

ETIOLOGY OF BEET SEEDLING
NECROSIS AS A BASIS FOR CONTROL

by

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ETIOLOGY OF BEET SEEDLING NECROSIS AS A BASIS FOR CONTROL

INTRODUCTION

Beet seedling necrosis is analogous to beet seedling diseases described in the literature under the names damping-off (7, pp. 37-46), black root (16, pp. 364-380), and black leg (63, pp. 402-404). The general characteristics and symptomology have been sufficiently described by Beach (7, pp. 37-46), Chester (14, pp. 238-243) and Walker (66, pp. 62-64). While the disease is widespread and the symptomology is essentially the same wherever it occurs, the suggested means for control are often specific for different geographic areas. Among the factors which could be considered responsible for this disparity are differences in soil type and fertility, environmental influences, source of infection and variability in pathogenicity and the number of organisms associated with the disease. The purpose of this investigation was to determine those factors which are essential to the development of beet seedling necrosis in the Willamette Valley and to use this information as a basis for formulating an adequate means of control.

Beet seedling necrosis is the cause of poor stands of beets and ensuing reduced yields. Losses resulting from the cost of replanting, diminished yields and

delayed planting dates have made it necessary for some growers to substitute other crops.

Personal observations and interviews with growers and representatives from beet canneries resulted in the following inferences. Severe incidence of seedling necrosis is not dependent upon the culture of beets year after year in the same location. Some fields were severely affected the first year beets were planted. In these cases the disease continued to be devastating in subsequent years. Beet crops in some fields are affected intermittently suggesting the influence of seasonal variations. The disease is of minor importance in some locations and apparently absent in others. Large areas of some fields may be affected by seedling necrosis, while in other instances the disease seems to recur in many small restricted areas within a field.

Seedling necrosis is characterized by the occurrence of both preemergent and postemergent deaths. In some geographic areas one phase of the disease is more prevalent than the other; a condition which greatly influences the effectiveness of control measures. Although both phases of the disease occur in Willamette Valley soils death of seedlings after emergence causes the greatest economic losses.

The incidence of beet seedling necrosis is most

severe with late plantings. Early spring plantings during cool weather are less affected. Only a limited amount of control could be expected by planting early, since planting dates for a particular crop must be spaced over an extended period of time to accomodate the facilities of canneries.

On the basis of these preliminary observations, a plan for experimentation was formulated and effectuated.

LITERATURE REVIEW

The etiology and control of beet seedling necrosis are specific problems for different geographic areas. The following compilation illustrates both the disparity and agreement which exists between data presented by various workers. Much of the literature on beet seedling diseases is confined to sugar beets rather than to garden beets. These two agronomic groups are not distinguished botanically and records of associated organisms often do not discriminate between them (68, p. 138).

Berkeley (9, pp. 67-123) in reviewing the root rots of sugar beets, listed five different fungi which in general are associated with black root, namely; Aphanomyces cochlioides, Fusarium species, Phoma betae, Pythium debaryanum, and Rhizoctonia solani. In addition to these, Alternaria tenuis, Aphanomyces laevis, Macrosporium cladosporioides (21, pp. 19-23), Pythium aphanidermatum (27, pp. 23-43) and Pythium ultimum (33, p. 788) have been reported as causing beet seedling diseases.

Afanasiev (1, pp. 205-212) reported beet seedlings were artificially inoculated with six different fungi. Fusarium, Macrosporium and Rhizoctonia were only slightly pathogenic, Phoma and Pythium were moderately so, and Aphanomyces cochlioides was most pathogenic on sugar beet

seedlings. Since the symptoms produced by Aphanomyces cochlioides on artificially inoculated beets resembled most closely those manifested by diseased seedlings in the field, this fungus was believed to be responsible for most of the diseases in Montana. According to Leach (33, p. 788) the three pathogens most frequently responsible for damping-off of sugar beets in California are Pythium ultimum, Rhizoctonia solani, and Phoma betae. The first two are common in field soils, while Phoma appears to originate only from imported seed. Reddy (52, p. 69) reported that species of the same three genera of fungi were the cause of sugar beet seedling diseases in Iowa. In Germany according to Esmarch (21, pp. 19-23) Phoma betae, Pythium debaryanum, and Aphanomyces laevis rank in the order given as the principal parasites on seedling beets. Hildebrand (27, pp. 23-43) in Canada, isolated Pythium aphanidermatum from sugar beet seedlings, as well as Pythium ultimum and Aphanomyces cochlioides.

Buchholtz (12, pp. 490-496) compared the relative importance of Pythium debaryanum and Aphanomyces cochlioides and found that Pythium debaryanum killed thirty-three per cent of beet seedlings grown in lightly infested soil, ninety per cent in heavily infested soil, and sixty per cent in soil where both fungi were present. The number of seedlings killed by Pythium debaryanum was

nearly complete fifteen days after planting. Infection of the remaining seedlings by Aphanomyces cochlioides began when the seedlings ceased dying from Pythium debaryanum. Buchholtz (11, pp. 448-475) also studied the factors influencing the pathogenicity of Pythium debaryanum on sugar beet seedlings in northern Iowa and found damping-off to be more severe on acid soils (below pH 6.5) at moderately high soil temperatures (above 15°C.). Pythium debaryanum caused over ninety-five per cent of the field damage to germinating, emerging and emerged seedlings. Seed borne Phoma betae acted slowly and was observed on and isolated from seedlings from infected seed when Pythium was relatively inactive. Rhizoctonia was isolated rarely from field grown seedlings. Temperatures below fifteen degrees Centigrade were unfavorable both to the growth of Pythium debaryanum in pure culture and to its pathogenicity to sugar beet seedlings.

A study of sugar beet seedling diseases by Warren (67, pp. 883-892) showed that Aphanomyces cochlioides was primarily responsible for black root in Ohio, with Pythium debaryanum and Rhizoctonia solani being less important. While Rhizoctonia solani was not isolated regularly from infected seedlings, some of the isolates were more virulent than either Aphanomyces cochlioides or Pythium debaryanum. Afanasiev (1, pp. 205-212) discovered the same type of

situation with *Fusarium* species. In determining the pathogenicity of forty-seven isolates of *Fusarium*, the average number of diseased sugar beet seedlings was 14.9 per cent, but this percentage varied from 3.8 to 95.5 for individual cultures.

Kotila (32, pp. 741-743) in describing the perfect stage of *Rhizoctonia* on sugar beets, found this fungus induced a postemergence type of damping-off in sugar beet seedlings, reducing stands to 61.5 per cent of controls. It proved to be non-pathogenic to older roots. Maxson (43, pp. 38-45) reviewed the occurrence of *Rhizoctonia solani* on sugar beets.

In a study of the effects of field conditions and field practices on the development of black root in sugar beets, the plants in fields planted late or replanted and those with low fertility or poor drainage showed relatively poor recovery from black root. Among individual treatments, immediate aeration with a rotary hoe, weeder, or spike-tooth harrows saved 68 per cent of the crop, the use of a roller or cultipack saved 34 per cent. (57, p. 466).

According to Kadow and Anderson (31, pp. 291-384), the chief variables affecting the pre-emergence phase of damping-off of vegetable seedlings were soil moisture and temperature. High humidity and high temperature were

the main factors during post-emergence, though soil moisture was also important. Young (71, pp. 127-129) found that the lowest incidence of black root and the best sugar beets developed at 54° and 61° Fahrenheit in slightly dry and slightly wet soils. Seedling diseases in general were much less prevalent in light, well aerated soils than in heavy ones. Coons, Stewart, and Kotila (17, pp. 38-44) also reported on the advantages of adequate drainage, prompt and thorough stirring and loosening to aerate and dry the soil. Le Clerg (38, pp. 1-7) states that damping-off of sugar beets is a destructive disease in early plantings on moist soils in Minnesota. Black leg, caused by Phoma betae, is reportedly favored by cold wet conditions (49, p. 464), while Stirrup (63, pp. 402-404) states that black leg, caused by Phoma betae, species of Rhizoctonia and Pythium, is favored by poor tilth in the seed bed and low soil temperature. Leach (36, pp. 161-179) found that the temperature ranges in which the most severe preemergence infection of sugar beets occurred were: Pythium infested soil, 12° to 20° Centigrade; Rhizoctonia, 16° to 30°; and from seed infested with Phoma betae, 4° to 20°. In the absence of any chemical soil treatment against damping-off, Osmum (47, pp. 18-24) found that stands of vegetable seedlings under glass may be improved by post-

poning the first watering. Stands of eight commonly grown vegetables including beets, were better when the soil was not watered until three to five days after seeding. Chester (14, pp. 238-243) states that damping-off caused by *Pythium* can be much reduced by planting in soil with no more than 30 per cent moisture and not watering until four to five days after planting.

With heavy seedings of beet seed balls, preemergence damping-off may go largely unnoticed, but when segmented seed and very low seeding rates are employed to make use of mechanical thinning methods, serious lowering of stands may be experienced. Treatment of segmented sugar beet seed with New Improved Ceresan or Yellow Cuproside greatly reduced damping-off of seedlings (23, pp. 12-13).

Controlled tests by Leach (35, pp. 8, 29) have shown that sheared beet seed can be effectively protected against *Pythium* and *Rhizoctonia* by treating with either Ceresan or New Improved Ceresan, but the seed should be planted within a few days to avoid injury. Seed treatment failed to control *Aphanomyces*, but early planting avoided infection. (34, pp. 65, 68). Afansiev and Morris (3, pp. 477-486) reported that seed treatments with Ceresan and New Improved Ceresan proved to be only slightly beneficial for controlling sugar beet seedling diseases in Montana.

Greenhouse experiments by the same workers (5, pp. 331-340) on coating of beet seeds with fungicides and different amounts of treble superphosphate or with various combinations of treble superphosphate and sodium nitrate, using glue as a sticker, showed that neither the fungicides nor the fertilizers produced a satisfactory control of sugar beet seedling diseases. It was concluded that an adequate and properly balanced fertilization of soil is much more important in controlling seedling diseases of sugar beets than either of the above mentioned treatments. A review of black root diseases of sugar beets in the United States during 1941 by Coons, Kotila and Bockstahler (15, pp. 436-437) indicated that with disease incidence minor, treatment of seed with commonly used copper and mercurial fungicides at Beltsville, Maryland, gave no significant results. In similar experiments at East Lansing, Michigan, with somewhat increased disease incidence, initial stands were significantly improved by all fungicide treatments employed in comparison with untreated plots.

Using naturally infested soil, Hildebrand and Koch (26, pp. 557-567) made studies in the greenhouse on the incidence and severity of black root of sugar beet seedlings in relation to aeration by cultivation, row application of commercial fertilizer, seed treatment and

incorporation of green manure in the form of a cover crop. Black root was substantially greater in cultivated than in non-cultivated soil whether or not fertilizer was added. Germination was poorest in non-fertilized, cultivated soil and highest in cultivated soil with fertilizer added. Black root was less prevalent when a 2-16-10 NPK commercial fertilizer was applied in the row at the rate of 400 pounds per acre, half with the seed and half placed an inch and a half below the seed. Seed treatment with Ceresan increased germination and protected seedlings against preemergent damping-off, but failed to protect them during the post-emergent phase of the disease to ensure a profitable stand.

Incidence of black root was lower in root rot soil with which cover crops of corn or soybean had been incorporated than in lots of the same soil sterilized with steam. The vigor of seedlings in the soil which had a soybean cover crop was markedly greater than that of any other series including corn. Black root infected seedlings developed into mature beets, which, in the aggregate, gave a yield only fifteen per cent less than that resulting from the use of initially healthy seedlings. At Holgate, Ohio, (15, pp. 436-437) effects of crop sequence were tested in an experiment involving sweet clover, corn, and soybeans followed by sugar beets. Stands

were significantly better when sugar beets followed corn or soybeans but not when they followed sweet clover.

Campbell (13, p. 785) found that calcium cyanamide added at the rate of 135 pounds per acre a week before planting resulted in an average of 231 per cent increase in healthy plants and gave an excellent growth of annual beets. Formaldehyde dust, New Improved Ceresan, and hydrated lime, added the proper amounts at planting time, were only slightly less effective in the control of black root.

Only a few fungicidal materials are used for soil treatment. Most commercial fungicides are intended for the treatment of seeds or for the spraying of foliage and fruit. Among the relatively new organic fungicides, a few have been used, at least experimentally, for the treatment of soil. For example, soil treatment with Arasan was effective against damping-off of cucumber, pepper, spinach, and tomato, although control was more complete when seed and soil treatment with this fungicide were combined (44, p. 15). Dithane applied to soil at the rate of 75 pounds per acre gave better control of damping-off of peas than did seed treatment with Spergon (25, pp. 295-297); and there was marked reduction in the severity of root rots of bean when Dithane D-14 was applied as a soil treatment in the planted row at the time of seeding (37, p. 363). Wettable Spergon at 0.7 grams per square foot was effective in protecting tomato seedlings against

damping-off when applied to soil immediately after transplanting. (7, pp. 1-26).

Doran (20, pp. 1-28) showed that certain fungicides could be conveniently and effectively applied to soil in commercial fertilizer used as a carrier. Phygon applied to the soil in fertilizer gave good results with eggplant, pepper, beet, cucumber, and tomato. Stands of beet, cucumber, and tomato were also improved by Dithane D-14, Dow Seed Protectant No. 9, MTD, and Tuads similarly used. The possibility of such combinations of fungicides and fertilizer had been suggested before (18, pp. 100-103 and 19, p. 848). More recently Hildebrand, McKeen and Koch (27, pp. 23-43) controlled black root of sugar beet seedlings using three or four pounds of Arasan in 300 to 400 pounds of fertilizer per acre, applying it in the row and above the seeds.

Afanasiev and Morris (3, pp. 477-486 and 4, pp. 1-23) concluded that seedling diseases of sugar beets in heavy, irrigated Montana soils can be effectively controlled with good stands and high yields by creating conditions conducive to rapid and healthy development of young sugar beets, through sufficient and balanced fertilization and improvement of the physical condition of the soil. Seed treatments proved to be only slightly beneficial for controlling seedling diseases but soil treat-

ments, regardless of seed treatments, proved highly important. The effect of phosphorus and nitrogen added to the soil in seven different ratios in the form of equivalent amounts of various fertilizers was studied in relation to seedling diseases of sugar beets grown under glass. One of three series of experiments received manure. Seedling diseases, in general, were lowest in the series where treble superphosphate, ammonium sulphate, and manure were applied, and highest with treble superphosphate and ammonium phosphate. Where treble superphosphate and calcium nitrate were used the amount of seedling diseases was intermediate. The amount of seedling diseases was well correlated in all series; the lowest was in the P:N ratio 1:3 and 1:2, and the highest with 3:0 and the check (2, pp. 407-411).

As a result of field, greenhouse, and laboratory studies, Young (72, pp. 212-218) claimed that black root of sugar beets could be controlled by the use of ample manure or other organic matter. Where severe, use of 400-500 pounds of superphosphate (0-20-0) per acre in the row with the seed, supplemented by plowing down nitrogen and potash, or a complete fertilizer at the rate of 300-400 pounds per acre in the row below the seed was effective. Hutchins and Krantz (30, pp. 19-23) and Nuckols (46, pp. 121-137), are among those who have also

reported on the beneficial effects of manure and other organic matter on the growth and yield of beets.

Lehr (39, pp. 237-244) considers sodium an indispensable nutrient element for beets, approaching potassium in importance. Bower and Wadleigh (10, pp. 218-223), Hopkins (28, pp. 333-336), and Sayre (55, pp. 235-239) have also reported on the tolerance and response of table beets and sugar beets to sodium. Sayre (54, pp. 153-161) found that beets gave striking responses to various levels of potassium and sodium. Sodium chloride can be substituted for a portion of the K_2O in mixed fertilizers where the supply of K_2O is inadequate for the maximum growth of the beets. At all K_2O levels tested the substitution of sodium chloride for one fourth of the K_2O resulted in no reduction in yield and in most cases significantly increased the yield of beets. The optimum fertilizer recommendation for beets appeared to be 75 pounds of K_2O and 500 pounds of sodium chloride per acre. For beets, sodium chloride should be considered more as an important ingredient of the fertilizer mixture rather than as a substitute for part of the K_2O .

According to Lehr (41, pp. 399-411) sodium can replace potassium to a large extent without detriment to the beet root. Calcium, when present in relatively large

quantities, very soon has a detrimental effect on the production of both foliage and root. For practical beet culture sodium should be preferred as a secondary ion of fertilizers. It tends to have a potassium-conserving effect. Fertilizer experiments by Steenbjerg and Dorph-Peterson (61, pp. 668-672) showed that the effect of sodium on beets lessens as potassium increases. Harmer and Benne (24, pp. 952-979) stated that the use of sodium chloride in phosphate-potash mixtures increased the growth of both roots and tops, decreased damping-off and black rot of roots, and caused healthier, glossier leaves which appeared more resistant to the attacks of such diseases as leaf spot of beets. In the absence of K_2O fertilization, applications of sodium chloride gave very low yields of beets, unhealthy growth of roots, chlorosis of the foliage and markedly decreased sugar content.

Experiments by Lehr (40, pp. 373-379) with fodder beets grown on sandy soil show that secondary ion or cation, sodium in this case, has a very marked effect on the fertilizing value of the anion, NO_3 . Sodium nitrate gave a substantially higher yield of beets than either calcium nitrate or ammonium nitrate plus calcium carbonate. The last two sources of nitrate gave approximately equal yields. The effect of the secondary ions in these cases is

as important as the effect of the main component. The effect of the secondary ion is most pronounced in weakly buffered soils. The effect is not negligible on clay soils, although not as great as on sandy soils. In another report, Lehr (42, pp. 479-486) states that the addition of $\text{Ca}(\text{NO}_3)_2$ even in mixture with NaCl does not give greater yields than the addition of chilean NaNO_3 alone. Sayre (53, pp. 453-456), testing for the effects of side dressings of different sodium and nitrogenous salts on the yield of beets, found that sodium as either sodium nitrate or sodium chloride supplemented with nitrogen apparently supplied a definite nutrient need. The highest yields came from the three treatments that had either 500 or 1000 pounds of sodium chloride plus 160 pounds of ammonium sulphate; sodium carbonate or smaller amounts of sodium chloride were less beneficial. Skuderna (58, pp. 138-146) found that sodium nitrate proved to be a better source of nitrogen for sugar beets than ammonium sulphate. From a series of field experiments by Schulze (56, pp. 452-458) which included beets, the conclusion was drawn that the inferiority of ammonium sulphate as compared with sodium nitrate is due in large measure to the effect of the sodium in the nitrate. The author recommends that ammonium sulphate should always be used in combination with an amount of salt

sufficient to supply sodium equal to that in an equivalent application of sodium nitrate. The results of experiments by Strohmer and Fallada (64, pp. 425-441) indicate that the sodium chloride-ammonium sulphate mixture may profitably replace sodium nitrate under certain conditions.

Raleigh (50, pp. 433-436), studying the relative effects of sodium and chloride ions in the nutrition of table beets in culture solutions, found that the addition of chlorides, in general, gave a much more consistent increase in growth than did additions of sodium.

Tolman and Stoker (65, pp. 1072-1079) describe the manifestations of both nitrogen and sulfur deficiencies, which are evident on sugar beets grown for seed in the Willamette Valley in Oregon. They found an interaction response of sulfur and nitrogen, both on plant development and on seed reproduction. Sulfur application did not affect seed production on plots where nitrogen was not applied. The response to nitrogen was much greater in the presence of the sulfur treatment.

When placed either in contact with sugar beet seed or in close proximity in greenhouse tests with Yolo loam, Pendleton and Robbins (48, pp. 1-26) found all nitrogen-carrying fertilizers strongly depressed germination of sugar beet seed. Phosphorus fertilizers depressed germination somewhat less, but were injurious. One inch of soil

separating seed from fertilizer was insufficient for protection. Seed planted two and one-half inches deep germinated only thirty per cent as well as that planted one and one-half inches deep. Baur, Walters, and Cumings (6, pp. 142-146) in comparing eleven placements of four hundred pounds per acre of 10-20-20 fertilizer showed that placement of fertilizer had a marked effect on stand, which in turn affected the size of the beets as well as total yield. The best stand was obtained where the fertilizer was broadcast or placed one to two inches directly below the seed.

GENERAL METHODS AND MATERIALS

Several materials and techniques were used repeatedly throughout the course of this investigation. Methods which were designed to suit the purposes of specific experiments are presented with the description and results of the work. Such arrangement is convenient and appropriate for this type of investigation, which involves a sequence of separate, but related experiments.

Culture media. Potato dextrose agar was used for culturing the microorganisms associated with beet seedling necrosis and was prepared with the following formula:

Ingredients per liter:

Infusion from 200 g. of potatoes

Dextrose, 20 g.

Yeast extract, 2 g.

Agar, 20 g.

Distilled water to volume

A beet agar medium was prepared by surface sterilizing fresh, healthy, beet seedlings, rinsing them in sterile water and suspending them in two per cent water agar just before solidification. While Pythium ultimum would not sporulate on the potato dextrose agar, it was found that reproductive structures were produced in abundance on the beet agar medium. Consequently this medium was

employed in the identification of this organism.

Fungi were introduced into sterile soil by first growing the organisms on sterile oat grain and then mixing the infested grain with the soil. Three grams of whole oat grains were placed in a six inch test tube, covered with distilled water and allowed to soak over night. Cotton plugs were placed in the tubes and they were steam sterilized at fifteen pounds pressure for twenty minutes. The prepared grain tubes were stored in a refrigerator until used.

Surface sterilization. Surface sterilization of all plant parts was accomplished by immersing the parts in commercial sodium hypochlorite, diluted one to five with water, for varying lengths of time, depending upon the nature of the plant tissue involved. Infected hypocotyls of young beet seedlings were treated in the disinfectant from one-half to two minutes. Beet seed balls were treated from three to five minutes. The rapid penetration of the disinfectant into infected seedling hypocotyls made it necessary to control the time of treatment so that organisms within the tissues would not be killed.

Surface treatment with sodium hypochlorite was usually followed with rinsing of plant parts in sterile water. This step was not always employed when the time

for treatment was not critical, since the potency of the compound is lost with the volatilization of the chlorine.

Purification of fungus cultures. Bacteria free cultures of fungi were attained by the method described for this purpose by Raper (51, p. 342). Three glass beads were fused to the bottom of a Van Tiegham cell so that its lower rim was elevated about one-sixteenth of an inch when the cell was placed in a Petri plate. The elevated cell was first sterilized by dipping in ninety-five per cent ethanol and flaming and then placed upright in a sterile Petri plate. Melted potato dextrose agar was poured into the plate until the medium was about half way up the side of the Van Tiegham cell. The agar flowed under the lower rim filling the inside of the cell to the same level as that outside of it. After solidification, hyphae from a contaminated fungus culture were transferred to the surface of the agar inside the cell. The fungus grew down through the agar, under the lower rim and onto the surface of the agar outside the cell. A new hyphal transfer was made from here to another Petri plate.

Bacteria should not be able to penetrate down through the agar and escape the confines of the cell. It was found, however, that certain bacteria would follow the growth of the fungus hyphae through the agar and would still be with the fungus outside the cell. This situation

was rectified by acidifying the agar medium with lactic acid to inhibit the growth of the bacteria. Acidification of the agar alone or the use of the modified Van Tiegham cell alone often was not sufficient to purify some of the fungus cultures.

Cultures of Pythium ultimum free from bacteria were often attained by merely growing the fungus on two per cent water agar. The absence of nutrients in the media was sufficient to inhibit growth and movement of bacteria.

Single spore isolations of *Fusarium* species were accomplished by first preparing a dilute spore suspension in sterile water. A wire loopful of the suspension was distributed over the surface of a thin layer of water agar in a Petri plate. The spores were allowed to germinate and were then located with a compound microscope. Well-isolated spores were brought directly to the center of the visible field in the microscope. The diaphragm on the microscope was closed down so that a small beam of light passed directly up through the agar where the isolated spore was located. The objective of the microscope was raised, moved out of position and the spore located by the position of the light beam.

The illuminated section of agar was cut out and transferred to a Petri plate containing potato dextrose

agar. The spore was again located by means of the microscope to verify the transfer of a single spore.

Single spore cultures were employed when identifications of fungi were made. Mass cultures were used for all inoculation experiments.

Source of Beet Seed. Detroit Dark Red Table Beet seed were used in all of the experiments. This variety is used almost exclusively at the present time by commercial beet growers in Oregon.

Beet seed was obtained from the following sources:

Ferry-Morse Seed Company, Mountain View,
California.

California Packing Corporation, San Francisco
California.

Lorin Jones, Corvallis, Oregon

The Ferry-Morse Company provided decorticated seed and natural seed from the same seed lot. These two seed samples were used to test the effects of decortication upon the incidence of seedling necrosis.

Hot water treatment of beet seed. A shallow metal pan was placed on a tripod stand and filled with water. An electric stirrer was used to agitate the water vigorously. The water was heated by a gas flame and a continuous record of the temperature maintained by suspending a thermometer in the water. The temperature was kept

constant at 55 degrees Centigrade by adjusting the intensity of the flame. Twenty-five grams of beet seed balls, tied loosely in a thin cheesecloth bag, were immersed in the heated water for one half hour. After treatment the seed balls were spread out on paper towels until dry and then stored in stoppered bottles for future use.

Preparation of fungicides and fertilizers. Six commercial fungicides were employed in this investigation. They were as follows:

Ceresan M, Ethyl mercury p-toluene sulfonanilide,
Du Pont-Semesan.

Manzate, Manganese ethylene bisdithiocarbamate,
Du Pont.

Arasan, Tetramethyl thiuramdisulfide, Du Pont-Semesan.

Fermate, Ferric Dimethyl dithiocarbamate, Du Pont.

Orthocide 406, N-trichloromethylthio tetrahydro-phthalimide, California Spray Chemical.

Niagara C.O.C.S., Copper oxychloride sulphate,
Niagara Chemical.

All of the fungicides were incorporated into one per cent dusts using one of the following materials as a carrier:

Attaclay, Attapulgius Clay Company, Philadelphia
Friarite, California Industrial Mineral Company,

Friant, California.

The fungicide dusts were prepared by the Gresham Berry Growers Association, Gresham, Oregon.

The following fertilizers were used in the field experiments:

| | |
|-----------------------|------------------------|
| Calcium nitrate | 15.5 per cent N |
| Ammonium sulphate | 21.0 per cent N |
| Muriate of potash | 60.0 per cent K_2O |
| Treble superphosphate | 42.0 per cent P_2O_5 |

In addition to those listed above, the following fertilizers were also used in greenhouse experiments.

| | |
|------------------------|------------------|
| Chilean sodium nitrate | 16.0 per cent N |
| Sodium chloride | 39.4 per cent Na |
| Potassium chloride | 52.4 per cent K |

Two fertilizer combinations were used in the field tests during 1952. Fertilizer one was composed of 64.52 pounds of calcium nitrate, 16.67 pounds of muriate of potash and 28.57 pounds of treble superphosphate. Fertilizer two was composed of the same amount of muriate of potash and treble superphosphate with 47.62 pounds of ammonium sulphate as the nitrogen source.

The components of each fertilizer combination were mixed in a concrete mixer. The fertilizers used in greenhouse experiments were applied separately without prior mixing.

Rate of application of fungicides and fertilizers.

A Scott's model 25 lawn seed and fertilizer spreader was used to apply the fungicide dusts and fertilizers to the surface of the soil in the field. The rate of application was regulated by adjusting the size of the delivery holes which allowed the material to drop from the hopper to the ground and by attempting to maintain a constant speed while pushing the spreader.

The amount of fungicide or fertilizer to apply to a given area of ground was calculated by determining the fraction of an acre in the area to be treated and multiplying this by the desired number of pounds per acre of the material. The size of the delivery holes in the Scott spreader were adjusted so that a given number of pounds of fungicide or fertilizer were applied over a given area at a given rate of speed. The adjustment was made by applying fungicides or fertilizers with the spreader to a strip of wrapping paper three feet wide and twenty feet long. The width of application by the spreader was eighteen inches. The material applied to the paper was removed and weighed. The adjustment of delivery hole size was continued until the correct weight of material was released by the spreader. A scaled adjustment lever made it possible to regulate the flow of materials at any desired rate.

In greenhouse experiments, the rate of application of fungicides and fertilizers was determined by first calculating the fraction of an acre in a certain sized clay pot. For example, a clay pot with a top diameter of 0.5 feet would have an area of 0.1964 square feet. Assuming 43,560 square feet per acre, the surface of the soil in such a clay pot would occupy 4.52×10^{-6} acres. The weight of fungicide or fertilizer to apply was calculated by multiplying this fraction of an acre by the weight of the material desired per acre.

Since large numbers of pots were treated in the greenhouse experiments, a rapid method of measuring out fungicides and fertilizers was devised. The number of grams of fungicide dust or fertilizer were weighed out and then poured into a narrow test tube. The material in the tube was settled by tapping the bottom of the test tube against a firm surface five times. The volume occupied by the material in the tube was marked with a fine line on the outside of the tube. The amount indicated by the mark on the test tube served as the means for measuring out a given amount of fungicide or fertilizer.

The calculated amounts of fungicides and fertilizers were incorporated with an amount of soil which would fill the top four inches of the clay pots employed. Mixing

was accomplished with a trowel in a shallow pan. The amount of soil used for each pot was kept constant by measuring out the correct volume of soil in a graduated container. The amount of soil used to cover planted seed was standardized in the same way.

Soil. Fields were chosen where the incidence of beet seedling necrosis was known to be extremely high. Samples of soil were collected from these fields for greenhouse experiments. The infested soils were stored in large, covered, metal cans until used.

Before using, all soils were screened through three eighths inch mesh hardware cloth and thoroughly mixed by coning to create as uniform a distribution of nutrients and microorganisms as possible.

Experimental design and error. All of the experiments except one were set up in replicated randomized blocks. The treatments in the experiment to determine the effects of soil temperature upon the incidence of beet seedling necrosis were completely randomized.

The ground chosen for the field experiment during 1952 had a slight slope in one direction. The replicates were arranged in the direction of this slope.

In addition to the usual sources of experimental error, there are problems which are peculiar to experiments concerning seedling diseases and in some cases

specific for beet seedling necrosis.

The variability of factors in the soil which control the incidence and severity of seedling necrosis was by far the greatest source of error. There was no precise way of knowing the true cause of variation which could be attributed to poor distribution of nutrients or microorganisms, variable soil moisture or drainage or perhaps a combination of several or all of these. No matter how thoroughly infested soil samples were mixed, a certain amount of error was always experienced from soil variations.

Beet seed balls contain an indeterminate number of seed, varying from one to five per seed ball. Selecting seed balls at random from one pound seed lots and planting thirty or more seed balls per pot were sufficient to overcome this source of error.

Controlling soil moisture in greenhouse experiments was difficult and a constant source of error. Irrigating pots by flooding the surface of the soil after planting tended to compact the soil and delay or inhibit the emergence of seedlings. Subirrigation by leaving the bases of pots immersed in water kept the soil too wet and did not allow for natural leaching. Fertilizer concentrations which stimulated seedling development when pots were irrigated by surface flooding retarded their growth

when the pots were subirrigated. Periodic subirrigation helped to prevent excessive soil moisture, but the water ascended at different rates in separate pots, making it difficult to control the amount of water applied to each pot.

Observations. The yield of beets from separate treatments in the field experiment were obtained by hand pulling the beets in each replicate, twisting off the tops and weighing them in sacks with a spring scale. Each sack of beets was run through a grading machine at the Eugene Fruit Growers' cannery. The weights of beets in pounds in each of the three grades of marketable beets were recorded.

The number of seedlings that emerged, died, and survived in greenhouse experiments were observed by removing and recording the number of dead seedlings and counting those which survived after a certain length of time. Adding the number of seedlings that survived to those which died gave the total number that emerged.

PATHOGENIC ORGANISMS

In order to obtain an insight into the pathogenic organisms associated with beet seedling necrosis, isolations were made from infected beet seedlings collected in different fields at different times during the growing season. Certain fungi were consistently and continually present in seedlings wherever the disease occurred. As a result of these and other field observations, the following experiments were conducted to determine the relationship between these organisms and the incidence of the disease.

Soil temperature. The best stands of beets were obtained with early plantings. The later the planting date, the more severe the disease was apt to be.

The parasitic organisms associated with the disease and the effects of soil temperature upon their pathogenicity were determined by growing beet seedlings in infested soils maintained at three different temperature levels. The infested soils were collected from two different beet fields where the disease had been severe. The beet seedlings were grown in water tight, gallon cans filled three quarters full with infested soil. Thirty beet seed balls were placed one half inch deep in each can. The cans were put in the greenhouse hot-bed and surrounded by clean damp sand. The

temperature of the soil in the cans was maintained by a soil heating cable running between the cans and regulated by means of a thermostat. The maximum fluctuations were plus or minus five degrees Fahrenheit, so that the temperature intervals maintained were: 50°-60°, 60°-70°, and 70°-80°. Twenty cans of each soil were completely randomized at each temperature interval. The emergence, number of plants that died, and the ultimate stand after twenty-eight days were recorded.

In addition to these observations, organisms were isolated from the diseased seedlings and the frequency of their presence recorded. The infected parts of diseased seedlings were surface sterilized for one minute, rinsed in sterile water and placed on potato dextrose agar in Petri plates. Isolates were obtained of the organisms that grew from the infected seedlings.

The results of this experiment, presented in Tables I and II, indicate that the incidence of beet seedling necrosis increases as the soil temperature increases. The average number of emergences at 50°-60° F. was much less than at 60°-70° F., but the number of seedling deaths was also less at the lower temperature, resulting in stands which were not significantly different. The increase in disease incidence at 70°-80° F. killed nearly all of the plants which had emerged. The interval, 60°-70° F. was

TABLE I

The Effects of Soil Temperature Upon the Incidence
of Beet Seedling Necrosis in Two Different Soils

| Soil Temp. | Soil (1) | Average seedlings from 30 seed balls | | |
|---------------|-------------|--------------------------------------|--------|------------------------|
| | | Emergences | Deaths | Stand after 28 days |
| 50°-60°F. | I | 7.2 | 1.6 | 6.6 |
| | II | 9.0 | 4.2 | 4.7 |
| 60°-70°F. | I | 12.0 | 6.2 | 5.8 |
| | II | 24.2 | 17.8 | 6.4 |
| 70°-80°F. | I | 9.8 | 9.0 | 0.8 |
| | II | 6.6 | 6.2 | 0.4 |

(1) Soil I: Sandy loam, Springfield, Oregon

Soil II: Clay loam, Junction City, Oregon

TABLE II

The Frequency of Isolations of Organisms from Infected Beet Seedlings Grown at Different Soil Temperatures

| Organism | Soil (1) | Soil temperature in degrees F. | | |
|------------------------|-------------|--------------------------------|---------|---------|
| | | 50°-60° | 60°-70° | 70°-80° |
| <u>Pythium ultimum</u> | I | 12/25 ⁽²⁾ | 28/40 | 6/10 |
| | II | 19/25 | 34/40 | 8/14 |
| <u>Fusarium solani</u> | I | 0/25 | 7/40 | 4/10 |
| | II | 0/25 | 3/40 | 1/14 |
| <u>Fusarium roseum</u> | I | 0/25 | 9/40 | 2/10 |
| | II | 0/25 | 3/40 | 3/14 |
| Fusarium spp. | I | 0/25 | 4/40 | 1/10 |
| | II | 0/25 | 2/40 | 5/14 |

(1) Soil I: Sandy loam, Springfield, Oregon
Soil II: Clay loam, Junction City, Oregon

(2) Numerator: Number of times isolated
Denominator: Number of seedlings from which isolations were attempted.

apparently the most favorable temperature level for development of the host and least favorable for the incidence of preemergence deaths. It seems reasonable to suspect that control by seed or soil treatment would be most effective at this temperature interval.

The organisms isolated most frequently from the diseased seedlings were Pythium ultimum Trow, Fusarium solani (Mart.) Snyder and Hansen f. Beta, and Fusarium roseum (Lk.). Rhizoctonia solani Kuhn, was isolated from seedlings only twice. Pythium ultimum Trow was identified from the key and description presented by Middleton (45, pp. 1-171). The taxonomic system devised by Wollenweber and Reinking (69, pp. 833-834 and 70, pp. 1-355) for classifying species of Fusarium was employed to place the species of Fusarium into the sections Martiella and Roseum. In keeping with the species concept proposed and adhered to by Snyder and Hansen (59, pp. 738-742 and 60, pp. 657-666), the species placed in section Martiella was classified as Fusarium solani (Mart.) Snyder and Hansen f. Beta, and the species placed in section Roseum was classified as Fusarium roseum (Lk.). Rhizoctonia solani Kuhn was determined from the key and description given by Stevens (62, pp. 434-438).

The results in Table II show that Pythium ultimum was isolated most frequently from diseased seedlings at

all three temperature levels. The presence of *Fusarium* species was less frequent and they were entirely absent from infected seedlings below 60°F. The severity of the disease increased with a rise in temperature and the presence of *Fusarium* species became more frequent with a corresponding rise in temperature. Consequently, the results of this experiment could be interpreted in two ways. The increase in disease incidence could be due to an interaction between *Pythium ultimum* and the *Fusarium* species at the higher temperature or to an increase in the virulence of *Pythium ultimum* alone.

Pathogenicity. The pathogenicity of *Pythium ultimum*, *Fusarium solani*, *F. roseum* and *Rhizoctonia solani* was demonstrated. The fungi were obtained free from bacteria and were grown on oat grain media for seven days. The infested grain was incorporated with sterile soil in six inch, clay pots. The contents of one grain tube were used for each pot of soil. The infested soil was allowed to stand for three days and then thirty beet seed balls were planted one half inch deep in each pot. Five pots of soil were used for each fungus. The cultures of fungi employed were isolated from infected seedlings obtained from fields in the following places.

Pythium ultimum:

Isolate 1- Springfield, Oregon

Isolate 6- Harrisburg, Oregon

Isolate 9- Coburg, Oregon

Fusarium solani:

Isolate 1- Coburg, Oregon

Isolate 2- Coburg, Oregon

Fusarium roseum from Harrisburg, Oregon

Rhizoctonia solani from Springfield, Oregon

The isolates of Pythium ultimum were morphologically the same. The two isolates of Fusarium solani differed in their growth rates on potato dextrose agar and in the amount of color produced in the substrate. Isolate 1 of Fusarium solani grew more rapidly at room temperatures than isolate 2. Isolate 1 produced a dark blue to purple color in the substrate while isolate 2 produced a very slight lavender coloration.

The temperature in the greenhouse during this experiment was maintained above 60°F.

The emergence, number of deaths, and the ultimate stand of seedlings obtained are presented in Table III. The isolates of Pythium ultimum employed caused the death of all seedlings before they emerged. Isolate 1 of Fusarium solani was almost as virulent as Pythium ultimum.

TABLE III

The Pathogenicity of Fungi Isolated from
the Hypocotyls of Diseased Beet Seedlings

| Fungus | Average seedlings from 30 seed balls | | |
|---------------------------|--------------------------------------|--------|------------------------|
| | Emergences | Deaths | Stand after 21 days |
| <u>Pythium ultimum</u> | | | |
| Isolate 1 | 0 | 0 | 0 |
| " 2 | 0 | 0 | 0 |
| " 3 | 0 | 0 | 0 |
| <u>Fusarium solani</u> | | | |
| Isolate 1 | 4.4 | 3.4 | 1.0 |
| " 2 | 39.6 | 9.0 | 30.6 |
| <u>Fusarium roseum</u> | 26.0 | 10.6 | 15.4 |
| <u>Rhizoctonia solani</u> | 38.6 | 0 | 38.6 |
| Sterile soil | 41.8 | 0 | 41.8 |

A small number of plants emerged and only a few of these survived. Isolate 2 of Fusarium solani was much less pathogenic, causing a small number of preemergent and postemergent deaths. The isolate of Fusarium roseum was less virulent than Pythium ultimum and was intermediate between the two isolates of Fusarium solani. Rhizoctonia solani did not produce any of the characteristic symptoms of pathogenic beet seedling necrosis and there were no postemergent deaths. However, the seedlings were conspicuously stunted and remained in the cotyledonary stage for about twelve days after the plants in the control pots had formed secondary leaves.

It is evident from the results of this experiment that no interaction between Pythium ultimum and Fusarium species is necessary for the maximum severity of the disease. Since Pythium ultimum can cause the maximum disease incidence possible, the increase in disease severity at high soil temperatures could be due to the increased virulence of the fungus.

Linear growth rate of Pythium ultimum. To support this contention, the effect of temperature upon the linear growth rate of Pythium ultimum was determined by growing the fungus at constant temperatures ranging from five to forty degrees Centigrade at five degree intervals. Ninety-six eight inch test tubes were fitted with low dams two

inches from their mouths by heating the neck of each tube and pressing in one side. This made it possible to form a horizontal layer of nutrient agar three-fourths the length of the tubes. Ten millimeters of Difco glucose potato agar ⁽¹⁾ were added to each tube, sterilized and the tubes placed in a horizontal position until the agar media became firm. Each tube was inoculated at the open end with a small, uniform piece of inoculum, cut from a Petri dish culture of Pythium ultimum. The colony was allowed to grow at room temperature for sixteen hours, and then a mark was placed on the outside of the tube at the margin of growth to serve as a reference point for future measurements. Four cultures of Pythium ultimum isolated from infected seedlings collected from different fields were used in this experiment. Three tubes of each isolate were placed at each temperature and the linear growth of the fungus was measured to the nearest millimeter after twenty-four and forty-eight hours of growth. The mean growth increment for a twenty-four hour period at each temperature was determined.

The results of this experiment are shown in Table IV. Visible but meager growth was obtained at 5°C. The

¹ Formula. Ingredients per liter. Infusion from 200 g. of potatoes, 20 g. bacto-dextrose, 2 g. yeast extract, 15 g. bacto-agar.

TABLE IV

Linear Growth Rate of Pythium ultimum on
Potato Dextrose Agar at Different Temperatures

| Isolates (1) | Average growth increment for 24 hours in mm. | | | | | | | |
|-----------------|--|------|------|------|------|------|-----|-----|
| | Degrees Centigrade | | | | | | | |
| | 5° | 10° | 15° | 20° | 25° | 30° | 35° | 40° |
| 1 | 0.7 | 12.0 | 20.0 | 27.0 | 35.2 | 41.5 | 3.9 | 0 |
| 2 | 3.0 | 12.9 | 19.5 | 26.0 | 34.8 | 39.5 | 4.0 | 0 |
| 6 | 2.0 | 12.4 | 17.7 | 24.4 | 31.0 | 34.5 | 2.0 | 0 |
| 7 | 1.0 | 10.9 | 16.7 | 24.2 | 32.0 | 36.7 | 2.5 | 0 |

(1) The isolates were obtained from infected seedlings collected from different infested fields.

apparent optimum for growth of Pythium ultimum was between 25° and 30°C. and the maximum between 35° and 40°C. These results coincide closely with those of Middleton (32, pp. 1-171) who found the minimum temperature for growth of Pythium ultimum to be 1°C., the optimum 28-31°C. and the maximum between 35°-40°C.

The results indicate that the increased severity of beet seedling necrosis at higher temperatures could be due to the greater activity of Pythium ultimum. This conclusion also helps explain the greater losses from the disease in fields which are planted late, when the soil temperature is more favorable for the growth of the fungus.

It should be noted that the response to temperatures was not always the same with all isolates of Pythium ultimum tested. For example, the growth increments for isolate 1 and 6 at 30° C. differed by seven millimeters. The difference was not due to experimental error, since measurements between replicates coincided almost exactly. In general, however, the responses of the isolates are in fairly close agreement, the minimum, optimum, and maximum temperatures for growth being the same.

SOURCE OF INFECTION

Preliminary experiments indicated that many fungi including *Fusarium* species were present in beet seed balls. After *Fusarium solani* and *F. roseum* were isolated from diseased beet seedlings and it was demonstrated that they were capable of causing beet seedling necrosis, it was considered necessary to determine the relative importance of seed and soil borne infection.

Samples of commercial beet seed, obtained from three different sources, were used to determine the number of fungi present in the beet seed and the effectiveness of a hot water treatment (29, pp. 48-51) in eliminating these fungi. For convenience, the different seed sources will be designated by numbers. Sources one and two represent seed grown outside the state of Oregon; three, seed produced in the Willamette Valley. Part of the seed from source one was decorticated, the rest was natural. A portion of each seed sample was treated in water at 55°C. for one-half hour, the rest left untreated. Fifty seed balls from each sample of treated and untreated seed were surface sterilized for three minutes and placed on potato dextrose agar in Petri plates.

The results, presented in Table V, show the number of fungus colonies which grew from the beet seed balls during a period of ten days and the effectiveness of the

TABLE V

The Effect of Hot Water Treatment upon the
Fungus Population of Beet Seed Balls

| Seed source | Type | Treatment | Fungus colonies from 50 seed balls |
|-------------|--------------|-------------------|---------------------------------------|
| 1 | Natural | Hot water treated | 0 |
| | | Untreated | 22 |
| | Decorticated | Hot water treated | 0 |
| | | Untreated | 8 |
| 2 | Natural | Hot water treated | 1 |
| | | Untreated | 23 |
| 3 | Natural | Hot water treated | 0 |
| | | Untreated | 18 |

ADVANCE BOND

SMALL BROWN BONES

hot water treatment in eliminating these fungi. It will be noted that fewer fungi grew from the decorticated seed than from the natural seed. The genera of fungi isolated from the seed in the order of greatest occurrence were *Chaetomium*, *Aspergillus*, *Stemphyllium*, *Cladosporium*, *Fusarium*, *Phoma*, *Penicillium*, and *Rhizopus*. From this experiment and previous tests, the presence of *Fusarium* species was found to vary from 0 to 15 per cent.

Using seed samples from the same sources, the relative importance of seed and soil borne infection was determined by growing hot water treated seed in sterile soil, untreated seed in sterile soil, hot water treated seed in infested soil, and untreated seed in infested soil. The seed were planted in sterile six inch clay pots, thirty seed balls per pot. Each treatment was replicated eight times in randomized blocks. The observations recorded were the total number of emergences and the stand of seedlings obtained after eighteen days.

Comparing the emergences (Table VI) from untreated seed in sterile soil with those from hot water treated seed in infested soil, the number of preemergence deaths caused by seed borne organisms was not significant. The greater number of emergences from untreated seed than from hot water treated seed indicated an inhibition of seed germination by the hot water treatment. There was a

TABLE VI

The Effect of Seed and Soil Borne Organisms
upon the Emergence of Table Beets

| Treatment | Seed source | | | |
|---|--------------|-------------------|--------------|--------------|
| | Natural 1 | Decorticated 1 | Natural 2 | Natural 3 |
| Hot water treated seed in sterile soil | 334 | 340 | 581 | 346 |
| Untreated seed in sterile soil | 358 | 371 | 624 | 380 |
| Hot water treated seed in infested soil | 92 | 104 | 177 | 66 |
| Untreated seed in infested soil | 119 | 104 | 200 | 102 |

Figures based on number of plants from 240 seed balls.

significantly greater number of emergences from the seed of source two than from the other two seed sources.

The figures in Table VII represent the percentage of seedlings that died after emerging. All of the plants from hot water treated seed in sterile soil remained healthy. The number of seedling deaths caused by seed borne organisms was very small. There was no significant difference in the number of seedling deaths between plants grown from hot water treated seed and untreated seed in infested soil. There was a significantly greater amount of seedling necrosis where seed from source two was used.

Table VIII presents the stand of beet seedlings obtained after eighteen days. There was no significant difference in the stands obtained between seed sources. Where a greater number of plants emerged as with seed source two, a correspondingly greater number of seedlings died. The use of decorticated seed did not increase the incidence of the disease or increase the stand of seedlings obtained. For the seed samples tested, the amount of seed borne infection was relatively unimportant when compared to the source of infection from naturally infested field soil. When untreated seed were grown in sterile soil, the source of infection did not move through the soil from diseased to healthy seedlings. One or two plants would die after three weeks and the rest of the

TABLE VII

Percentage of Postemergence Damping-off
of Table Beets after 18 Days

| Treatment | Seed source | | | |
|---|--------------|-------------------|--------------|--------------|
| | Natural 1 | Decorticated 1 | Natural 2 | Natural 3 |
| Hot water treated seed in sterile soil | 0 | 0 | 0 | 0 |
| Untreated seed in sterile soil | 2.0 | 0 | 0.5 | 0.8 |
| Hot water treated seed in infested soil | 54.4 | 47.1 | 54.8 | 43.9 |
| Untreated seed in infested soil | 41.2 | 48.1 | 59.5 | 43.1 |

TABLE VIII

The Effect of Seed and Soil Borne Organisms
upon the Stand of Table Beets after 18 Days

| Treatment | Seed source | | | |
|---|--------------|-------------------|--------------|--------------|
| | Natural 1 | Decorticated 1 | Natural 2 | Natural 3 |
| Hot water treated seed in sterile soil | 334 | 340 | 581 | 346 |
| Untreated seed in sterile soil | 351 | 371 | 621 | 377 |
| Hot water treated seed in infested soil | 42 | 55 | 80 | 37 |
| Untreated seed in infested soil | 70 | 54 | 81 | 58 |

Figures based on the number of plants from 240 seed balls.

plants remained healthy. In one instance, three seedlings emerged from one seed ball, one of the seedlings died and the other two remained healthy.

CONTROL

Experiments¹ conducted at the Oregon Agricultural Experiment Station previous to this investigation disclosed that recommended seed treatments with fungicides were not sufficient to control beet seedling necrosis. Other methods had been tested without satisfactory results. Fungicide dusts were sifted onto the top of the soil above the seed immediately after planting. Fungicides were also raked into the soil above the seed immediately after planting. Spraying fungicides on top of the soil above the seed was tried at the following seven times:

- (a) Immediately after seeding.
- (b) Immediately after seeding and just before emergence.
- (c) Immediately after seeding, just before emergence, and ten days after first emergences.
- (d) Just before emergence.
- (e) Just before emergence and ten days after first emergences.
- (f) During emergence of seedlings.
- (g) During emergence of seedlings and ten days later.

¹Vaughan, Edward K., Unpublished report, Oregon State Horticultural Society, November 29, 1951.

Fungicide dusts blown into the planting furrow did not give effective control. Since the source of infection was demonstrated to be primarily in the soil, investigations were made to determine the effectiveness of different soil amendments, incorporated into the soil before planting, in controlling beet seedling necrosis.

Field test. On May 30, 1952, a field experiment was begun at Junction City, Oregon, to determine the effects of fungicides and fertilizers incorporated with the soil upon the incidence of beet seedling necrosis.

The tests consisted of soil treated with fungicide dusts, soil treated with two different fertilizers, soil treated with fungicides and fertilizers combined and infested untreated soil. The fungicide dusts and fertilizers were distributed evenly over the surface of the soil in strips twenty feet long and eighteen inches wide, and were then incorporated into the top four inches of soil with a rototiller. Decorticated Detroit Dark Red beet seed was planted with a Planet Junior Seeder, Model 300-A, at a uniform rate in the treated soil, in rows spaced forty-two inches apart. Each treatment was replicated six times in randomized blocks.

The two fertilizers were prepared and applied at a rate that would furnish 120 pounds of P_2O_5 per acre as treble

superphosphate, and 100 pounds of K_2O per acre as muriate of potash. They differed only in the source of nitrogen. One fertilizer supplied 100 pounds of nitrogen per acre as calcium nitrate, the other 100 pounds of nitrogen as ammonium sulphate. The fertilizer containing calcium nitrate will be referred to as the nitrate fertilizer. The one containing ammonium sulphate will be referred to as the ammonium fertilizer.

The fungicides employed were Ceresan M, Manzate, Arasan, Fermate, Orthocide 406, and C.O.C.S., all applied at the rate of five pounds of commercial product per acre. Ceresan M, Manzate, and Arasan were applied alone as well as in combination with each of the two fertilizers. Fermate, Orthocide 406, and C.O.C.S. were applied in combination with the nitrate fertilizer.

The results of this experiment were observed in terms of the rate of emergence, the seedlings obtained thirty-two days after planting and in the yield and grade of beets obtained at harvest time. Only the results for Ceresan M, Manzate, and Arasan are recorded in the tables. The results for Fermate, Orthocide 406, and C.O.C.S. were not significantly different from those of the other three fungicides. Since these treatments were not included in the complete factorial they are omitted from the tables of results.

The emergence of seedlings was rated ten days after planting on the basis of whether there were few emergences, a fair stand, or a good stand. The results, presented in Table IX, show that none of the fungicides had any significant effect upon the rate of emergence while the fertilizers retarded the emergence of seedlings. The poorest stands after 10 days were observed in the plots which received calcium nitrate.

The average stands of seedlings obtained thirty-two days after planting are recorded in Table X. It was observed that none of the fungicides had any significant effect upon the stands of seedlings, whether they were applied alone or in combination with fertilizers. Differences observed between treatments were due only to the effects of the fertilizers containing calcium nitrate. Stands in the plots which received ammonium sulphate were not significantly different from those where no fertilizer was applied.

The plants in the plots receiving the nitrate fertilizer had green foliage in contrast to the red foliage of plants in the other treatments. This distinction was less obvious as the plants became larger.

A comparison of the stands obtained from the various treatments indicated that there was some interaction between the nitrate fertilizer and the two fungicides Ceresan

TABLE IX

Effects of Fungicides and Fertilizers⁽¹⁾ Incorporated
Into the Soil upon the Rate of Emergence of Table Beets

| Fungicide 5 lbs. per acre | Nitrogen, 100 lbs. per acre, applied as | | |
|--|---|------------------------------|---------------|
| | $\text{Ca}(\text{NO}_3)_2$ | $(\text{NH}_4)_2\text{SO}_4$ | No fertilizer |
| Average emergence rating ⁽²⁾ per 20 ft. row 10 days after planting | | | |
| Ceresan M | 1.000 | 1.833 | 3.000 |
| Manzate | 1.333 | 2.000 | 2.833 |
| Arasan | 1.333 | 2.000 | 2.833 |
| None | 1.167 | 2.000 | 2.667 |

L.S.D. .05 between treatments means = 0.6346

- (1) All fertilizers contained the following per acre:
 100 lbs. N. as $\text{Ca}(\text{NO}_3)_2$ or $(\text{NH}_4)_2\text{SO}_4$
 120 lbs. P_2O_5 as treble superphosphate
 100 lbs. K_2O as muriate of potash

- (2) Emergence ratings:
 1,000 - Almost no emergences
 2,000 - Fair stand
 3,000 - Good stand

TABLE X

Effects of Fungicides and Fertilizers⁽¹⁾
Incorporated with the Soil upon Stands of Table Beets

| Fungicide 5 lbs. per acre | Nitrogen, 100 lbs. per acre, applied as | | |
|---|---|------------------------------|---------------|
| | $\text{Ca}(\text{NO}_3)_2$ | $(\text{NH}_4)_2\text{SO}_4$ | No fertilizer |
| Average number of plants per 20 ft. row 32 days after planting | | | |
| Ceresan M | 379 | 200 | 206 |
| Manzate | 346 | 207 | 206 |
| Arasan | 261 | 213 | 200 |
| None | 323 | 224 | 181 |

L.S.D. .05 between treatment means = 63

- (1) All fertilizers contained the following per acre:
 100 lbs. N as $\text{Ca}(\text{NO}_3)_2$ or $(\text{NH}_4)_2\text{SO}_4$
 120 lbs. P_2O_5 as Treble superphosphate
 100 lbs K_2O as muriate of potash

M and Manzate. Although the interaction was not statistically significant, the results indicated that additional experimentation with these materials was warranted.

When the beets were harvested, the yields were weighed and recorded. The results of these observations are presented in Table XI. None of the fungicides had any significant effect upon the yield of beets. It was found, however, that the average yield was significantly greater where the ammonium fertilizer was employed. When the yields were segregated into the three grades of marketable beets,¹ the results presented in Table XII were observed. A significantly greater number of grade one beets was produced in the plot which received nitrate fertilizer, but a significantly larger number of grade two and three beets where the ammonium fertilizer was used. Grade one beets are of the highest quality and bring the highest price, but a certain tonnage of larger beets is necessary to provide a profitable crop.

Greenhouse tests. The effect of nitrogen source and concentration upon beet seedling necrosis was studied further by growing beet seedlings in field soil in the greenhouse and observing the emergence and stand

¹Grades of marketable beets based upon their diameter in inches. Grade one: 1-1.75; grade two: 1.75-3.0; grade three: 3.0 and larger.

TABLE XI

Effects of Fungicides and Fertilizers¹
Incorporated with the Soil upon Yield of Table Beets

| Fungicide 5 lbs. per acre | Nitrogen, 100 lbs. per acre, applied as | | |
|--|---|------------------------------|---------------|
| | $\text{Ca}(\text{NO}_3)_2$ | $(\text{NH}_4)_2\text{SO}_4$ | No fertilizer |
| Average yield of marketable beets per 20 ft. row, in pounds | | | |
| Ceresan M | 22.8 | 24.8 | 14.3 |
| Manzate | 23.5 | 27.5 | 14.3 |
| Arasan | 21.1 | 25.8 | 15.1 |
| None | 23.4 | 26.3 | 12.8 |

L.S.D. .05 between treatment means = 4.5

- ¹ All fertilizers contained the following per acre:
 100 lbs. N as $\text{Ca}(\text{NO}_3)_2$ or $(\text{NH}_4)_2\text{SO}_4$
 120 lbs. P_2O_5 as Treble Superphosphate
 100 lbs. K_2O as Muriate of Potash

TABLE XII

Effects of Nitrogen Fertilizer Incorporated
with the Soil upon Grade of Table Beets

| Nitrogen Source (1) | Average yield of marketable beets per 20 ft. row by grades (2) | | | | | |
|--|---|----------|-------------|----------|-------------|----------|
| | Grade 1 | | Grade 2 | | Grade 3 | |
| | <u>lbs.</u> | <u>%</u> | <u>lbs.</u> | <u>%</u> | <u>lbs.</u> | <u>%</u> |
| $\text{Ca}(\text{NO}_3)_2$ | 7.8 | 34.4 | 13.0 | 57.1 | 1.9 | 8.4 |
| $(\text{NH}_4)_2\text{SO}_4$ | 5.0 | 19.2 | 15.1 | 57.9 | 6.0 | 23.0 |
| None | 5.2 | 37.3 | 7.9 | 0.8 | 0.8 | 5.8 |
| L.S.D. .05 between fertilizer means | 1.0 | | 1.6 | | 1.2 | |

(1) 100 lbs. of N per acre.

(2) Grades of marketable beets bases upon their
diameter in inches. Grade 1: 1-1.75; Grade
2: 1.75-3.0; Grade 3: 3.0 and larger

of seedlings 21 days after planting. Calcium nitrate and ammonium sulphate were thoroughly mixed with the top four inches of soil in six-inch clay pots at the rates of 0, 100, 200, and 400 pounds per acre. Thirty-five beet seed balls were planted in each pot. Each treatment was replicated ten times in randomized blocks.

The results presented in Table XIII indicate that calcium nitrate significantly increased the emergence and survival of beet seedlings while ammonium sulphate had no such significant effect. Also, 100 and 200 pounds of nitrogen per acre, applied as calcium nitrate, gave better control of beet seedling necrosis than 400 pounds per acre. 100 pounds per acre seemed to be slightly more effective than 200 pounds, but the difference was not statistically significant.

The relative effect of nitrogen and phosphorus upon the emergence and survival of beet seedlings in infested soil was determined by planting beet seeds in six-inch clay pots containing untreated field soil, soil treated with calcium nitrate alone, and with treble superphosphate alone, with calcium nitrate and treble superphosphate together. The calcium nitrate was applied to furnish 100 pounds of nitrogen per acre, and the treble superphosphate to furnish 120 pounds of P_2O_5 per acre. The fertilizers were mixed with the top four inches of soil and each pot

TABLE XIII

Effects of Source and Concentration
of Nitrogen in the Soil upon Emergence
and Survival of Table Beets in the Greenhouse

| Nitrogen Source and Concentration | Number of beet seedlings from 350 seed balls, 21 days after planting | | |
|---|---|---------------------|----------------------|
| | Number emerged | Number surviving | Per cent survival |
| <hr/> | | | |
| Ca(NO ₃) ₂ | | | |
| 100 lbs. N per acre | 328 | 283 | 86.3 |
| 200 lbs. N per acre | 288 | 230 | 80.0 |
| 400 lbs. N per acre | 248 | 201 | 81.0 |
| (NH ₄) ₂ SO ₄ | | | |
| 100 lbs. N per acre | 256 | 171 | 66.8 |
| 200 lbs. N per acre | 236 | 109 | 41.9 |
| 400 lbs. N per acre | 226 | 127 | 56.2 |
| No nitrogen | 201 | 145 | 72.1 |
| <hr/> | | | |
| L.S.D. .05 between treatment means | 68 | 60 | |

planted with 35 seed balls. Treatments were replicated ten times in randomized blocks.

Counts made 21 days after planting indicated that phosphorus, applied as treble superphosphate, either alone or in combination with calcium nitrate, had no significant effect upon emergence or survival of beet seedlings. On the other hand, calcium nitrate did increase the emergence and survival of seedlings, Table XIV.

The beneficial effects of sodium on the nutrition of beets were discussed in the literature review. With these facts in mind, it was considered important to determine the relative effects of calcium nitrate, sodium nitrate, and sodium chloride upon the incidence of beet seedling necrosis. Infested soils from three different fields were collected in October after the beets had been harvested and were stored in large paper sacks in the greenhouse for three months before use. When the experiment was begun, sodium chloride, calcium nitrate, and sodium nitrate, at 100 pounds of nitrogen and 169 pounds of sodium per acre, were incorporated into the top four inches of soil in five inch, clay pots. Thirty beet seed balls were planted one-half inch deep in each pot. The treatments were replicated ten times in randomized blocks.

The average stands of seedlings obtained sixteen

TABLE XIV

Effects of Nitrogen and Phosphorus in the Soil on
Emergence and Survival of Table Beets in the Greenhouse

| Fertilizer in Pounds per Acre | | Number of beet seedlings from 350 seed balls, 21 days after planting | | |
|---|---|---|---------------------|----------------------|
| P ₂ O ₅ as treble superphosphate | N as Ca(NO ₃) ₂ | Number emerged | Number surviving | Per cent survival |
| 120 | 100 | 209 | 171 | 81.8 |
| None | 100 | 218 | 192 | 88.1 |
| 120 | None | 117 | 73 | 62.4 |
| No fertilizer | | 116 | 72 | 62.1 |

days after planting were very poor (Table XV). The response that had been observed in previous experiments from calcium nitrate in reducing seedling necrosis was not apparent, nor was any such response observed in the treatments with sodium nitrate or sodium chloride. In previous experiments, infested soils were used soon after their collection from the field. The results of this experiment indicated that soil conditioning had an important effect upon the ability of certain soil amendments to reduce beet seedling necrosis.

To further substantiate this hypothesis, beet seed balls were planted in six inch clay pots containing untreated field soil and soil treated with sodium nitrate, potassium chloride and treble superphosphate. The fertilizers were incorporated with the top four inches of soil at the following rates: 200 pounds of N per acre as sodium nitrate, 120 pounds of P_2O_5 per acre as treble superphosphate and 100 pounds of K_2O per acre as potassium chloride. The treatments were duplicated in two different soil samples. Both soil samples were collected from the same location in the same field, but at different times. One sample was collected in October after beets had been harvested from the field. The sample was air-dried in a metal bin for four months in the greenhouse. The other soil sample was collected the following February and used

TABLE XV

Effects of Calcium Nitrate, Sodium Nitrate
and Sodium Chloride in Different Soils on the
Survival of Table Beets in the Greenhouse

| Soil Source | Nitrogen, 100 lbs./acre as: | | Sodium, 169 lbs./acre as: | |
|---|--------------------------------|-----------------|------------------------------|---------|
| | $\text{Ca}(\text{NO}_3)_2$ | NaNO_3 | NaCl | Control |
| Average stand of seedlings obtained from 35 seed balls, 16 days after planting | | | | |
| L. Stump | 6.8 | 5.4 | 2.8 | 3.0 |
| E. Strome | 8.2 | 6.3 | 5.4 | 6.8 |
| C. Chase | 4.2 | 7.3 | 3.6 | 2.6 |

immediately for this experiment.

In addition to these treatments the fresh sample of soil was used to test the effects of sodium nitrate and potassium chloride, both alone and combined together in the soil, upon the incidence of beet seedling necrosis. Thirty-five beet seed balls were planted in each pot. The treatments were replicated seven times in randomized blocks.

The average number of seedlings twenty-one days after planting are presented in Table XVI. The results substantiate the contention that the inorganic fertilizers are effective in reducing beet seedling necrosis only when the soil is in a state of normal biological equilibrium.

Both sodium nitrate and potassium chloride, at the rate applied, increased the stands of seedlings obtained. Combining the two compounds together in the soil resulted in stands which were significantly greater than those where the compounds were applied separately. The addition of treble superphosphate to the combination did not increase the effectiveness of the fertilizers to reduce the disease.

At this point in the investigation, the results obtained thus far were evaluated to determine the objectives for future experiments. It was apparent that the reduction of beet seedling necrosis by certain inorganic compounds in the soil could be attributed to three phenomena. The

TABLE XVI

Effects of Nitrogen and Potassium in the Soil
and the Influence of Soil Condition upon the
Survival of Table Beets in the Greenhouse

| Fertilizer(1) | Infested soil collected from same location | |
|--|--|---------------------------------------|
| | Soil fresh from field | Soil air-dried in bin for 4 months |
| Average seedlings from 35 seed balls, 21 days after planting. | | |
| NaNO_3 | 8.3 | - |
| KCl | 5.1 | - |
| $\text{NaNO}_3 + \text{KCl}$ | 18.3 | - |
| NPK | 14.6 | 5.0 |
| No fertilizer | 1.5 | 3.0 |

- (1) Fertilizers applied to furnish:
 200 lbs. of N per acre as NaNO_3
 80 lbs. of K per acre as KCl
 120 lbs. of P_2O_5 per acre as treble superphosphate

inorganic substances could (1) affect the metabolism of the host in such a way as to render them more resistant to infection; (2) alter the biological equilibrium in the soil to the extent that the pathogens associated with the disease would be inhibited by the antibiotic activities of other soil organisms; (3) have a direct inhibitory effect upon the growth of pathogens themselves. Experimental evidence presented already has indicated that the presence of nitrate fertilizers, at the concentrations tested, retarded the germination and emergence of beet seedlings (Table IX). However, seedlings in the presence of such treatment were less susceptible to the disease, indicating some direct influence upon the host itself. The results of subsequent experiments (Tables XV and XVI) showed that the inorganic compounds in the soil were less effective in reducing the incidence of beet seedling necrosis unless the soil was in a state of normal biological equilibrium. This indicated that the fertilizers, to a large extent, were achieving effective antibiosis in the soil.

The possibility that these fertilizers might have a direct effect on the pathogen also could not be overlooked. The nitrate fertilizers were applied at rates high enough to delay the early development of beet seedlings. Therefore it was within the realm of possib-

ility that the activities of a pathogen could be curtailed in a similar manner. According to Foster (22, pp. 481-531) ammonium nitrogen is more directly available to fungi in general than nitrate nitrogen, while the opposite is true in most of the higher plants. This circumstance might explain the reduction of seedling necrosis with nitrate fertilizers in the soil while no reduction in the disease was observed when ammonium fertilizer was used. (Tables X and XIII).

To determine whether certain concentrations of sodium nitrate and potassium chloride could inhibit the growth of Pythium ultimum and still allow an adequate development of beet seedlings, Pythium ultimum was grown in Petri plates on two per cent wateragar containing different concentrations of sodium nitrate and potassium chloride, both alone and together. Beet seed balls were placed in media containing the same concentrations of sodium nitrate and potassium chloride also. In the first of two experiments, sodium nitrate was incorporated with the media at 1000, 2000, 4000, and 8000 parts per million. In another set of media potassium chloride at 400 parts per million was added to the same concentrations of sodium nitrate. Two per cent water agar served as a control. Petri plates were inoculated with uniform pieces cut from a fresh culture of

Pythium ultimum on potato dextrose agar. Each treatment was replicated three times. Seven beet seed balls were placed in each of three Petri plates for each treatment. The beet seed balls were hot water treated and then surface sterilized for three minutes to prevent contamination of the plates with seed borne microorganisms. The seed balls were pushed below the surface of the agar to insure direct contact with the substances dissolved in the agar.

The results of this experiment, presented in Table XVII, show that sodium nitrate, at all concentrations tested except one, increased the linear growth rate of Pythium ultimum. Growth of the fungus was not increased at 8000 parts per million of sodium nitrate except when potassium chloride was present in the medium. Sodium nitrate at 8000 parts per million reduced the number of seed that germinated and seed balls with viable seed, but this reduction was abated with the introduction of potassium chloride into the medium. The number of seed that germinated at 1000 parts per million of sodium nitrate with potassium chloride was low, while the number of seed balls with viable seed was not. The size of the seed sample and the number of replicated were apparently too small.

For a second experiment, sodium nitrate was incorporated into a two per cent water agar medium at 10,000,

TABLE XVII

Effects of Different Concentrations of Sodium Nitrate, Alone and in Combination with Potassium Chloride, upon the Growth of Pythium ultimum and the Germination of Beet Seed

| Treatments(1) in ppm | | <u>Pythium ultimum</u> | Table beets | |
|-------------------------|-----|--------------------------------------|--|-------------------------|
| NaNO ₃ | KCl | Average growth for 24 hrs. in mm. | Seed balls with viable seed | Seed that germinated |
| | | | Average from 7 seed balls after 12 days | |
| 1000 | - | 41.7 | 6.3 | 16.7 |
| 1000 | 400 | 41.8 | 6.3 | 12.3 |
| 2000 | - | 43.7 | 5.3 | 16.7 |
| 2000 | 400 | 44.2 | 6.7 | 18.0 |
| 4000 | - | 45.3 | 6.3 | 16.0 |
| 4000 | 400 | 44.6 | 5.7 | 16.0 |
| 8000 | - | 37.6 | 4.7 | 12.0 |
| 8000 | 400 | 45.7 | 5.7 | 15.3 |
| - | - | 38.1 | 6.0 | 15.3 |

(1) The solutes were dissolved in 2 per cent agar media

12,000 and 16,000 parts per million. These concentrations were duplicated with the addition of potassium chloride at 400 parts per million. Two per cent water agar, with and without potassium chloride at 400 parts per million, served as controls. The methods of the previous experiment were repeated to transfer Pythium ultimum and beet seed balls to the prepared media. The number of replicates was increased to four with eight seed balls in each Petri plate.

The same procedure was employed to determine the effects of Manzate at 120 parts per million upon the linear growth rate of Pythium ultimum and germination of beet seed. 1.2 grams of Manzate were dissolved in a liter of aqueous solution. Ten milliliters of this solution were added to ninety milliliters of two per cent water agar solution containing 0.5 per cent sucrose just before solidification. It was assumed that this concentration of fungicide approximated the amount present in soil when applied at the rate of twenty pounds per acre.

The linear growth rate of Pythium ultimum was steadily decreased with increasing concentrations of sodium nitrate (Table XVIII). As in the previous experiment, the presence of potassium chloride decreased the inhibitory effects of sodium nitrate. Potassium chloride alone at 400 parts per million had no significant effect on the linear

TABLE XVIII

Effects of Different Concentrations of Sodium Nitrate, Alone and in Combination with Potassium Chloride and of Manzate, upon the Growth of Pythium ultimum and the Germination of Beet Seed

| Treatments (1) in ppm | | <u>Pythium ultimum</u> | Table beets | |
|--------------------------|-----|--------------------------------------|--|-------------------------|
| NaNO ₃ | KCl | Average growth for 24 hrs. in mm. | Seed balls with viable seed | Seed that germinated |
| | | | Average from 8 seed balls after 12 days | |
| 10,000 | - | 42.2 | 6.2 | 9.5 |
| 10,000 | 400 | 45.4 | 6.8 | 14.0 |
| 12,000 | - | 34.2 | 6.5 | 11.8 |
| 12,000 | 400 | 45.1 | 5.2 | 8.0 |
| 16,000 | - | 29.6 | 2.0 | 2.8 |
| 16,000 | 400 | 37.6 | 3.0 | 4.2 |
| - | 400 | 51.2 | 6.2 | 16.5 |
| - | - | 50.7 | 6.2 | 14.2 |
| Manzate, 120 ppm. | | 0.0 | 7.0 | 18.0 |

(1) The solutes were dissolved in 2 per cent agar media

growth rate of Pythium ultimum. The number of beet seed that germinated was severely attenuated by increasing concentrations of sodium nitrate. Even at the highest concentration of sodium nitrate, the presence of potassium chloride in the medium reduced the effects of sodium nitrate.

Manzate at 120 parts per million prohibited the growth of Pythium ultimum.

The results of the field experiment (Table X) indicated that additional tests were needed to determine the effects of Manzate and Ceresan M at higher concentrations upon the incidence of beet seedling necrosis. Manzate and Ceresan M were incorporated with the top four inches of soil in five inch clay pots, at ten and twenty pounds per acre. At the same concentrations, the fungicides were also incorporated with a fertilizer which furnished 200 pounds of N per acre as sodium nitrate, 120 pounds of P_2O_5 per acre as treble superphosphate, and 100 pounds of K_2O per acre as potassium chloride. Thirty-five beet seed balls were planted in each pot. The treatments were replicated seven times in randomized blocks.

The results, recorded in Table IXX, show that Manzate at twenty pounds per acre greatly enhanced the emergence of seedlings and the presence of the fertilizer retarded the rate of emergence. Where the two were combined

TABLE IXX

Effects of Fungicides and Fertilizers
Incorporated with the Soil upon the Emergence
of Table Beets in the Greenhouse

| Fungicides | Average number of emergences from 35 seed balls after: | | | |
|--------------|---|------------------|---------|------------------|
| | 6 days | | 18 days | |
| | NPK (1) | No Fertilizer | NPK | No Fertilizer |
| Manzate | | | | |
| 10 lbs./acre | 0 | 9.3 | 18.4 | 12.1 |
| 20 lbs./acre | 0 | 35.1 | 24.3 | 46.4 |
| Ceresan | | | | |
| 10 lbs./acre | 0.1 | 2.0 | 15.6 | 3.0 |
| 20 lbs./acre | 0.3 | 3.4 | 31.4 | 5.6 |
| None | 0 | 2.7 | 22.4 | 6.4 |

(1) Fertilizer applied to furnish:

200 lbs. of N per acre as NaNO_3

120 lbs. of P_2O_5 per acre as treble superphosphate

100 lbs. of K_2O per acre as KCl

together, the fertilizer prevented the acceleration of germination. Ceresan M had no significant effect upon the number of emergences when applied alone, but did increase the number when applied at twenty pounds per acre with the fertilizer. Such interaction was not observed between Manzate and fertilizer.

The best stands of seedlings after eighteen days were recorded from the pots receiving Manzate alone at twenty pounds per acre and Ceresan M at twenty pounds per acre with fertilizer (Table XX).

The effects of Manzate on the emergence and survival of seedlings was studied further by incorporating the fungicide at twenty pounds per acre with the top four inches of soil in five inch, clay pots, both alone and in combination with a fertilizer.

It was considered that the severe retardation of seedling emergence observed in the previous experiment was due to an excess of sodium nitrate. Consequently the rate was decreased from 200 to 50 pounds of N per acre as sodium nitrate. The levels of phosphorus and potassium remained the same as before. Each treatment was replicated seven times in randomized blocks. Forty beet seed balls were planted in each pot,

A far greater number of emergences was attained when Manzate was used alone. Fertilizer alone or in combination

TABLE XX

Effects of Fungicides and Fertilizers
Incorporated with the Soil upon Stands of
Table Beets in the Greenhouse

| Fungicides | Average stand of seedlings from 35 seed balls, 18 days after planting. | |
|----------------|---|---------------|
| | NPK(1) | No fertilizer |
| Manzate | | |
| 10 lbs./acre | 8.8 | 4.6 |
| 20 lbs./acre | 14.6 | 20.8 |
| Ceresan | | |
| 10 lbs./acre | 8.3 | 1.8 |
| 20 lbs./acre | 21.0 | 1.7 |
| None | 14.0 | 2.5 |

(1) Fertilizer applied to furnish:

200 lbs. of N per acre as NaNO_3

120 lbs. of P_2O_5 per acre as treble superphosphate

100 lbs. of K_2O per acre as KCl

with Manzate was not as effective as the fungicide alone in increasing the number of emergences, Table XXI. Of the seedlings that emerged, 53.7 per cent died when Manzate was used alone, 51.4 per cent when the fertilizer was used alone, and 49.7 per cent when the fungicide and fertilizer were used together. Therefore the increased stands obtained using Manzate alone are attributed to a control of preemergent deaths. The fertilizer, at fifty pounds of N per acre as sodium nitrate, nullified the beneficial effects of Manzate just as thoroughly as the same fertilizer at two hundred pounds of N per acre. The use of the fertilizer alone at the lesser concentration of nitrate still doubled the stands of seedlings obtained.

TABLE XXI

Effects of Manzate Incorporated with Soil,
Both Alone and with Fertilizer, upon Emergence
Death and Survival of Table Beets in the Greenhouse

| Soil Treatments | Average number of seedlings from 40 seed balls | | |
|-------------------------------------|---|---------|--------------|
| | Emergences after: | | Stand after: |
| | 7 days | 18 days | 18 days |
| Manzate 20 lbs./acre | 47.3 | 52.0 | 24.1 |
| NPK (1) | 28.6 | 35.8 | 17.4 |
| Manzate 20 lbs./acre plus NPK | 22.4 | 30.4 | 15.1 |
| Control | 18.3 | 20.1 | 8.1 |

(1) Fertilizer applied to furnish:

50 lbs. of N per acre as NaNO_3

120 lbs. of P_2O_5 per acre as treble superphosphate

100 lbs. of K_2O per acre as KCl

DISCUSSION AND CONCLUSIONS

A review of the literature indicated that species of microorganisms associated with beet seedling necrosis were not always the same wherever the disease was studied. Similarly, discussions of predisposing factors and control measures by different workers frequently were in disagreement.

Pythium ultimum was the primary cause of beet seedling necrosis in the Willamette Valley of Oregon. The variable pathogenicity and low frequency of isolation of Fusarium species indicated that they were of secondary importance.

Soil temperatures between sixty and seventy degrees Fahrenheit favored the development of beet seedlings and minimized the incidence of beet seedling necrosis. Soil temperatures below sixty degrees were unfavorable for the host as well as the pathogens. Fusarium species were never isolated from diseased seedlings grown in soil maintained below sixty degrees. Soil temperatures above seventy degrees favored the incidence of beet seedling necrosis. Eighty degrees was the limit of the experiment and the effects of higher soil temperatures on disease incidence were not determined. The linear growth of Pythium ultimum was greatest at thirty degrees Centigrade (86° F.), indicating that high soil temperatures accelerated certain

activities of the fungus.

Species of *Fusarium*, isolated from diseased seedlings collected from infested fields were also present in commercial beet seed. Other species of fungi including Phoma betae were also found in beet seed. Experiments demonstrated that the most important source of infection was from infested soil and not from microorganisms in beet seed balls. Consequently it was necessary to determine an adequate means of controlling soil borne infection.

Greatly improved stands of table beets were obtained by incorporating calcium nitrate or sodium nitrate with the soil before planting. The effectiveness of a nitrate fertilizer was enhanced by the presence of potassium chloride, but not by treble superphosphate. Ammonium sulphate did not increase the stands of seedlings but produced larger beets than calcium nitrate. The concentration of calcium nitrate in the soil or the competition between crowded plants could have been responsible for the smaller beets. In comparison, fewer plants and less competition could explain the growth of larger beets in the rows receiving ammonium sulphate. Perhaps ammonium sulphate was more available and readily utilized by maturing beets than calcium nitrate. While emergences of seedlings was delayed, they appeared green and vigorous with calcium nitrate in the soil. Emergences were delayed less when ammonium sulphate was in

the soil, but seedlings had red leaves and appeared less vigorous. This lack of vigor and discoloration could be attributed to injury by infectious soil organisms which caused less impairment to seedlings in soil with calcium nitrate.

Experimental evidence showed that a decrease in beet seedling necrosis was not accomplished by simply increasing the vigor and resistance of seedlings with the application of nitrate fertilizers to the soil. Decreases in disease incidence were observed only when nitrate and potassium fertilizers were added to fresh field soil. When infested field soil was allowed to dry out for four months in the greenhouse, little attenuation of seedling necrosis was observed when the same fertilizers were added to the soil. The principal effect was apparently on the soil microflora, which had to be in normal biological equilibrium before any significant reduction in disease incidence could be brought about.

The occurrence of beet seedling necrosis in soils where beets were grown for the first time and the reduction in disease incidence when nitrate and potassium fertilizers were added to the soil indicated that such soils were lacking in certain nutrients which would tend to accelerate the antibiotic activity of saprophytic organisms and impair the infectivity of parasites. It

was apparent that a rectification of such deficiencies was fundamental in achieving adequate control of the disease.

In addition to this indirect approach to control, the effects of fungicides on infectious organisms in the soil were also studied. Greenhouse experiments demonstrated that Manzate, mixed with the soil at twenty pounds per acre, reduced the number of seedling deaths before emergence. Although a considerable number of subsequent deaths occurred, stands were significantly increased by this treatment. Ceresan M at the same concentration was equally effective when nitrate and potassium fertilizers were present in the soil. The presence of the same fertilizer completely nullified the effects of Manzate. The reasons for the contrasting interaction between fungicide and fertilizer were not understood.

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