 hours, the periphery of growth was outlined on the bottom of each plate with wax pencil. This line was used as the starting point for measuring the growth rate of the isolates in terms of millimeters per 24 hours.

The original data summarized here are available in the Department of Plant Pathology. Statistical analysis of them is in progress, and will be used in an anticipated publication. The conclusions drawn here are based on the analyses to the extent already completed.

EXPERIMENTAL RESULTS

Symptoms of Flax Seedling Blight

Rhizoctonia blight of flax is typically a seedling disease, although plants may be killed at later stages of growth. Although the most conspicuous phase of the disease is the post-emergence killing of seedlings, under certain conditions, the number of plants may be greatly reduced by pre-emergence killing. Usually, the first observable symptoms on newly emerged seedlings are small reddish-tan lesions on the hypocotyl just below the soil surface (Figure 1). These lesions enlarge and spread in both directions so that the cortex of the entire hypocotyl becomes involved in a soft rot (Figures 1 and 2). The plants thus infected become flaccid, and quickly wither and fall to the ground (Figure 3). Often the hypocotyl becomes thread-like following fungus invasion, but the roots usually remain unaffected except for the presence of occasional sclerotia and small, brown, discolored areas which may form on the surface of the roots (Figure 2). Severely diseased plants, after falling to the ground, turn buff in color, are detached readily by wind action at the soil line, and are blown away. In a few days, scarcely any evidence of the diseased plants remains to indicate that flax seedlings were once present (Figure 4).

The disease characteristically appears in scattered areas in a field. It may involve a few plants within a row or all plants in several linear feet of row (Figure 5). When the disease reaches epiphytotic

Figure 1. Typical Seedling Blight Symptoms Caused by R. solani on Marine Flax Seedlings Grown Under Field Conditions. (A) Flax seedlings in cotyledonary leaf stage. (B) Four-week-old flax seedlings. (At the time of collection of these specimens, the above ground parts of the plant on the left were normal. The leaves of the plant in the middle were flaccid, and the leaves of the plant on the right were completely wilted and were beginning to turn buff in color.)

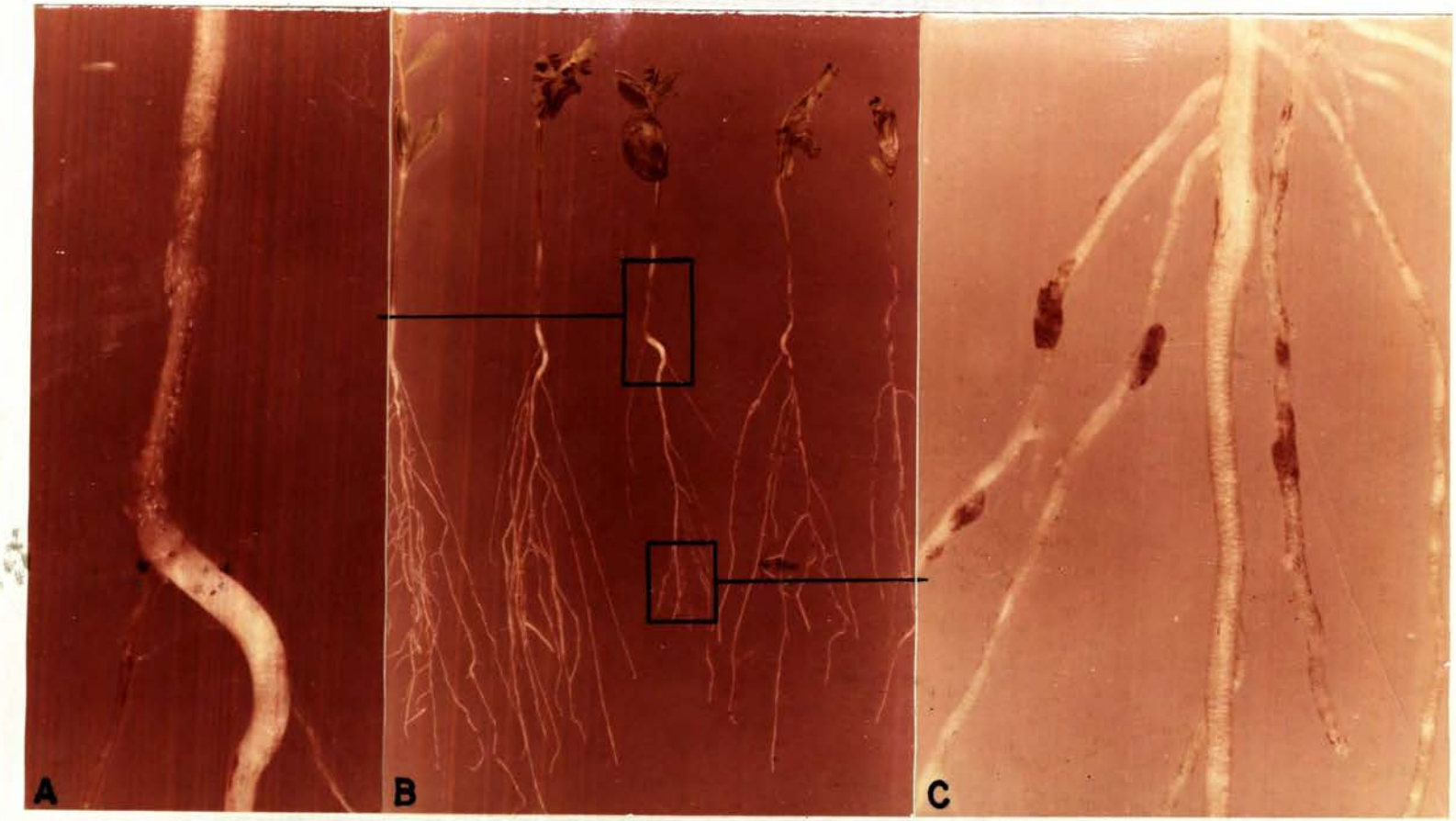

Figure 2. Symptoms of Seedling Blight Appearing on Marine Flax Grown in Steamed Soil Inoculated with R. solani. (A) Rotted hypocotyl region of the flax seedling. (B) Representative diseased seedlings arranged from left to right in increasing order of severity of disease. (C) Sclerotia of R. solani appearing on the roots of a flax seedling.

Figure 3. Seedling Blight Symptoms Reproduced with a Pure Culture of R. solani in Steamed Soil. (The inoculum was placed at point X when the seedlings were in the cotyledonary leaf stage.)

Figure 4. Thinning of Flax Stands Caused by Rhizoctonia Seedling Blight. The disease usually attacks the plants in the early stages of development.

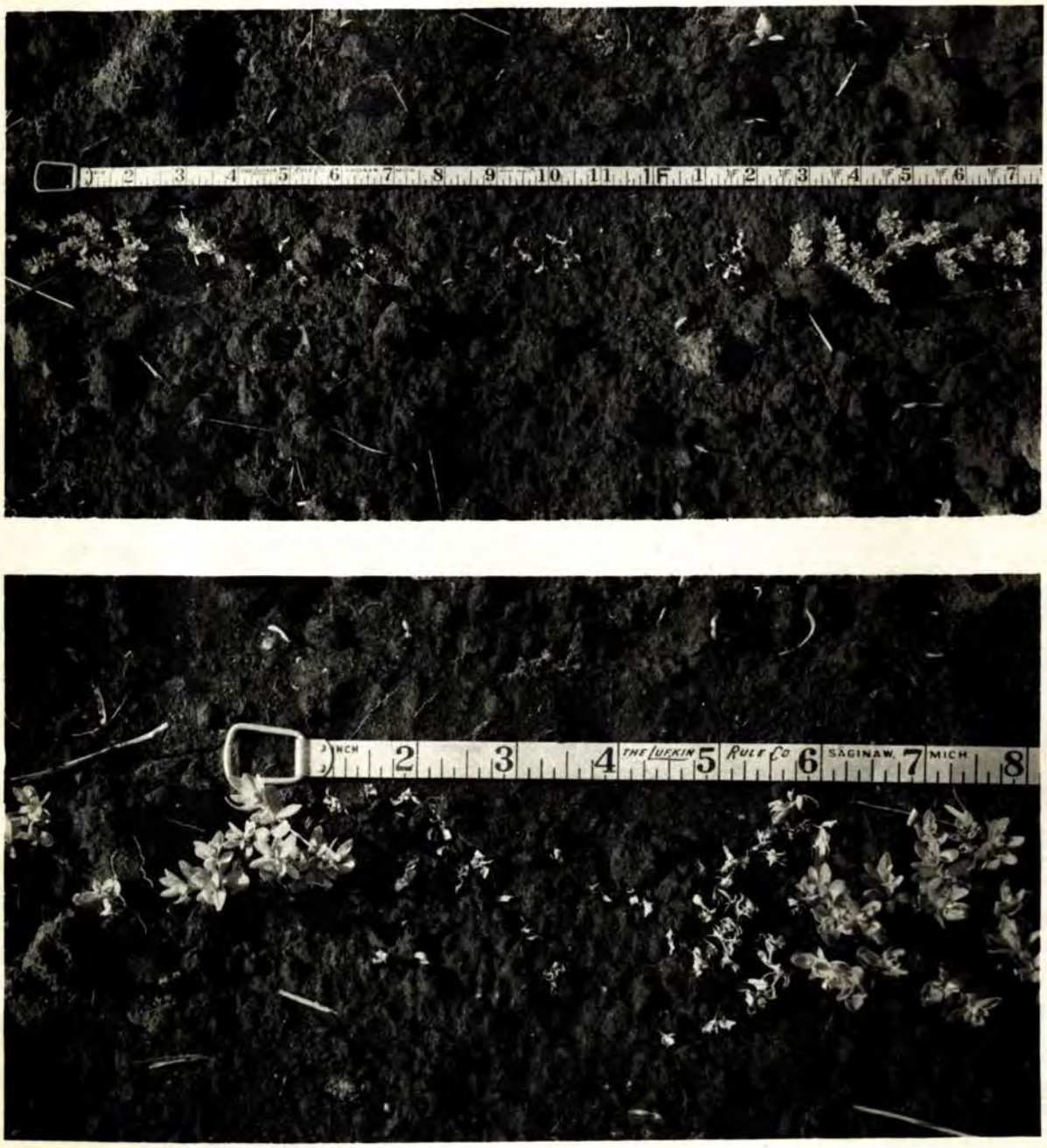

Figure 5. Typical Field Symptoms of Flax Seedling Blight Caused by R. solani.

proportions, the entire field may be affected with stand losses up to 50 per cent or more.

The most striking feature of the disease is the rapidity of its progress. Seedlings seemingly healthy one day may be non-existent a few days later. Spread of the disease within a row of flax is rapid once the first infected plants are noticed. The disease may spread one or two inches in the row from a given point within a 24 hour period. On the other hand, it is not unusual for the disease spread to be checked abruptly by changes in the environment adverse to the growth of the fungus.

Fungi Associated with Diseased Flax Seedlings

The fungi most frequently isolated from diseased seedlings during 1955 and 1956 were Rhizoctonia solani and species of Fusarium and Pythium. Species of Alternaria, Colletotrichum, Helminthosporium, Penicillium, and Trichoderma were also isolated to a lesser extent. Tests of their pathogenicity were conducted with two-week old soil-cornmeal cultures of representative isolates. Fifty milliliters of this inoculum were diluted 1 : 1 with sterile soil and applied over 25 seeds on steamed soil in pots. Two replicate pots for each isolate were prepared for each of three temperatures. Flax seedlings were counted in the two-leaf stage. After four weeks, plants were removed from the pots and the roots were washed and examined for symptoms.

Within two weeks after planting, only R. solani and Pythium spp. caused pronounced reduction of stand, largely as seed rot and pre-

emergence and post-emergence killing. However, Fusarium, Colletotrichum, and Alternaria species had limited pathogenic effects, causing discoloration, lesions, or rot of the roots.

The consistency with which R. solani was isolated from diseased flax plants selected from typical areas in the field and the reproducibility of these field symptoms under greenhouse conditions with pure culture isolates, lead to the assumption that this fungus was primarily responsible for the loss of flax stands in farmers' fields in South Dakota.

Cultural Characteristics, Growth Rates and Pathogenicity of R. Solani Isolates

Twenty-four R. solani isolates were selected for a study of their cultural characteristics, growth rates at different temperatures, and pathogenicity to flax. Most of these isolates were from diseased flax seedlings collected mainly in fields from scattered areas in the state, while a few were from other plant species. The sources of nine of these isolates are listed in Table I.

The gross cultural appearance of the 24 isolates grown at room temperature on potato-dextrose agar are shown in Figure 6. Most of these isolates differed in one or more characteristics, such as amount and type of growth, color, and abundance and size of sclerotia formed in culture.

The growth rates of nine of these isolates are presented in Figure 7. The minimum temperature for growth was approximately 6° C.

Table I. Sources of Nine Isolates of R. solani Used in the Present Investigations

Isolate Number	Host Plant	Location
534	Flax	Greenhouse
632	Field bind weed	Sisseton
667	Flax	Watertown
684	Flax	Clear Lake
688	Flax	Aberdeen
691	Flax	Watertown
695	Flax	Greenhouse
697	Soybean	Brookings
698	Oats	Sisseton

The maximum for some isolates was 35° and for others 40° C., whereas the optimum was 25° for some isolates and 27° to 30° C. for others.

The pathogenicities of the above nine isolates were tested on Marine flax in the greenhouse. However, in this test, the usual practice of using colonized soil-cornmeal medium as inoculum was abandoned for the reason that the more rapidly growing isolates were considered to provide a higher concentration of inoculum in a given length of time than the slowly growing ones. Therefore, a method was chosen which would minimize the effect of such growth rate differences on the degree of virulence of each isolate. Consequently, each isolate was grown on 100 milliliters of potato-dextrose liquid medium for six days. The mycelium was removed from the medium and two grams of mycelium from each isolate were macerated for one minute in 100 milliliters of water in a Waring Blendor. Twenty-five milliliters of this suspension and the same amount of an additional 1:100 diluted suspension were sprinkled evenly over the soil in each of three pots for each isolate.

The emergence and survival of seedlings for nine of these

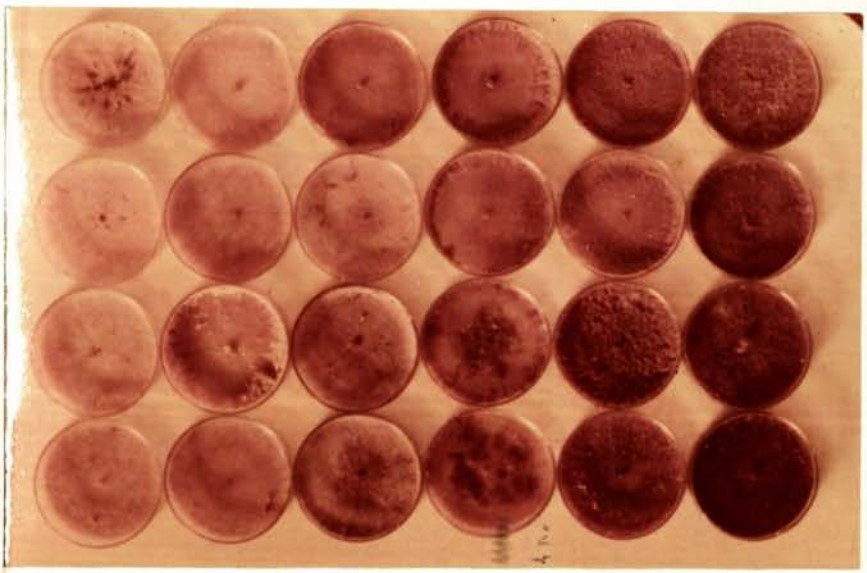

Figure 6. Growth of 24 Isolates of R. solani Grown on Potato-Dextrose Agar.

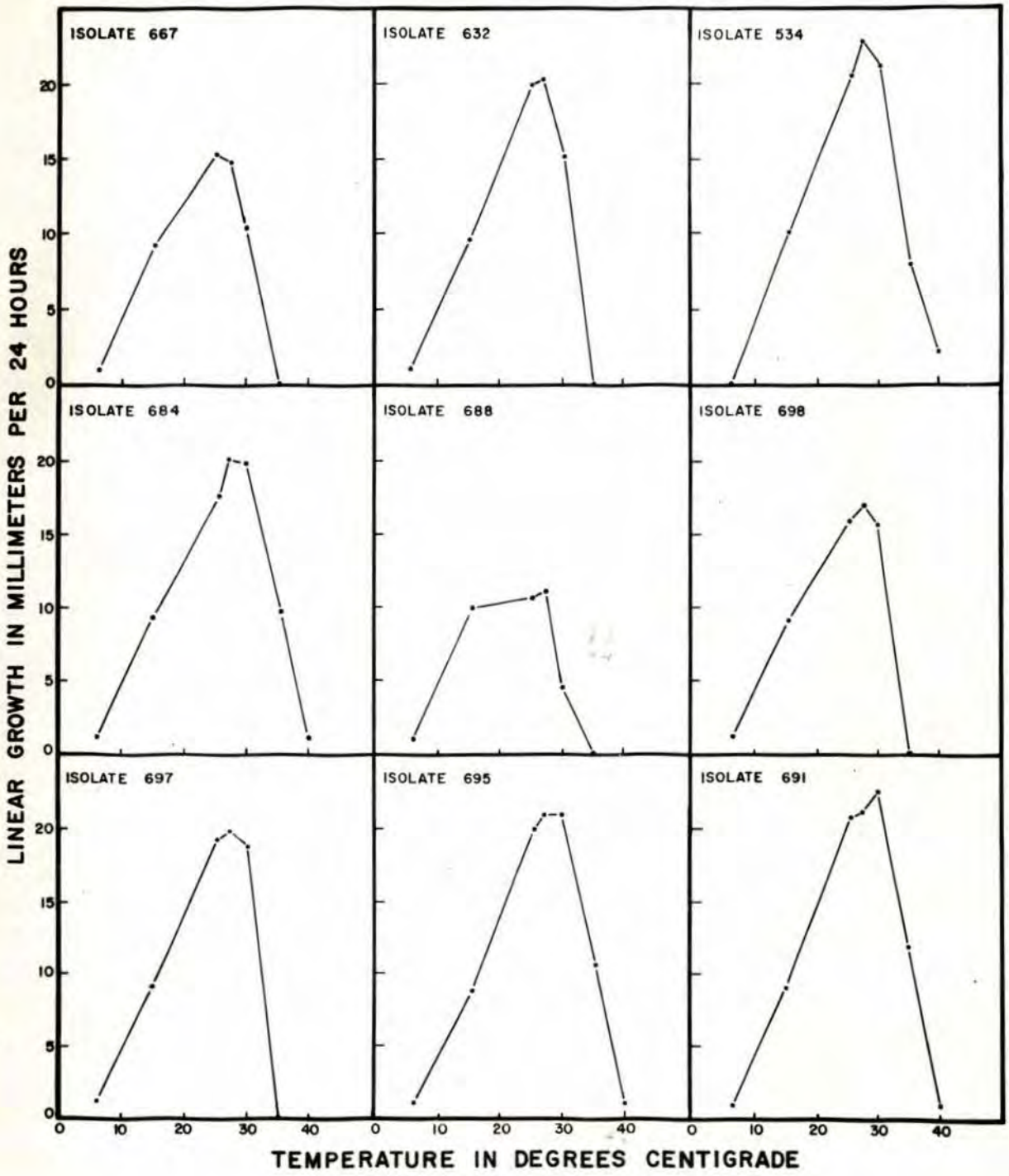

Figure 7. Average Growth Rates of Nine Different Isolates of R. solani Grown at Seven Different Temperatures on Potato-Dextrose Agar.

isolates are presented in Figure 8. The isolates used were the same as those for which growth rate curves are shown in Figure 1. The degree of pathogenicity based on flax stands varied from zero to 80 per cent in soil inoculated with the undiluted suspension of R. solani. There appeared to be no important correlation between virulence and the rate of growth of these isolates. The fast-growing isolate number 534 was most pathogenic; but isolate 695, which also was fast-growing, was one of the least pathogenic. Isolate 688, on the other hand, was slow-growing but quite virulent. It is noteworthy that isolates from flax varied as widely in virulence as isolates obtained from soybeans, oats, or field bind weed.

Host Range

A limited test of the host range of R. solani involved the planting of several plant species in pots of steamed soil inoculated with the virulent isolate 534. The results of this test are presented in Table II. In general, of the plants tested, monocotyledons appeared to be less susceptible than the dicotyledons. Larkspur appeared to be an exception, however, because stands in inoculated soil were almost as good as stands in steamed soil.

Parasitism of the graminous hosts included pre-emergence blighting by destruction of the elongating plumule and browning and necrosis of the coleoptile and lower leaf sheath. Most of the emerged grasses in inoculated soil appeared stunted with pronounced yellowing of the lower leaves. Very few of these seedlings died after emergence

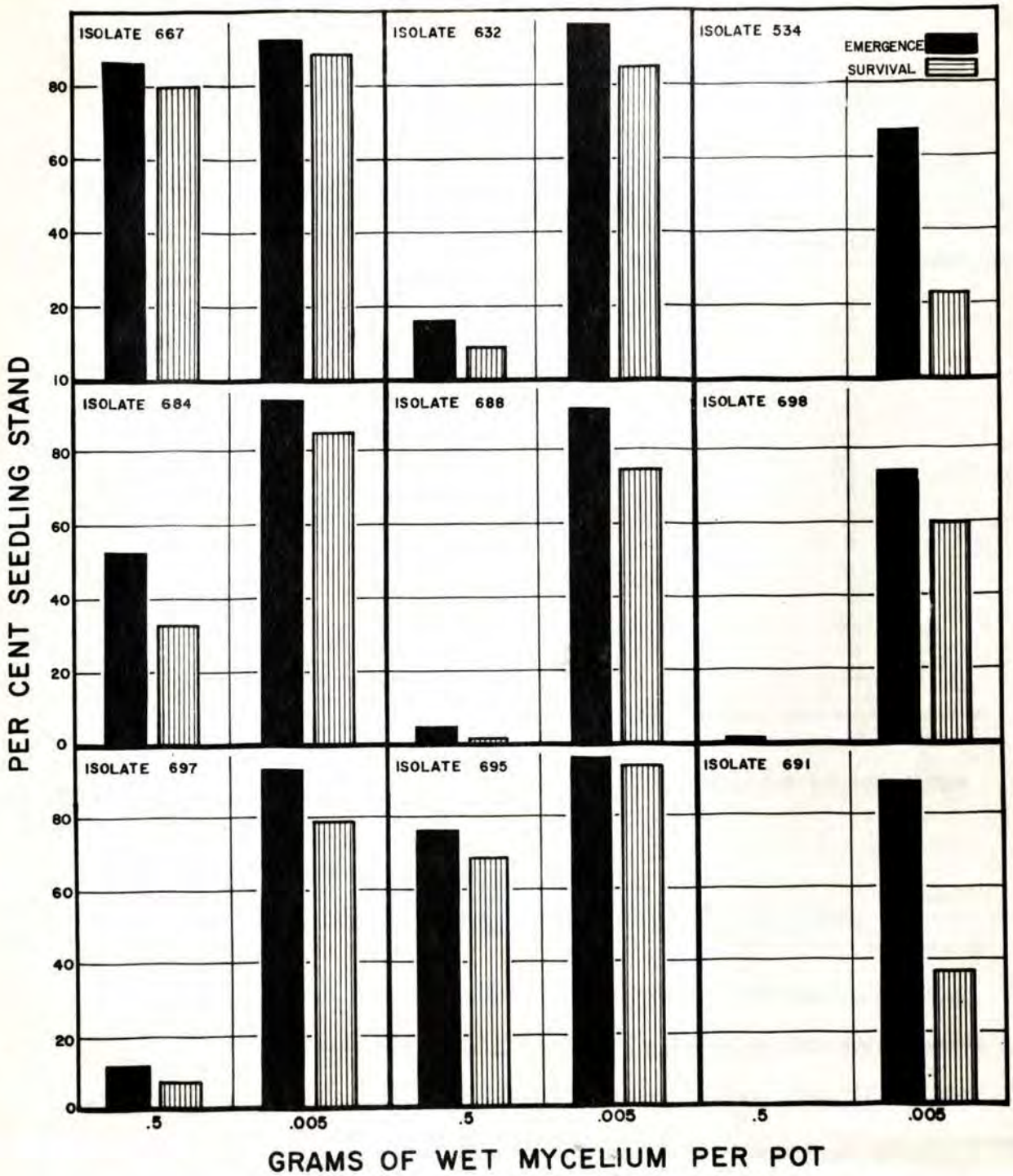

Figure 8. Per Cent Seedling Stands of Marine Flax Grown in Steamed Soil Inoculated with Two Concentrations of Each of Nine Isolates of R. solani. Each column represents per cent emergence or survival based on the mean of three replications.

Table II. Per Cent Survival of Seedlings of 15 Different Plant Species When Grown Under Greenhouse Conditions in Steamed Soil and in Steamed Soil Inoculated with R. solani

	Per cent Stand in Steamed Soil	
	Uninoculated	Inoculated
Buffalo Burr	38	1
Common Western Wheat Grass	84	48
Cucumber	81	0
Flax	71	1
Hedge Bind Weed	18	0
Homesteader Brome	92	45
Kentucky Blue Grass	72	21
Larkspur	20	18
Lettuce	86	7
Onion	49	0
Radish	99	6
Squash	52	0
Tall Wheat Grass	43	28
Watermelon	48	0
Wild Buckwheat	19	0

in contrast to the frequent post-emergence destruction of seedlings of flax and other dicotyledons.

Changes in the Flax Seedling Blight Potential of a Soil

Three tests were conducted to determine the changes in the flax seedling blight potential of a soil from one of the land ranges at the Agronomy Farm where flax stands were reduced over 50 per cent by seedling blight. In the first of these tests, the soil was collected in July, 1956, and brought to the greenhouse where part of it was planted to flax in four-inch pots. After the plants had grown for one month, all of them were pulled and the soil was left to dry on the greenhouse bench for an additional two months. In the meantime, the

other part of the soil was subdivided and stored dry at 8° and 20°-25° C. for three months. Marine flax was then planted in the three different lots of soil. The results are presented in Figure 9. Dry storage for three months apparently did not alter the disease potential of the soil. However, cropping the soil lowered the disease potential, but whether this was due to the crop or to the moist condition of the soil was not known.

In the second test, an attempt was made to ascertain whether a R. solani culture added to this soil would persist or decline at different temperatures under dry or moist storage conditions. Soil was collected from the field in October. To one portion of the soil, a dry soil-cornmeal culture of R. solani was added in the proportion of 1:100, while no inoculum was added to the second portion. The soils were stored in ten-inch clay pots at 8° and at 20°-25° C. Part of the soil at each of these temperatures was maintained in a moist condition by watering periodically during the storage period, while the other remained unwatered. In January, soil was taken from each of the pots and planted with Marine flax seed. The stands obtained were compared with the stands obtained in the same soils before the storage period. The results of the experiment are presented in Figure 10. At high moisture, stands were improved in both inoculated and uninoculated field soil, whereas when the soil was kept dry and at moderate temperatures, stands were not improved after four months of storage. At low temperatures, stands were improved in inoculated dry field soil, whereas in uninoculated dry field soil, such improvement of stands

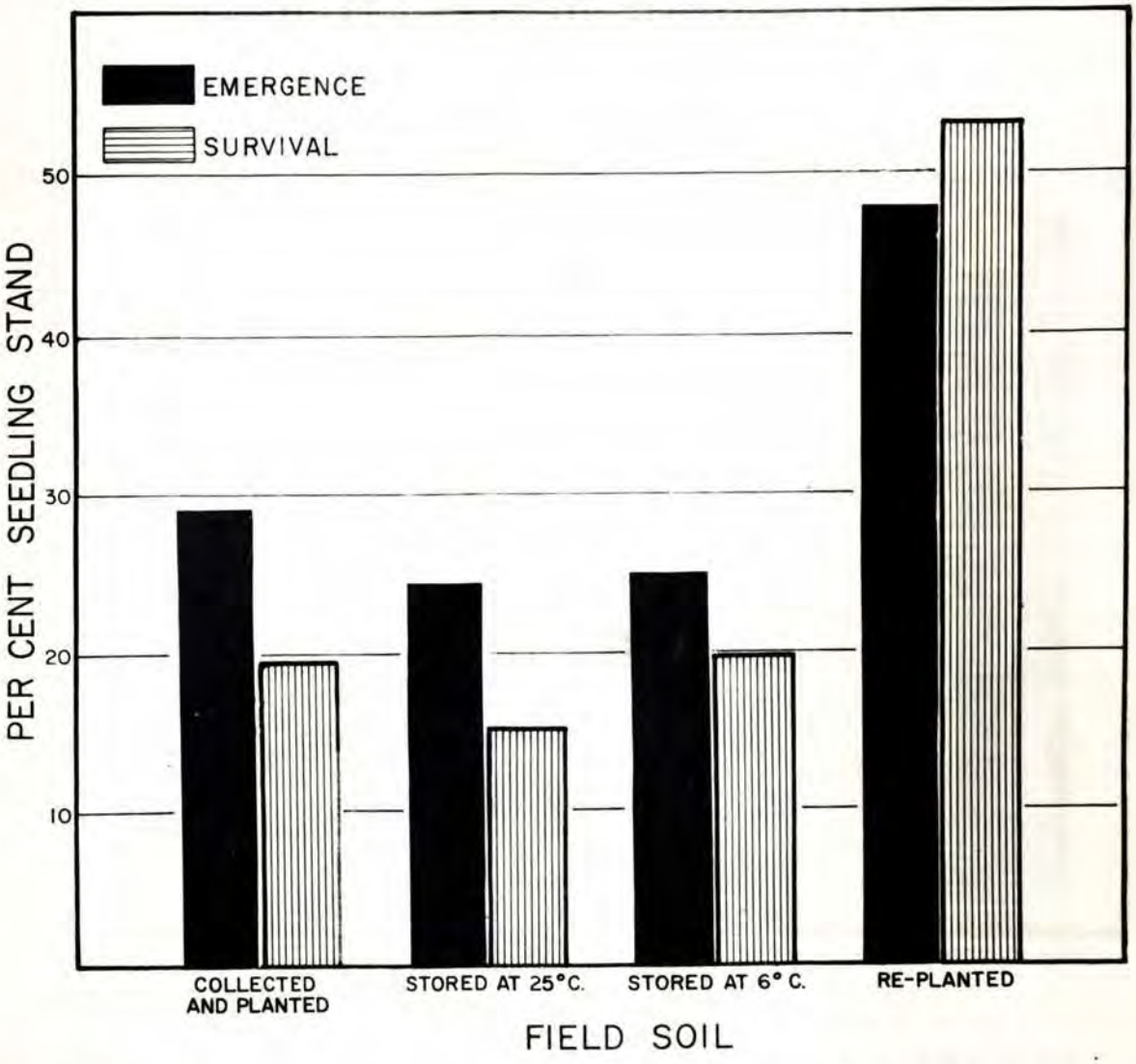

Figure 9. Per Cent Emergence and Survival of Marine Flax Seedlings Grown Under Greenhouse Conditions; Planted in Field Soil Immediately After Collection, in Field Soil Stored Dry for Three Months at 6° and 25° C., and in Field Soil Stored Dry for One Month After Supporting the Growth of Flax Plants for Two Months. Each column represents per cent emergence or survival based on the mean of four replications.

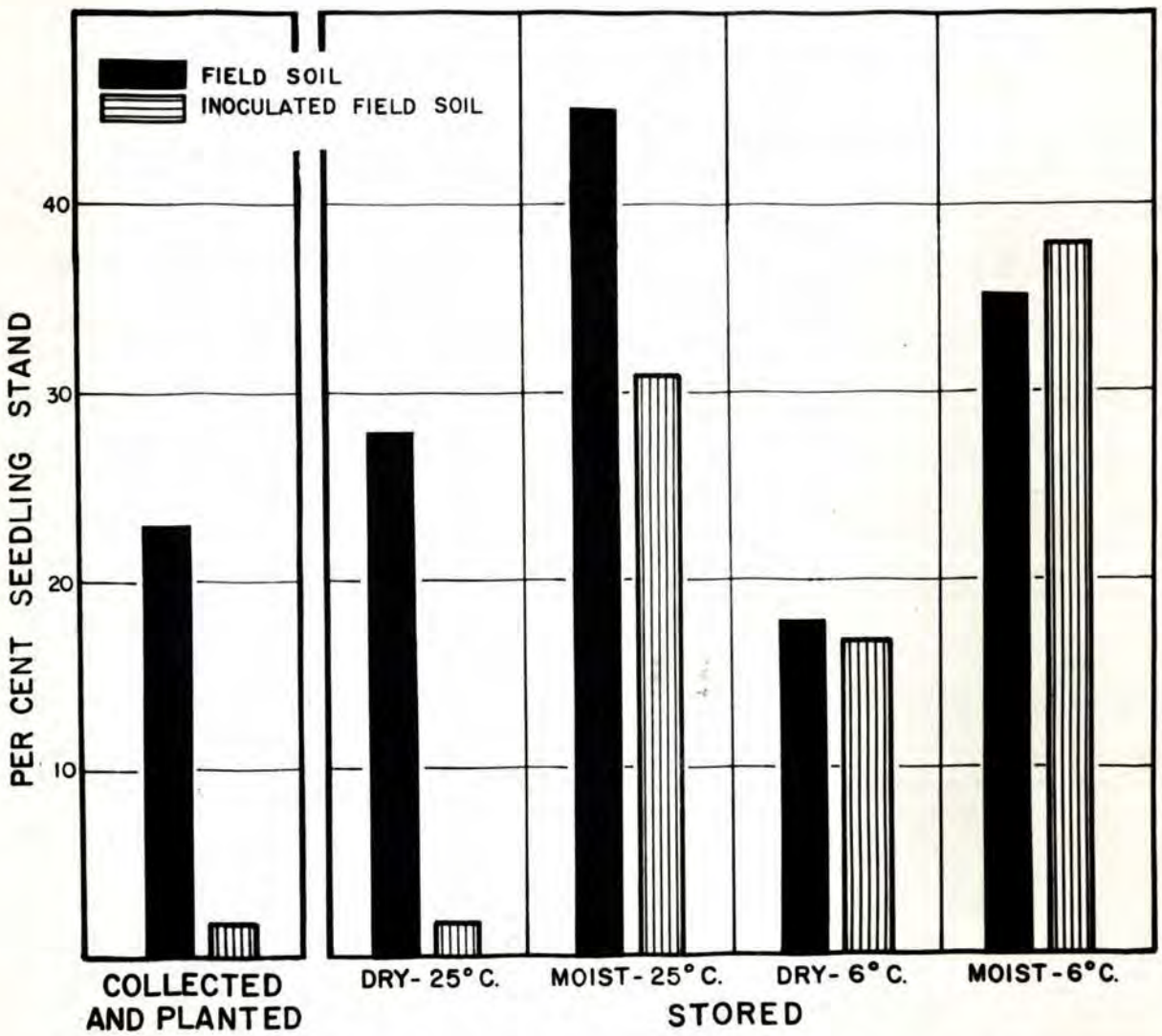

Figure 10. Per Cent Stands of Marine Flax Seedlings Grown Under Greenhouse Conditions; Planted in Field Soil and R. solani Inoculated Field Soil Immediately After Collection, and Planted Three Months Later Following Storage at Two Temperatures at Two Moisture Levels. Each column represents per cent survival based on four replications.

did not occur. From these experiments, it appears that R. solani, and possibly other pathogens that cause reduction of stands in field soil, persist for at least four months when the soil is stored dry at moderate temperatures.

In the third experiment, the changes in seedling blight potential over a period of time in the field were evaluated by collecting soil samples in July and October, 1956, and in February and April, 1957, and planting Marine flax in these soils in the greenhouse. The stands obtained on these samples are presented graphically in Figure 11. No marked change in stands occurred from one planting to the next, but the trend was toward a slight reduction of disease potential during the nine-month period. Freezing and thawing of the soil surface evidently have little effect on modifying the soil to reduce the incidence of seedling blight.

Factors Affecting Disease Development

Inoculum Dosage

The effect of inoculum dosage on stands of flax was studied by inoculating steamed soil with culture 534 of R. solani in a graded series of .25, 2.5, 25, and 250 milligrams of wet mycelium per pot. The basic concentration was obtained by macerating one gram of a mycelium mat grown on a liquid medium with 100 milliliters of water in a Waring Blendor. The other concentrations were prepared from this suspension by appropriate dilutions with water. Twenty-five

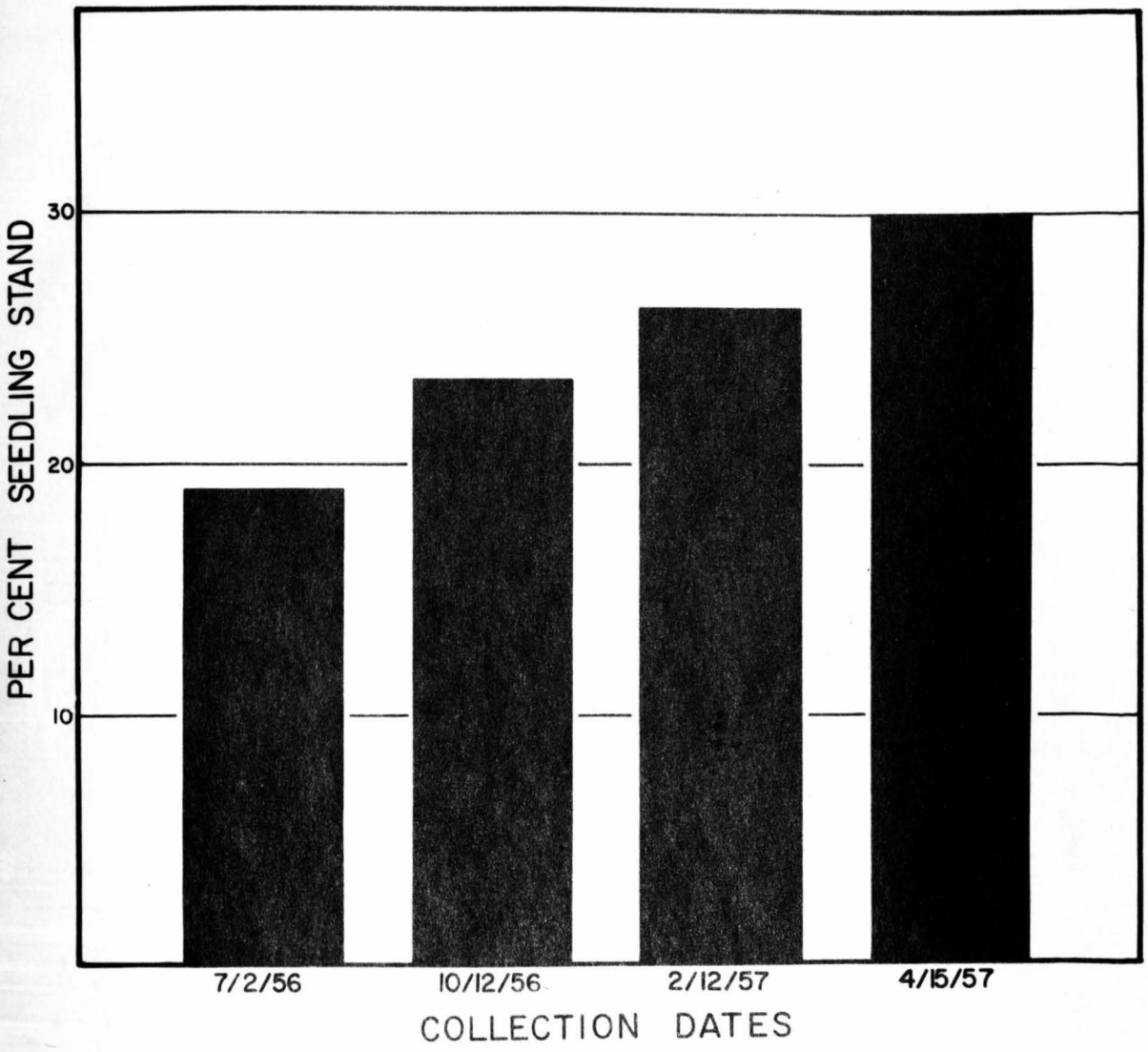

Figure 11. Per Cent Stands of Marine Flax Seedlings Planted in Soil Collected from the Field at Four Different Dates. Each column represents per cent survival based on the mean of four replications.

milliliters of each dilution were sprinkled evenly over the soil in three-inch pots. Seed was planted and covered uniformly with steamed soil to a depth of one centimeter. The data obtained from this experiment are recorded in Table III. These data are the averages of the per cent stands from 12 varieties of flax presented in Table IX.

The relation between inoculum concentration and emergence reduction was curvilinear, with the greatest stand reduction occurring at the higher concentration of inoculum.

Soil Temperature

The relation of soil temperature to emergence and survival of flax seedlings grown from cracked and uncracked seed of Marine flax was investigated. Field soil, steamed soil, and steamed soil inoculated with *R. solani* were used in the experiment. Inoculation of steamed soil was accomplished by thoroughly distributing a soil-cornmeal culture of *R. solani* number 534 throughout the soil in the proportion of 1:1000. Eight-inch glazed crocks were used as containers for the soils which were placed in six automatically controlled temperature tanks of the Wisconsin type. The temperature range provided by this series of tanks was from 10° to 35° C. The average temperatures provided by the various tanks were as follows: 12°, 16°, 20°, 23°, 27°, and 32° C. Fifty seeds of cracked and fifty seeds of uncracked Marine flax were planted in each crock of soil, replicated twice. The results are presented in Table IV.

Table III. Per Cent Emergence and Survival of Seedlings of Flax Grown in Steamed Soil Inoculated with Suspensions of R. solani Mycelium

Milligrams of Wet Mycelium Per Pot									
0	.25	2.5	25	250	0	.25	2.5	25	250
Per cent Emergence					Per cent Survival				
85	74	69	20	2.8	86	74	65	20	3.8

The emergence and survival of flax seedlings from uncracked seeds in steamed soil were high at all temperatures. In inoculated steamed soil, the emergence was nearly the same from 20° to 32° C., but less at 12° and 16° C. In this soil, all emerged seedlings survived at 12° C., about one-half survived at 16° C., and none at 20° to 32° C. The plants at 23°, 27°, and 32° C., died within two weeks after emergence, with those at 27° and 32° C. dying more rapidly than those at 23° C. The seedlings at 20° C. died at the rate of a few each day following emergence. Those at 16° C. appeared healthy for at least one week after emergence, but each day thereafter a few seedlings wilted and died. It is noteworthy that the temperature at which most post-emergence killing of seedlings occurred in the inoculated soil closely approximated the optimum temperature for the growth in pure culture of R. solani isolate 534.

In field soil, seedling emergence was much less than in the preceding soils, especially at the temperatures of 20°, 23°, and 32° C. Seedling survival at the higher temperatures of 20°, 23°, and 27° C.

Table IV. Per Cent Seedling Emergence and Survival from Cracked and Uncracked Marine Flax Seed Grown in Steamed Soil, *R. solani* inoculated Steamed Soil, and in Field Soil at Six Different Soil Temperatures

Soil Temperatures °C.	Per cent Emergence		Per cent Survival	
	Cracked	Uncracked	Cracked	Uncracked
		Steamed soil		
12	73	89	73	89
16	65	92	62	90
20	61	91	64	92
23	89	93	81	94
27	64	99	62	94
32	69	87	61	85
	R. solani inoculated steamed soil			
12	60	74	60	74
16	55	72	19	39
20	67	86	2	9
23	58	85	0	0
27	39	81	0	0
32	58	79	0	0
		Field soil		
12	7	59	7	65
16	8	40	9	43
20	6	27	6	22
23	7	29	4	16
27	3	43	3	31
32	14	27	10	29

was better in this soil than in the inoculated soil. After emergence, approximately one-half of the seedlings died at these temperatures, whereas none died at 12°, 16°, or at 32° C. The low stands at 32° C. may have been due to factors other than disease development. This temperature was observed to be sub-optimum for the proper development of flax. Although germination was more rapid than at the other temperatures, the plants soon began to appear stunted and yellow. This effect was apparent in steamed soil as well as in field soil.

Cracked seeds yielded poorer stands than uncracked seeds. The difference was greater in field soil where stands were reduced more than 90 per cent. The difference in stands in steamed and in inoculated steamed soil ranged from 14 to 54 per cent over-all for the various temperatures. It is significant that emergence from cracked seed was markedly reduced only at 27° C. in inoculated steamed soil.

It appears that cracked seed was at a disadvantage because of the presence of R. solani, which was able to increase most rapidly at this temperature.

The most favorable temperatures for optimum development of flax seedlings appeared to be 20° to 23° C. It appears significant that the most serious reduction in stand also occurred at these temperatures in field soil. In field soil, typical post-emergence killing of flax seedlings was also noted at temperatures of 20°, 23°, and 27° C., but not at 12°, 16°, or at 32° C.

Depth of Planting

Because R. solani attacks the hypocotyl just below the soil surface and also prevents emergence by killing flax seedlings, it was thought that depth of planting the seed might have some influence on the expression of the disease. Seeds were planted at depths of 0.5, 1.0, 2.0, and 3.0 centimeters in steamed soil, R. solani inoculated steamed soil, and in field soil. The inoculum consisted of R. solani isolate 534 increased in a sterile soil-cornmeal medium. The inoculum was thoroughly incorporated into the steamed soil in the proportion of 1:100.

The averages of the results from four replications of the experiment, presented graphically in Figure 12, show that stands were markedly reduced in the inoculated soil with progressively deeper plantings. In field soil where R. solani is generally present, the reduction in stands due to increased increments of planting depth is not as great proportionally as in the inoculated soil. Evidently, other factors modify the relationship between depth of planting and stands in field soil.

Age of Flax

An experiment was performed to determine whether applying inoculum of R. solani to steamed soil at various stages of flax development would influence the severity of disease. The soil in each of three flats was divided at right angles through the center to form four equal rectangular sections, constituting four replications. Five rows of ten seeds each were planted in each section. The rows were two inches

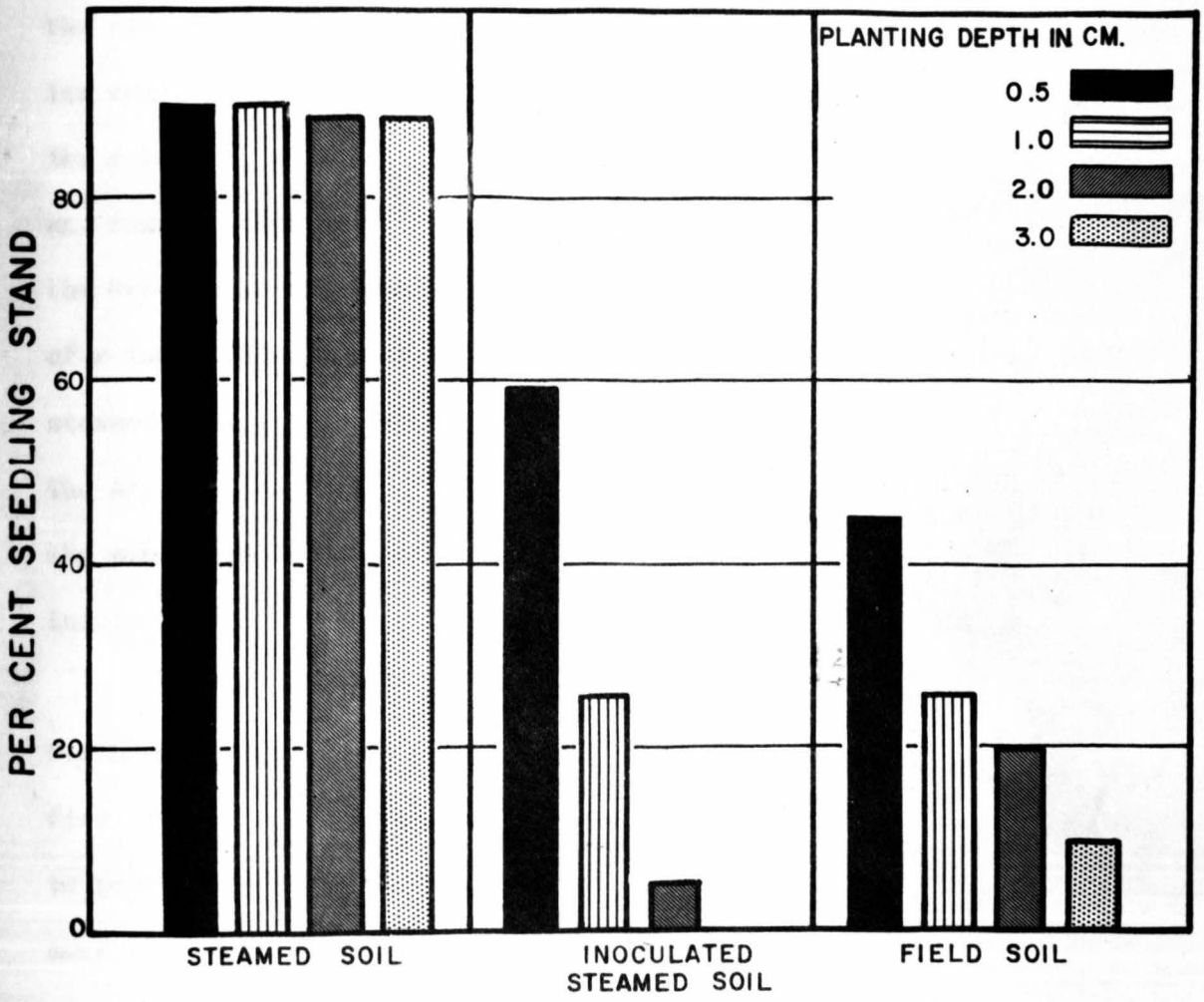

Figure 12. Per Cent Seedling Stands of Marine Flax Planted at Four Different Depths in Steamed Soil, R. solani Inoculated Steamed Soil, and in Field Soil. Each column represents per cent emergence based on the mean of four replications.

apart and the seeds were one-half inch apart within rows. Two of the rows were planted at one end and across the width of each rectangular section, while the other three rows were planted lengthwise in the remaining area to within two inches of the two rows. The inoculum was spread in an even band one inch wide and five inches long between the two groups of rows. Inoculum consisted of 50 cubic centimeters of a two-month-old soil-cornmeal culture of R. solani diluted with steamed soil 1:10. Inoculum was applied to the soil in one flat when the seed was planted. Soil in the second flat was inoculated when the seedlings had emerged, and in the third when the flax was three inches tall.

The results of this experiment are illustrated in Figure 13. Death of most of the seedlings in the first flat occurred during the first five-day period after application of the inoculum, due either to pre-emergence or post-emergence killing. In the other two flats, more time was required for optimum disease expression to be reached, as most seedlings died during the second five-day period. There was a rapid decline in death of seedlings during and after the third five-day period. During this period, disease expression was more erratic. Some plants growing somewhat distant from the point of inoculum placement suddenly wilted and died, while others remained healthy.

More plants were killed in the flat where inoculum was applied at the time the seeds were planted than in the other two flats. However, one reason for the lesser amount of disease in the second and third flats may have been the presence of saprophytic organisms such

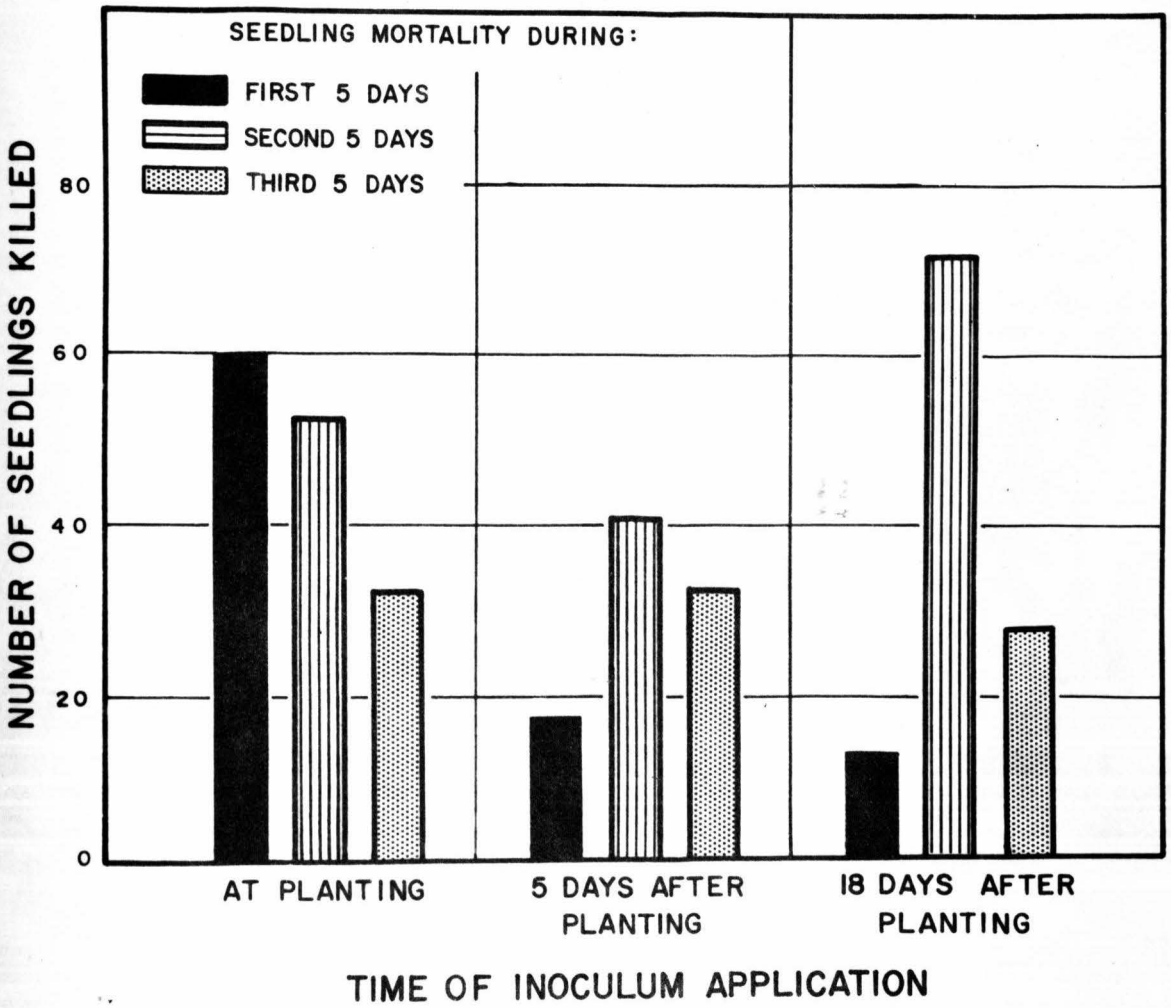

Figure 13. Number of Marine Flax Seedlings Killed During Three Intervals of Five Days Each When R. solani was Added to the Soil at Time of Planting, and Five and Eighteen Days After Planting. Each column represents the number of plants killed based on the total of four replications.

as Pyronema spp. which reinvaded the soil and possibly were antagonistic to the growth and parasitism of R. solani.

Stand Density and Inoculum Dosage

An experiment was conducted to determine whether the amount of inoculum and the density of seed planting would have any bearing on the growth rate or total distance the mycelium of R. solani would spread in steamed soil. Four flats containing steamed soil were seeded with Marine flax in rows two inches apart across the width of the flat. The rows were separated by strips of glass embedded in the soil. The seeds were planted 0.25, 0.5, 1.0, and 2.0 inches apart within rows. Two rows of similarly spaced seeds were planted in each flat. Agar cubes cut to 1, 2, and 3 cubic millimeters in size from a one-week old culture 534 of R. solani growing on potato-dextrose agar medium in a Petri dish, served as inoculum. One piece of the inoculum was placed midway along the length of each row of seed at planting time. The results of this experiment are presented in Figure 14.

The fungus was able to grow to the edge of the flats as rapidly from small pieces as from large pieces of inoculum, and seedlings equidistant from both small and large pieces of inoculum were killed at about the same time. However, the number of seedlings that died in these rows was greater from the larger than from the smaller pieces of inoculum. Higher percentages of seedlings died in rows where plants were spaced more closely together than where the plants were farther apart.

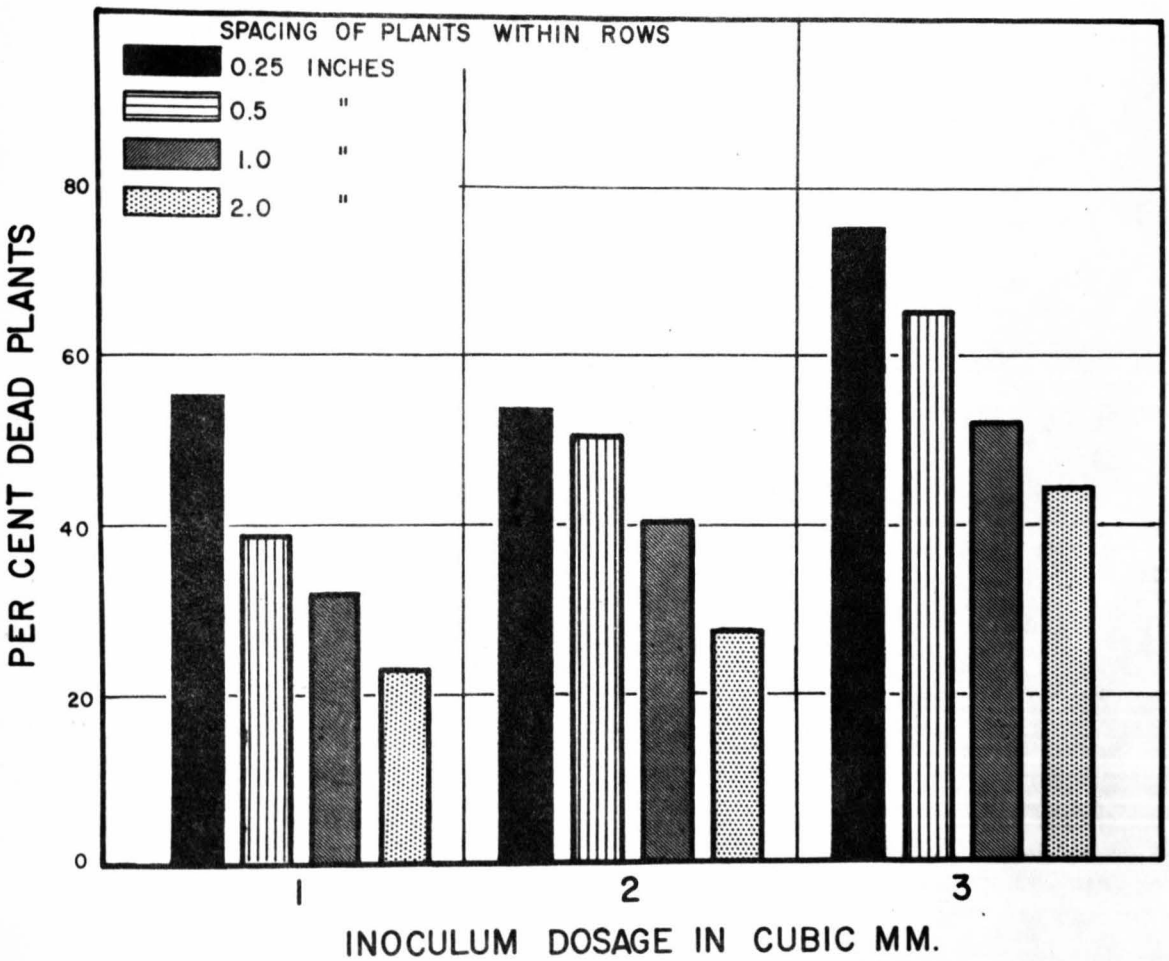

Figure 14. Per Cent of Marine Flax Seedlings Killed when Planted at Four Different Spacings Within Rows with Three Different Inoculum Dosages. Each column represents per cent dead plants based on the mean of two replications.

Control of Flax Seedling Blight

Seed and Soil Treatment

One field and two greenhouse experiments were conducted to determine whether seed and soil treatments would control flax seedling blight. Chemical fungicides, chemical soil fumigants, and mycelium and spores of *Trichoderma lignorum* were used in these experiments.

The field experiment was conducted in 1955 at three locations in eastern South Dakota as follows: a farmer's flax field eight miles east of Sisseton, the northeast sub-station twelve miles north of Watertown, and the Plant Pathology research plots at Brookings. Fourteen seed treatments and six chemicals used as soil drenches were used at the rates indicated in Table V. The chemicals used as soil drenches were suspended in water and were applied with a watering can to the soil in a band six inches wide immediately after planting. The various plantings consisted of randomized blocks replicated four times, with four row plots, one rod long. They were planted on the following dates: Sisseton, May 4; Watertown, April 28; and Brookings, April 19, May 3, and May 17. Stand counts were taken in two linear feet of the two central rows of each four-row plot when the flax was four to eight inches tall. The counts varied markedly and were inconsistent; therefore, they were not incorporated into this manuscript. The two middle rows of each plot were later harvested for yield.

Typical symptoms of seedling blight caused by *R. solani* appeared in the Watertown and Sisseton plantings. On May 23, numerous gaps in

Table V. Fungicides, Active Ingredient, and Dosage of Chemicals Used in Treating Flax Seed and Soil for the Control of Seedling Blight of Flax

Fungicides	Active Ingredient	Oz. per Bushel	Gm. per sq. ft.
Agrox	phenylmercuriurea	1.5	
Arasan	bis(dimethylthiocarbamoyl) disulfide	3.0	2.0
C & C Experimental Fungicide 224	mercury zinc chromate ($O \cdot 4HgO \cdot 3ZnO \cdot CrO_3$)	1.5	
C & C Experimental Fungicide 640	copper zinc chromate ($4CuO \cdot ZnO \cdot CrO_3 \cdot XH_2O$)	3.0	
Ceresan D	ethylmercury-2,3-dihydroxy-propyl mercaptide; ethylmercury acetate	1.5	
Ceresan M	N-(ethylmercuri)-p-toluenesulfonanilide	1.5	1.0
Gallotox	phenylmercury acetate	1.5	
Orthocide 75	N-(trichloromethylthio)-4-cyclohexene-1,2-dicarboximide	2.0	
Panogen	cyano(methylmercuri)guanidine	1.5	
Phygon XL	2,3-dichloro-1,4-naphthoquinone	4.0	
Puraseed	N-phenylmercuriformamide; anilinocadmium lactate	1.5	1.0
Terrachlor	pentachloronitrobenzene	2.0	1.3
Yellow Cuprocide	cuprous oxide	3.0	2.0
1843B	(coded)	2.5	

the rows from six to twelve inches in length were present in which all of the seedlings were dead or in a severely wilted condition. Records made of the length of the gaps in the row did not show secondary spread of the disease following the original outbreak. Furthermore, no typical seedling blight damage could be observed at any later date during the growth and maturity of flax in those plots.

Typical seedling blight was not observed in any of the experimental plots on the Plant Pathology research grounds at the first two plantings. A few scattered wilted or dead plants were observed but isolations from diseased tissue resulted only in Pythium and Fusarium cultures. Typical disease symptoms were evident in only a few instances in the third planting.

Phytotoxic effects were observed from Ceresan M used as a soil drench. These effects could be noted when the flax was in bloom and setting seed. Some plants died, while others remained alive after falling to the ground; however, normal maturity was impaired and yield was reduced. This condition was not observed in the plots at Sisseton or in the first planting at Brookings. Phytotoxicity was evident in the third planting at Brookings, but the damage was light.

The yield data from the planting at Sisseton are not available since the flax was inadvertently plowed by the farmer before it could be harvested. Yields of flax obtained from the other field plots in the fungicide treatment experiments are recorded in Table VI. The data indicate that there was no significant increase in yield from seed or soil treatments.

Table VI. Yields of Marine Flax Treated with 14 Chemicals Either as a Seed Treatment or as a Soil Drench, at Three Dates of Planting at Brookings and One Date of Planting at Watertown.

	Flax Yields in Bushels Per Acre			
Fungicide	April 19 Brookings	May 3 Brookings	May 17 Brookings	April 28 Watertown
Check	9.2	15.1	14.4	18.3
Agrox	10.6	14.1	13.9	19.2
Arasan	10.9	13.8	14.9	20.0
Arasan drench	11.5	14.8	14.9	19.6
C & C 224	11.1	12.2	14.6	18.9
C & C 640	11.8	15.4	14.4	17.6
Ceresan D	11.5	14.5	14.7	20.0
Ceresan M	8.6	14.6	14.5	19.1
Ceresan M drench	9.3	7.7	13.4	7.1
Gallotox	10.4	14.8	15.5	18.9
Orthocide 75	11.7	13.3	15.0	18.2
Panogen	9.7	14.7	13.8	20.5
Phygon XL	9.1	17.0	15.1	19.0
Puraseed	9.6	13.1	15.5	19.4
Puraseed drench	9.4	13.3	14.1	16.0
Terrachlor	8.0	14.7	14.7	19.1
Terrachlor drench	9.5	12.9	12.9	17.6
Yellow cuprocide	10.9	12.3	15.1	18.7
Yellow cuprocide drench	12.1	13.3	14.7	16.9
1843B	11.0	13.9	14.0	18.6

Because the field experiment did not provide the control sought, further work on control was conducted in the greenhouse where environmental and soil factors could be better controlled. Accordingly, the first greenhouse experiment was designed to determine the efficacy of certain seed and soil treatments in improving the stands from cracked and uncracked Marine flax seed in steamed, R. solani inoculated steamed, and field soil. Cracked and uncracked seeds were used because, as noted

earlier, cracked seeds are less able to produce good stands of seedlings in field soil than are uncracked seeds. Steamed soil was inoculated by mixing into it a soil-cornmeal culture of the pathogen in the proportion of 1:1000. The seed and soil treatment fungicides tested were Panogen, Terrachlor, and Orthocide. In addition, dry spores of T. lignorum were applied to the seed and soil. This fungus is known to parasitize R. solani and to inhibit the growth of certain other fungi. The spores were prepared by culturing the fungus on sterilized moist oats in a 4000 milliliter wide mouth Erlenmeyer flask. After three weeks, the medium on which the fungus had sporulated profusely was removed and dried. The spores were then separated by shaking the medium on a 100 mesh screen. Three milligrams of the liquid chemical Panogen were mixed thoroughly with the seed in a test tube. The seed was removed, dried quickly, and placed in a small glass vial which was corked for 24 hours before the seeds were removed for planting. The fungicides, Terrachlor and Orthocide, and the spores of T. lignorum were applied to the seeds in excess. The excess was removed by shaking the seed on a 60 mesh screen after treatment. As soil treatments, one gram each of the dry fungicides and T. lignorum spores were mixed thoroughly into five kilograms of soil. Panogen was applied as Panodrench, a product which contains .6 per cent active ingredient cyano-(methylmercuri)guanidine. This application was made according to the manufacturer's recommendation. One teaspoonful of the liquid chemical in three gallons of water was applied to the soil as a drench before planting seed and again when the seedlings emerged. The averages of the

results from four replications used in this experiment are presented in Table VII.

Without treatment, better stands were obtained from uncracked seed than from cracked seed in each of the soils tested. The greatest difference occurred in field soil where stands from uncracked seed were 42 per cent greater than stands from cracked seed. In inoculated soil, the difference was 28 per cent, whereas in steamed soil the difference was only 8 per cent. Although the viability of cracked and uncracked seed was about equal, the ability of these seeds to produce stands was different in infested soils. In addition, seed-coat soundness or unsoundness did not alter the amount of post-emergence killing of seedlings. Apparently, seedlings were equally susceptible once they emerged, whether they grew from cracked or uncracked seed.

Soil treatment was more effective than seed treatment for controlling R. solani under pure culture conditions, but seed treatment with certain chemicals was more effective in improving stands in field soil. Some of these chemicals proved superior in preventing post-emergence killing of seedlings when mixed with the soil, but were generally less successful when applied to the seed.

Inferior stands were obtained from treated cracked seed planted in steamed soil. This result was presumably due to phytotoxicity which affected only cracked seed, for the stands from uncracked seed were as good as from the untreated check. Such reduction in stand did not occur when untreated seeds were planted in treated steamed soil.

Table VII. Per Cent Emergence and Survival of Seedlings from Cracked and Uncracked Seed in Field Soil, R. solani Inoculated Steamed Soil, and in Steamed Soil, When Three Fungicides and Spores of T. lignorum were Applied Either to the Seed or to the Soil.

	Per cent Emergence				Per cent Survival			
	Cracked		Uncracked		Cracked		Uncracked	
Treatment	Seed	Soil	Seed	Soil	Seed	Soil	Seed	Soil
				Field Soil				
None	4	4	46	46	8	8	25	25
Panogen	48	5	57	44	50	8	55	48
Terrachlor	6	5	39	41	5	4	26	30
Orthocide	41	39	63	62	25	36	45	63
Trichoderma spores	4	4	37	34	3	5	29	29
				R. solani Inoculated Steamed Soil				
None	15	15	43	43	3	3	2	2
Panogen	24	38	56	84	3	8	10	33
Terrachlor	30	82	41	98	6	80	3	96
Orthocide	43	70	73	82	6	54	22	83
Trichoderma spores	14	28	40	54	0	2	0	2
				Steamed Soil				
None	87	87	95	95	88	88	94	94
Panogen	50	85	92	96	49	78	93	96
Terrachlor	73	87	89	98	71	88	92	98
Orthocide	72	83	87	99	72	80	86	97
Trichoderma spores	68	90	91	90	66	86	91	90

The fungicides varied widely in their individual ability to protect seedlings in infested soils. Furthermore, they were not equally effective in the two soils. Panogen as a seed treatment was most effective of the seed treatments used in improving emergence and survival in field soil but, as a soil treatment, it did not prove effective. In inoculated soil, more improvement was derived from the use of Panogen as a soil treatment than as a seed treatment, but the protection was not extended, as post-emergence killing was almost as severe as in the check. Terrachlor and Orthocide were about equally effective in improving emergence of seedlings from both cracked and uncracked seed in inoculated soil. As seed treatments, however, these chemicals did not prevent post-emergence killing, whereas it was effectively prevented by soil treatment. There was some protection evidenced by the use of T. lignorum spores mixed with the soil, but this protection was limited since post-emergence killing of seedlings was fully as severe as in the check.

In field soil, Terrachlor was ineffective, either as a soil or seed treatment. As a seed treatment, Orthocide was about as effective as Panogen in increasing the emergence of seedlings from cracked and uncracked seed, and post-emergence killing of seedlings was prevented as well. Orthocide used as a soil treatment proved equally as effective as a seed treatment in improving emergence of seedlings from both cracked and uncracked seed. Furthermore, this protection was extended since practically no post-emergence killing of flax occurred. T. lignorum spores provided no protection, either as a soil or seed treatment in field soil.

In the second greenhouse experiment, an attempt was made to establish whether soil fumigants Shell DD or Chloropicrin had any preferential fungistatic effect on R. solani or T. lignorum when these fungi were mixed together as inoculum in steamed soil. The experiment was divided into two parts. In the first of these, 800 grams of moist soil in each of 60 quart-sized mason jars were steam sterilized in the autoclave. The 60 jars of soil were divided into five series of 12 jars each. Fifteen grams of one-month-old soil-cornmeal inoculum were added to the soil in each of the 12 jars of one series. Fifteen grams of a similar culture of T. lignorum were added to the soil in another series. In a third series of 12 jars, 15 grams of R. solani and three grams of T. lignorum inocula were added; and to the fourth series, 15 grams of T. lignorum and three grams of R. solani provided the inoculum. The fifth series was left uninoculated as a control. All jars were thoroughly agitated to mix the inoculum throughout the soil. These were allowed to incubate with the tops slightly loosened at room temperature, which averaged 23° C. for 16 days. At the end of this period, the soil in four jars of each series was treated with three drops of Shell DD. The soil in another four jars was treated with four drops of Chloropicrin, and the soil in the remaining four jars was left untreated. The drops of chemicals were administered to the center of the soil in each jar with a one milliliter pipette, after a hole had been probed with a sterile glass rod. All jars were left tightly capped for 24 hours; then they were loosened for 48 hours to allow the volatile gases of the treatments

to dissipate. Part of the soil from each jar was then diluted with steamed soil in the proportion of 1:10. Soils in the four jars of similar inoculation and treatment were mixed together and placed in three-inch pots for planting.

In the second part of the experiment, jars were set up in the same manner as described above. However, instead of waiting 16 days before treatment, the soils were treated with the chemicals immediately after inoculation with the various organisms. The averages of the results from the four replications used in the two parts of this experiment are presented in Table VIII.

The most evident result was that Chloropicrin was more effective in controlling the fungus than was Shell DD at the concentrations used. It could not be demonstrated, however, that either Shell DD or Chloropicrin had preferential fungicidal properties for either R. solani or T. lignorum. The improvement in stands in both Shell DD and Chloropicrin treated soils seemed to be due solely to the fungicidal effect on R. solani. The antagonistic activity of T. lignorum was apparent where it was used in combination with R. solani for inoculating steamed soil. T. lignorum did not, however, after 19 days incubation with R. solani, provide better stands than those obtained with combinations of T. lignorum and R. solani incubated together for only three days. On the contrary, stands were reduced in soil inoculated with three grams R. solani and 15 grams T. lignorum after incubation for 19 days. This indicates that under the moisture conditions of the soil, R. solani was evidently able to increase during the period despite the

Table VIII. Per Cent Emergence and Survival of Flax Seedlings in Soils Inoculated with R. solani, and with Combinations of R. solani and T. lignorum, When the Soil was Treated with Two Soil Fumigants at Two Dates After Inoculum was Added to the Soil

Soil Fumigant	Inoculum[a] Combinations	Per cent Emergence		Per cent Survival	
		Undiluted[b]	Diluted[c]	Undiluted[b]	Diluted[c]
	Soil Fumigated Immediately After Inoculation				
Shell DD	R	32	42	22	29
	R-T	51	79	46	70
	T-R	83	83	76	80
	O	90	77	83	74
Chloropicrin	R	81	90	70	87
	R-T	84	91	86	87
	T-R	79	90	84	89
	O	85	90	89	90
None	R	4	11	2	5
	R-T	6	64	4	45
	T-R	53	83	45	72
	O	90	89	91	89
	Soil Fumigated 16 Days After Inoculation				
Shell DD	R	20	17	20	7
	R-T	22	18	21	18
	T-R	38	46	36	45
	O	88	91	87	91
Chloropicrin	R	48	80	58	59
	R-T	63	95	69	97
	T-R	64	79	65	75
	O	72	89	75	83
None	R	15	19	6	18
	R-T	23	35	23	30
	T-R	30	65	24	59
	O	91	95	89	91

[a] R - 15 grams R. solani inoculum added to 800 grams steamed soil.
R-T - 15 grams R. solani plus three grams T. lignorum inoculum added to 800 grams steamed soil.
T-R - 15 grams T. lignorum plus three grams R. solani inoculum added to 800 grams steamed soil.
O - no inoculum added.

[b] Undiluted inoculated soil.

[c] Inoculated soil diluted 1:9 with steamed soil.

presence of T. lignorum. The data for stands in soil inoculated with T. lignorum alone are not included in the table as they did not indicate any differences from the stands obtained in the check.

Varietal Resistance

One field and three greenhouse experiments were conducted to evaluate flax varietal resistance to seedling blight. In the field experiment, eight varieties were planted on July 1, 1956, on one of the land ranges at the Agronomy Farm where over 50 per cent of the flax stands were lost because of seedling blight. One hundred seeds of each variety were planted in four replications. Stand counts were determined six weeks after planting. The results of this experiment are shown graphically in Figure 15. The best stands were obtained with Marine, B5128, and C.I. 1478, but Redwood proved inferior. These differences, however, were related to the initial viability of the seed and for that reason, they cannot be taken to indicate true varietal differences resulting from resistance or tolerance to the soil-borne pathogens of the field soil.

The first greenhouse experiment was performed with 12 varieties of flax grown in steamed soil and steamed soil inoculated with four different concentrations of R. solani. The methods used for preparation and application of inoculum have been described on pages 30 and 32. The averages of the results from four replications are presented in Table IX. With the exception of Raja and B5128, there seemed to be a tendency for those varieties which produced best stands in steamed

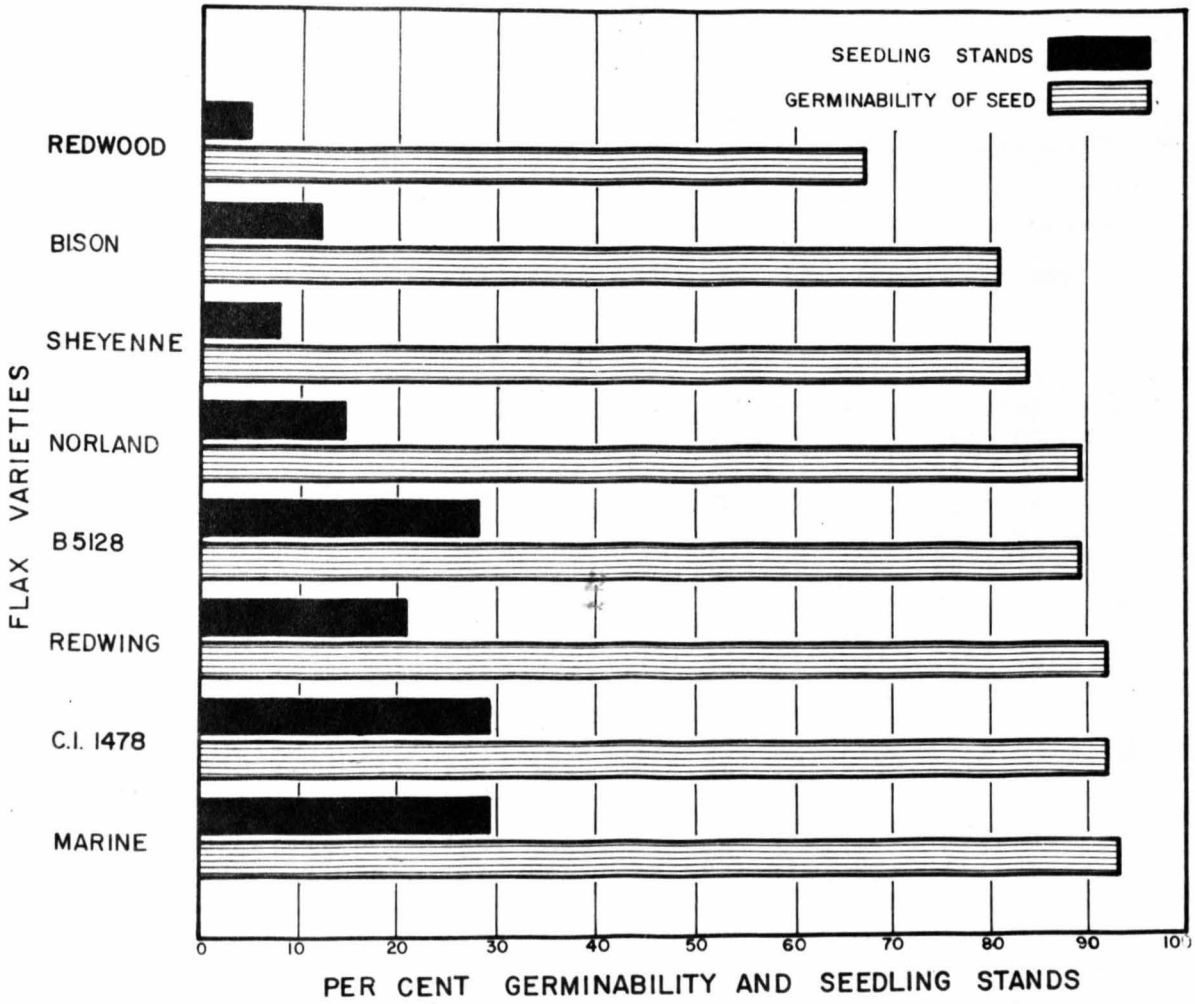

Figure 15. A Comparison of Germinability and Seedling Stands of Eight Varieties of Flax Grown Under Field Conditions at Brookings. Each bar represents per cent survival based on four replications.

Table IX. Per Cent Emergence and Survival of Seedlings of 12 Varieties of Flax Grown in Steamed Soil and Steamed Soil Inoculated with Suspensions of R. solani

	Milligrams of Wet Mycelium Per Pot									
Variety	0	.25	2.5	25	250	0	.25	2.5	25	250
	Per cent Emergence					Per cent Survival				
Marine	97	87	84	18	1	96	87	81	16	4
Cascade	92	89	74	21	4	95	83	68	23	4
Concurrent	90	76	76	30	7	93	80	68	33	8
Redwing	90	86	76	21	7	92	79	65	22	9
Norland	87	87	68	14	1	90	90	72	13	3
Bison	85	73	72	5	2	87	70	62	5	2
C.I. 1478	85	78	76	25	0	82	80	74	24	2
C.I. 1332	82	56	79	4	2	81	59	58	7	0
Raja	81	65	72	43	7	86	68	71	37	10
B 5128	81	70	79	41	2	81	74	74	41	3
Sheyenne	81	58	49	14	1	80	63	48	17	1
Redwood	69	57	39	6	0	66	59	41	6	0

soil to also produce the best stands in the inoculated soil.

It appeared that the initial viability of the seed varieties influenced the stands in the field; therefore, it was supposed that the amount of cracked seed in each variety might be related to the variation in germination and stand in field soil. Consequently, a second experiment was performed in the greenhouse to demonstrate this relationship. Thirty-six varieties were planted in field and in steamed soil and the percentage of cracked seed was determined for each of the varieties by microscopic examination of the seed lots. The averages of the results from four replications used in this experiment are presented in Table X. The varieties are listed in order from

Table X. Per Cent Stands in Steamed and Unsteamed Field Soil Compared with Percentage of Cracked Seed in Each of 18 Varieties of Flax

Variety	Per cent[a] Cracked Seed	Per cent Survival	
		Steamed	Unsteamed
C.I. 1427	9	81	31
Sheyenne	10	87	4
6916/40	12	68	6
Tammes Type 1	14	91	24
Sel. of Argentina	15	83	6
Buck 3	16	87	11
Marine	18	88	20
Redwing	20	92	8
Norland	20	90	9
Victory Sel. 3254	21	90	38
Bison	21	79	6
291 '27xSmokey Golden B41-2603	21	71	1
C.I. 1478	23	91	24
Redwood	24	89	9
C.I. 608	25	78	4
C.I. 1409	25	76	21
Rio (Long 79)	29	70	3
Kota	29	68	7

[a] Each figure represents the average percentage of cracked seed in three samples of 100 seeds of each variety.

lowest to highest per cent cracked seed. It did not appear that the amount of cracked seed of each variety influenced in any way the relative stands of these varieties in either steamed or unsteamed field soil. Furthermore, the stands in steamed soil did not appear to be as closely associated with stands in field soil as was the case when germination of eight varieties was compared with stands of flax grown under field conditions.

To evaluate more effectively flax varietal resistance to seedling

blight, a third greenhouse experiment consisted of separating seeds of 36 varieties into cracked and uncracked lots and planting these in steamed soil, R. solani inoculated steamed soil, and field soil. The averages of the results from three replications are presented in Table XI. The varieties are listed in order from highest to lowest percent stands from uncracked seed in steamed soil.

For most varieties, stands obtained from cracked seed were somewhat inferior to stands from uncracked seed in steamed soil. Difference in stand was not consistent among the varieties, however, for some varieties such as Smokey Golden, the difference in stand between cracked and uncracked seed ranged from 33 to 88 per cent, whereas for some other varieties, stands from cracked seed even exceeded stands obtained from uncracked seed. The difference in stands obtained from cracked and uncracked seed was most pronounced in field soil. There appears to be no association, however, between seed injury and severity of disease caused by R. solani in inoculated soil.

Varieties which produced the best stands in steamed soil also produced somewhat better stands in field soil. There were exceptions, however, which may indicate that there are real differences in varieties. Stands of C.I. 1427 were relatively low in steamed soil, but in field soil, stands were better than those produced by any other variety. Cascade seed, on the other hand, had a high percentage of viability; but stands in field soil were among the lowest.

Table XI. Per Cent Emergence and Survival of Seedlings from Cracked and Uncracked Seed of 24 Varieties of Flax Grown in Steamed, Inoculated Steamed, and Field Soil Under Greenhouse Conditions

	Viability		Inoculated Per cent Emergence		Inoculated Per cent Survival		Field Per cent Emergence		Field Per cent Survival[a]	
Variety	C	UC	C	UC	C	UC	C	UC	C	UC[b]
Cascade	80	96	66	84	0	1	5	9	5	9
Marine	89	95	96	82	3	1	17	20	13	14
Redwing	81	93	102	84	6	1	11	20	11	17
Kota	79	92	85	83	0	0	11	8	10	8
Tammes Type 1	73	92	94	73	4	3	18	25	16	22
C.I. 608	68	91	110	80	4	0	16	14	16	12
Concurrent	89	89	75	98	0	6	10	9	10	9
Benvenueto	72	89	100	107	4	13	22	19	13	17
Sel. of Argentina	83	88	83	90	0	1	20	14	18	10
Buck 3	68	88	90	89	1	1	13	15	10	15
Smokey Golden	33	88	88	86	9	5	9	9	9	9
Raja	64	87	105	75	5	0	14	23	17	22
C.I. 1478	75	85	67	81	0	14	9	19	9	14
Norland	88	84	91	87	1	4	8	23	8	19
Sheyenne	87	84	89	110	1	14	20	29	17	24
C.I. 1332	56	84	29	56	0	0	7	5	5	4
Victory	81	81	70	109	0	4	20	25	16	21
Redwood	73	81	97	110	7	4	11	35	11	31
Bison	65	79	111	106	6	4	3	19	3	15
B 5128	88	77	65	108	5	12	10	14	10	14
6916/40	69	73	75	103	6	9	0	33	4	29
C.I. 1409	68	64	82	94	0	0	30	20	16	17
C.I. 1427	67	63	91	133	0	0	30	62	28	44
Rio (Long 79)	53	52	89	125	2	6	9	13	6	8

[a] Each figure represents the average of three replications calculated as a percentage of the stands in the check.

[b] C - cracked seed UC - uncracked seed.

DISCUSSION AND CONCLUSION

Rhizoctonia solani causes a seedling blight of flax and is potentially one of the most important pathogens of that crop in South Dakota. It is almost universally present in the soil, and diseased seedlings have been found in almost every flax field inspected throughout the northeastern part of the state.

During investigations of seedling blight in South Dakota, several different organisms were found associated with diseased seedlings of flax. Experimental inoculations revealed that some of the organisms isolated were definitely parasitic on flax; others appeared to be weakly parasitic, and still others appeared unable to parasitize flax.

The specific organisms causing wilting or death of isolated seedlings were often difficult to identify on the basis of symptoms alone, but identification depended upon isolation studies in the laboratory. Superficial symptoms caused by several of the root parasitizing fungi are similar. Therefore, the importance of species of *Pythium* and *Fusarium* along with *R. solani*, in causing reduction of flax stands in South Dakota, must not be minimized. Isolation studies indicated the frequent coexistence of these fungi on diseased roots of flax. Environmental conditions undoubtedly play a very important part in determining which of the organisms are predominantly operative in causing stand losses in a given field.

Isolates of *R. solani* from flax varied considerably in culture. The isolates varied in color, zonation, and number and size of sclerotia

produced on potato-dextrose agar. About 27° C. appeared to be optimum for growth of most isolates on potato-dextrose agar, but some grew best at 25° C. and others at 30° C. Furthermore, the maximum and minimum temperature tolerance and rate of growth varied widely. Of the many isolates studied, at least 17 were sufficiently distinct from one another to be considered separate strains.

Isolates of R. solani from flax and other hosts varied greatly in their virulence on flax. Isolate number 534, obtained from diseased flax in the greenhouse, was so destructive that it killed entire stands of flax in relatively low concentrations of inoculum in steamed soil, whereas number 597 from soybeans was nearly as pathogenic, and was considerably more pathogenic than certain other isolates originating from flax. Host range studies indicated that, in general, monocotyledons were less susceptible than dicotyledons to one highly virulent R. solani strain. These studies indicate that crop rotation should be considered in the flax seedling blight problem.

It was found that R. solani inoculum survived for at least three months when mixed with field soil and stored in a dry condition at 20°-25° C. Furthermore, field soil in which about 30 per cent stands were obtained did not change in disease potential over a period of three months when stored in a dry condition. It is noteworthy that R. solani inoculum was reduced in virulence when it was stored in the dry state at 8° C. Stands were improved, however, in soil which was stored in a moist condition for three months. Microflora of soil in the field tested at intervals of three months over a nine

month period did not change appreciably, judging by the stands obtained.

These studies indicate that there may be a rather delicate balance among the constituents of a natural soil microflora, determined by such factors as cropping history, temperature, moisture, and mineral and organic content. When the balance is upset by a change in one or more of these factors, the level of invading pathogens may rise or fall, thus altering the disease potential of a soil. The presence of Trichoderma lignorum and other antagonistic organisms in natural soil will further serve to alter the disease potential of field soil in response to changes in environmental conditions.

Among the various factors affecting disease expression are depth and rate of planting seeds, soil temperature, amount of inoculum, and age of seedlings.

Experiments indicated that disease severity was positively related with depth of planting. Differences were most pronounced in R. solani inoculated soil. These differences were due either to the increase in inoculum during the time required for emergence of seed planted at greater depths, or the greater susceptibility of individual seedlings because of the necessity for increased elongation of the hypocotyl. It is probable that both of these factors contributed to the results obtained. These results serve to emphasize the current recommendation of shallow planting of flax in the field to avoid poor stands due to pre-emergence killing of seedlings.

It appeared from these studies that temperatures favorable for saprophytic growth of R. solani were most favorable for greatest disease expression represented by the rapidity of post-emergence killing of seedlings. Pre-emergence killing was also most severe at 27° C. in inoculated steamed soil, but in field soil pre-emergence killing was prevalent over a wide range of temperatures. Nevertheless, the temperature range at which most post-emergence killing of seedlings in field soil occurred remained at 23° to 27° C., which lies within the optimum range of temperatures for growth of most R. solani isolates. Under field conditions, the direct effect of temperature on the growth of the fungus in the soil from plant to plant may exercise an important influence under epiphytotic conditions.

It was observed that severity of disease was increased when the amount of R. solani inoculum was increased. It was further demonstrated that R. solani in increasing quantities in the proximity of the seed caused increased pre-emergence killing of flax, while R. solani inoculum distributed throughout steamed soil could increase saprophytically and cause post-emergence killing of seedlings several days after emergence.

It was observed in several experiments that there appeared to be a limit to the total amount of damage caused by a certain amount of R. solani inoculum in steamed soil. Seedlings apparently became "resistant" after they reached a certain stage of development. Other experiments proved that flax seedlings were killed rapidly at considerably later stages in their growth. It is the writer's opinion that part of the reason for the cessation of damage is due either to the

accumulation of staling products of R. solani itself which inhibit further saprophytic progress of mycelium in the soil, or to an increase of organisms antagonistic to the growth of R. solani.

Experiments showed that spacing of plants and amount of inoculum influenced the amount of disease. Apparently, the tissues of diseased seedlings acted as further sources of nutrient and the fungus grew saprophytically from these points and spread to other seedlings. The initial source of inoculum, however, appeared to be of primary importance in the spread and dissemination of the fungus, because this determined to a large extent the total number of seedlings killed in a given time.

Under field conditions, progress of the disease may quickly assume epiphytotic proportions, depending upon the denseness of the seedling population within a row. However, regardless of the denseness of seedlings, the rate of growth of R. solani in the soil under ideal conditions is very rapid. As a result, almost 100 per cent seedling mortality may be caused by R. solani originating from relatively few centers of inoculum in the soil.

The three general methods of control of seedling blight studied were the use of chemical fungicides for seed and soil treatments, the use of the antagonistic T. lignorum, and the use of resistant varieties.

Neither seed nor soil treatments improved flax yield in field tests, but certain fungicides were effective in improving stands in infested soil in greenhouse tests. Of the fungicides tested, Orthocide

appeared to be moderately effective in both field soil and inoculated field soil when used as a soil treatment. Terrachlor, on the other hand, was very effective as a soil treatment in inoculated soil. Panogen was more effective as a seed treatment in field soil, but was comparatively less effective in inoculated soil. These results serve to emphasize the influence which the complex microflora of the soil have on expression of disease and response to various treatments. It is apparent that there is need for exercising extreme caution in basing conclusions on results obtained from experiments involving steamed soil inoculated only with the pathogenic organisms.

There appeared to be little real difference in resistance or tolerance among 36 varieties of flax to R. solani or other pathogens in field soil. However, the vigor of seed lots of individual varieties appeared to be a factor affecting the stands. Cracks in the seed coat evidently predisposed the seed to invasion and injury by soil-borne pathogens, for emergence from cracked seed was markedly reduced in infested soils. This effect was most marked in field soil, but it was also evident in R. solani inoculated soil. However, the amount of seed injury was in no way related to the severity of post-emergence killing. It was thought, therefore, that stands in infested soil may have been related to the percentage of cracked seed in the sample; but this relationship could not be demonstrated experimentally.

It can be concluded from these studies that R. solani can attack flax plants independently of variety or seed condition. Furthermore, other workers (6, 24, 33) have concluded that R. solani is not

specifically pathogenic to certain varieties of plants. Thus, if a strain is virulent on one variety, it is virulent on all varieties. If this principle be true, it seems that there may be some hope of improving resistance by selection among varieties grown in infested soil.

SUMMARY

1. One of the most important diseases of flax in South Dakota is seedling blight, which under severe conditions may almost eliminate stands.
2. Certain of the associated fungi isolated from diseased seedlings were non-pathogenic in inoculation tests; some were weakly or only occasionally pathogenic; some were definitely pathogenic, especially Rhizoctonia solani, Fusarium spp., and Pythium spp.
3. Symptoms of R. solani parasitism on flax seedlings under field conditions were similar to those obtained in steamed soil inoculated with isolates of the fungus. Other fungi, such as species of Fusarium and Pythium, were recognized as possible complicating factors under natural conditions.
4. A number of different isolates of R. solani from flax and from other hosts were compared culturally, and also compared with respect to their parasitism on Marine flax.
5. Isolates of R. solani from flax and other hosts differed in color, zonation, amounts and size of sclerotia formed, and ability to parasitize Marine flax.
6. All isolates grew best on potato-dextrose agar at 25° to 30° C., but their rate of growth and tolerance to maximum and minimum temperatures varied widely.
7. Temperatures of 25° to 30° C. appeared most favorable for growth and parasitism of a particular R. solani isolate in steamed soil. The optimum temperature for post-emergence killing of flax

seedlings appeared to be closely correlated with the optimum temperature for the growth of the fungus in pure culture.

8. Pre-emergence killing of seedlings was extensive over a wide temperature range in field soil, but post-emergence killing appeared to be correlated with the optimum temperature range for the growth of R. solani in culture.

9. Under pure culture conditions in steamed soil, there was some indication that Trichoderma lignorum inhibited the growth of R. solani.

10. The disease potential of a field soil was not reduced by dry storage for three months, but it was reduced when soil was stored in a moist condition. Additional R. solani inoculum added to field soil apparently was inactivated when stored moist at both high and low temperatures and dry at 8° C., but was not inactivated when stored dry at 25° C.

11. The severity of stand reduction caused by R. solani was shown to be increased both by closer spacing of plants within a row and application of greater amounts of inoculum.

12. Flax plants appeared to be most susceptible to R. solani during and soon after seed germination, but declined in susceptibility as the seedlings developed.

13. Flax planted five-tenths centimeters deep in infested soil escaped disease more successfully than flax planted at greater depths.

14. Fungicides used as seed and soil treatments did not improve yields of flax in field tests, but stands of flax were improved with

certain seed and soil treatments in greenhouse tests when flax was planted in field soil and in steamed soil inoculated with R. solani.

15. Poorer stands generally were obtained from cracked seed than from sound seed. The mortality occurred primarily as pre-emergence killing of seedlings which was most marked in field soil, followed by R. solani inoculated soil, and steamed soil.

16. Both Chloropicrin and Shell DD were fungicidal, but preferential fungicidal activity in mixtures of R. solani and T. lignorum inoculated soil could not be demonstrated.

17. It is doubtful that any of the 36 varieties of flax tested in the greenhouse had appreciable resistance to the virulent R. solani isolate 534. Differences were noted among varieties when they were planted in the field and in the greenhouse in field soil, but these differences were attributed to initial viability of the individual seed lots. However, this initial viability apparently was not related to the amount of seed injury.

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