

INVESTIGATIONS ON ROOT ROT OF
BEANS CAUSED BY
FUSARIUM SOLANI F. PHASEOLI

Thesis for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
Don Morgan Huber
1963



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ABSTRACT

INVESTIGATIONS ON ROOT ROT OF BEANS CAUSED BY FUSARIUM SOLANI F. PHASEOLI

by Don Morgan Huber

The purpose of this investigation was to determine the effects of cultural practices and induced resistance on the expression of Fusarium root rot. This study was threefold: First, to evaluate the effect of various cropping sequences on the development of bean root rot and the associated microflora; second, to determine the effect of soil temperature and moisture on the microflora of a "root rot" soil; and third, to investigate the role of enzymes in root rot resistant and susceptible plants by comparing the characteristics of genetic and induced resistance to root rot.

The plate-profile technique, developed especially for this investigation, provided an excellent method for studying the effects of cropping sequence on the actively growing fungi and microbial associations in the soil. The data obtained with the plate-profile method was less variable than data obtained with other available techniques, and could be analyzed directly without the need for prior transformation even though only four replicates per treatment were used.

Eighty-seven genera of fungi were isolated in association with other fungi and bacteria and nematodes. The various microbial associations of possible significance in the biological control of Fusarium, included the parasitism of Fusarium by an Actinomycete, bacterial necrosis, and lysis. Bacterial necrosis was influenced by the present

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and previous crop, and was directly correlated with reduction in disease severity. It was found that barley preceding beans in the rotation significantly increased root rot, while corn reduced the severity of the disease. The studies on cropping sequence emphasized the significant influence of the present and previous crops on the soil microflora and disease severity.

Soil moisture was found to have a significant effect on many of the frequently isolated soil fungi, and may be responsible for some of the year to year variation observed.

The histochemical localization of tyrosinase, peroxidase, and beta-glucosidase in the endodermis, phloem, cambium, and xylem and their activation around a wound were associated with resistance to Fusarium. Naturally resistant and induced resistant plants responded to infection in a similar manner. Resistance to Fusarium was characterized by a non-specific wound response. This response consisted of the rapid deposition of inhibitory compounds around the infection site followed by the formation of a wound periderm which limited further penetration of the pathogen.

INVESTIGATIONS ON ROOT ROT OF BEANS CAUSED BY
FUSARIUM SOLANI F. PHASEOLI

By

Don Morgan Huber

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1963

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The author expresses his sincere appreciation to Dr. Axel L. Andersen for his continued assistance and many helpful suggestions throughout the course of this investigation. The suggestions of Dr. E. S. Benke, Dr. E. H. Barnes, Dr. J. L. Fairley, Dr. N. E. Tolbert, Dr. W. B. Drew, and Dr. A. H. Ellingboe contributed much toward the completion of this research.

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**Dedicated to My Two Daughters
Brenda and Joyce**

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INTRODUCTION

The common bean (Phaseolus vulgaris L.) is one of the most important and universal food crops of the world (181). Root rot caused by Fusarium solani (Mart.) Appel and Wr. f. phaseoli (Burk.) Snyd. and Hans. is one of the major diseases of the common bean. This disease was first reported by Burkholder (19) on beans in New York, in 1917. Since that time it has been recognized as a serious problem in all of the major bean producing areas of the world (167, 181). The significance of root rot is often overlooked unless plants are dug or the environmental conditions emphasize it. Losses from root rot are difficult to estimate because satisfactory control measures are not available. In severe infestations the plant may die, but generally only growth is retarded. Losses from the disease are generally greater under low moisture conditions because few secondary roots are produced; however, a partial yield will generally be obtained (21, 22, 63).

Variable degrees of control have been obtained by suitable cultural practices such as crop rotation or the direct addition of soil ammendments (17, 20). Specific crop sequences may be of greater value, however, than a long term rotation. Alteration of the microbial population has been considered the mechanism responsible for disease control (96), but the underlying mechanisms are not understood.

No commercial variety of bean is resistant to root rot. Resistance is, however, available in a few non-commercial types and in Phaseolus coccineus. Hence, breeding programs have been initiated to incorporate the resistance from P. coccineus and N203 (a black Mexican variety of P. vulgaris) into commercial lines.

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The purpose of this study was: First, to evaluate the effect of various cropping sequences on the development of bean root rot and the associated microflora; second, to determine the effect of soil temperature and moisture on the microflora of a "root rot" soil; and third, to investigate the role of enzymes in root rot resistant and susceptible plants by comparing the characteristics of genetic and induced resistance to root rot.

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LITERATURE REVIEW

Causal Organism

Fusarium solani f. phaseoli is the primary pathogen in the "bean root rot complex." Rhizoctonia solani Kuhn and Thielaviopsis basicoli Zoph. are ordinarily of secondary importance. Fusarium, Rhizoctonia, and Thielaviopsis are capable of direct penetration or entrance through natural openings or wounds (28, 34, 35, 36). These organisms are soil-borne and may be indigenous to the soil, or live over in crop debris, or remain as soil saprophytes after introduction (17, 98).

Ecology of Soil Fungi

Many authors, including Cook (39), Garrett (50, 51, 52, 53), Menzies (98), Parkinson and Waid (123), Sadasivan and Subramania (130), Stover (147), and Warcup (164), have reviewed in detail the ecology of root disease pathogens and other soil fungi. In general, little is known of the actual manner of existence of soil-borne fungi (110, 158). Waksman (159, 160) was the first to conclude the presence of a cosmopolitan fungal flora of the soil. Jensen (66) reported that distinct fungal communities are associated with different soils. Garrett (50) considered Waksman's soil inhabiting fungi as primitive or unspecialized parasites characterized by a wide host range and strong saprophytic ability. He indicated that fungi exist saprophytically with an incidental parasitic ability and that most of the root-infecting fungi are soil inhabitants, although there is no sharp dividing line between the soil inhabitants and the soil invaders. The soil invaders

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were characterized by more highly specialized parasites in a closer association with their hosts.

Several investigators have studied the *Fusaria* in soil material. Maloy (88) indicated that the mycelial form, rather than the conidial (more virulent) form of *Fusarium solani* f. *phaseoli* predominated in the soil in the prolonged absence of a host plant. He also mentioned (89) that the mycelial form was more tolerant of residues, antibiotics, and possibly crop rotations; although both forms grew well on root exudates from a number of non-host plants. Maloy (88) suggested that the "dual phenomenon" which is a change to a less virulent mycelial type from a more virulent conidial type (59), could explain the reduction in disease severity which resulted without a corresponding change in the population of the pathogen. On the other hand, Nash *et al.* (110) reported that *F. solani* f. *phaseoli* existed in the form of chlamydospores in the soil, which, according to Schroth and Snyder (133) were stimulated to germinate by root exudates. Using soil smears, Nash *et al.* (110) observed chlamydospores in natural soil and found that macrospores added to the soil generally formed chlamydospores directly or shortly after germination. They reported mutation to the mycelial type only in sterilized soil in which *Fusarium* grew profusely.

Techniques for Studying the Soil Fungi

The lack of more adequate techniques to study the complex interactions involved with the soil fungi has limited our knowledge of the soil fungi (158). Techniques for studying the soil fungi have been discussed by several researchers (71, 104, 164). Most of the techniques were designed to study specific groups of organisms or microbial activities in the soil, and are limited by the size of the area sampled, the media employed, and the time of the study (71).

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The dilution-plate technique and its modifications have been more widely used than most other techniques, even though many organisms are not isolated from the soil because of their diverse nutritional requirements or low frequency. Species that sporulate abundantly in the soil are isolated most frequently with this method (71). The effect of sporulation was demonstrated by Hack (58) by plating soil previously infested with a spore suspension of Didymella lycopersici. Her plate counts decreased as the spores germinated, but the counts immediately increased as the fungus sporulated. Park (122) noted that the level of the soil population of Fusarium oxysporum was too low to be studied by dilution plating even though it was a "pioneer" fungus. The predominant genera of fungi isolated by the dilution-plate method included Penicillium, Cylindrocarpon, Trichoderma, Mortierella, Mucor, Pythium, Cephalosporium, Rhizopus, and Aspergillus (104, 118, 175, 177).

Warcup (162) modified the dilution-plate techniques by plating soil rather than soil-dilutions since spore masses remain more intact under these conditions. Watson (166) washed spores from the soil prior to plating and obtained results similar to techniques based on the isolation of actively growing hypha. Dilution and soil plate techniques failed to give information on the activity of fungi in the soil without further analysis to determine the origin of the colonies on the plates.

Several techniques have been devised to isolate only actively growing hyphae from the soil. Thus, a screened immersion plate was devised by Thornton (150) in which fungi were required to grow across an air gap to agar before being isolated. He demonstrated that non-sporing fungi dominated the soil and that all soils were of a similar mycelial status (151). Chesters (29) prepared an immersion tube with glass capillaries for hyphal isolation. This technique was modified by Mueller and Durrell (106) who prepared immersion tubes from

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plastic centrifuge tubes. Martinson (93) used this technique to obtain a close correlation of the inoculum potential of Rhizoctonia solani and disease incidence. Andersen and Huber (6) devised a plate-profile technique to study microbial interactions as well as the distribution of fungi in the soil. These techniques reveal ecological information not obtained by dilution techniques but still may give misleading information since fungi that produce "fast-growing" hyphae may be the most frequently isolated (71).

Warcup (163) isolated fungal fragments directly from a wheat-field soil and found that many of the fungi he isolated were infrequent or absent from dilution plates. Microscopic examination of a grass-land soil was also used to demonstrate that fungi were present both as spores and mycelium (164).

Alteration of the Soil Microflora through Cultural Practices

Since the soil-borne pathogens causing bean root rot may be indigenous to the soil, live over in crop debris, or remain as soil saprophytes after introduction, the effects of cultural practices assume major importance.

Extensive literature reviews concerning the control of diseases through cultural practices have been made by Berkeley (9), Butler (25), Simonds (139), Woods and Tveit (178), Garrett (50, 57), and Menzies (98). Thus, crop rotation has been an integral part of the recommendations for the control of bean root rot since Burkholder (19) first studied the disease. Garrett (50) pointed out that adequate control could be accomplished through the reduction of the pathogen to a low level rather than by its complete elimination. Burke (16, 17) found that root rot was more severe in virgin land than in land previously under cultivation for some time. Cultivated soil possessed a

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"pathogen suppressing" property while virgin soil favored germination and growth of the pathogen. This "pathogen suppressing" property was caused by microbial activity, although no qualitative differences were observed. Williams and Hack (173) demonstrated the pathogen suppressing effect of non-sterile soil was the result of cropping and management rather than soil type. Burke (17) found that Fusarium solani f. phaseoli was resistant to competition or suppression by other microorganisms in vivo and that it exhibited the ability to suppress most of the other organisms common in field soils. Schroth and Hendrix (135) hoped that rotation with nonsusceptible plants would lower the inoculum potential of Fusarium in the soil and reduce the severity of root rot. Their results indicated that standard rotation practices enabled the pathogen to survive for long periods in the absence of a susceptible host. Snyder et al. (143) found that other crops stimulated chlamydospore germination which was followed by conidial production and an increase in spore load.

Maloy (88) speculated that the effects of crop rotations on the saprophytic growth of Fusarium may account for the reduction in severity of the disease without a reduction in population since the mycelial (less virulent type) was hardly affected by cultural alterations and antibiotics in the soil. Maloy (87) also reported little correlation between the length of time out of beans and the severity of root rot.

Since long term rotations have failed to satisfactorily reduce the population of Fusarium solani f. phaseoli in the soil (88, 89, 135) and since rotations have not always been dependable in reducing root rot severity (20), attention has recently been focused more on the effects of crop sequence. For instance, Maloy and Burkholder (89) reported that wheat and alfalfa in the rotation reduced bean root rot; but Maloy (88) later showed that wheat reduced root rot only when it preceded beans in the rotations. Barley preceding beans also reduced the severity of root rot even though the population of the pathogen increased (111).

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Organic amendments have been incorporated into the soil to bring about specific microbial alterations which were less conducive to disease development. Bean root rot was markedly reduced when sawdusts, cellulose, wheat bran, sorghum, ground alfalfa hay, oat straw, soybean hay, bean straw, wheat straw, corn shucks, pine shavings, or barley straw were applied to the soil (17, 40, 42, 43, 86, 142, 172). On the other hand, tomato, alfalfa, lettuce, bean seed meal, green barley hay, alfalfa, and soybean residues markedly increased bean root rot (86, 142). Cooke (40) found that chlamydospore germination and the subsequent mycelial development of Fusarium solani f. phaseoli was reduced in infested soil when barley straw was incorporated prior to planting. Green corn and oat residues reduced the amount of Rhizoctonia root rot as well as the population of Rhizoctonia in the soil (119, 120, 121).

The effect of soil amendments on root rot has been attributed to an increased carbon to nitrogen ratio (142). Increased nitrogen in the soil was correlated with increased infection by Fusarium (142, 172) and decreased Rhizoctonia (42, 43). Sugar amendments increased root rot (17). Residues, high in nitrogen, such as bean straw, bean-seed meal, and alfalfa hay increased early infection but reduced the total infection by the end of the season. High rates of nitrogen in the soil appeared to nullify the beneficial effects of carbonaceous materials such as barley, wheat, and oat straws (18). However, Tyner (154) concluded that the general chemical nature of organic materials added to infested soils was more important than the carbon: nitrogen ratio.

Effect of Soil Temperature and Moisture On Soil Microorganisms

The saprophytic survival and activity of microorganisms in the soil may be influenced by soil temperature and moisture. Papavizas

and Davey (1960) found that the fungus was better at a temperature of 60-65°C than at 60-65°C. Nadakavukaren (1960) found that microscopical examination of the holding capacity of the soil showed that Rhizoctonia was capable of growing in dry soil for several months.

Maximal growth occurred on soil and air-dried populations and other conditions favored by the techniques, and that temperature factor influenced the number of dry soil colonies per season. F

Microscopical examination, fixation, and temperature not be related and Ledingham (1960) found that spores did not grow in soil. Campbell (1960) found that sativum was

and Davey (120, 121) and Blair (11) found that Rhizoctonia survived better at a soil moisture of 20-50 percent of the moisture capacity, than at 60-90 percent. Activity was also greatest at 20-22°C. Nadakavukaren and Horner (109) reported maximum survival of microsclerotia of Verticillium at 5-15°C and 50-75 percent moisture holding capacity and poor survival above 25°C. Elmer (48) stated that Rhizoctonia survived saprophytically in soil only when adequate rainfall prevented dessication during the summer months, but that it was capable of surviving on infected host plants under conditions too dry for saprophytic survival.

Maximum sclerotial development of Macrophomina phaseoli occurred on susceptible plants in soil at a low soil moisture and high soil and air temperature (62). Clark (37) reported that microbial populations increased in proportion to the root growth of soybeans and other crops, and that microbial numbers and root growth were favored by lower moisture levels. By using microscopic fluorescence techniques, Seifert (136) found that soil moisture was the only decisive factor influencing the number of bacteria in humus-carbonate soils and that temperature had no effect. Thornton (149) indicated a greater number of microorganisms were isolated in the summer under warm, dry soil conditions as compared to the spring, winter, and autumn seasons. However, little qualitative change occurred during the year.

Microbial activity, based upon respiration, nitrification, nitrogen-fixation, and ammonification studies, was influenced by various soil temperature and moisture conditions (10, 56). However, activity may not be related to the numbers of organisms in the soil (129). Chinn and Ledingham (32) reported that the viability of Helminthosporium spores did not decline in dry soil but declined markedly in saturated soil. Campbell (26) found that the pathogenicity of Helminthosporium sativum was increasingly depressed by other soil fungi as the

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Flooding has been reported effective in eliminating F. oxysporum cubense from the soil (147). Growth and survival of this fungus decreased progressively as the soil moisture content increased from the optimum of 25 percent saturation (145, 146).

Biological Control of Soil-Borne Fungi

Reduction in disease severity with specific cultural practices is generally the result of altered or stimulated biological interactions. Control through specific microbial interactions is referred to as "biological control." Berkeley (9), Garrett (50, 51), Snyder (144), Timonin (152, 153), Katznelson et al. (72), Weindling (169), Weindling et al. (170), Wood and Tveit (178), and Kristic (74) present a detailed review of the literature concerning the biological control of plant diseases.

Antibiosis: Antagonistic organisms have been found effective against many plant pathogens. Norton (114) reported the antagonism of Macrophomina phaseoli in soil containing Trichoderma, Thielavia, Aspergillus, and Bacillus cereus. Simmonds et al. (140) demonstrated that Helminthosporium sativum conidia were inhibited by the microflora in the surface soil. Ludwig and Henry (85) considered the rapid development of Trichoderma viride in previously sterilized soil of significance in the suppression of Ophiobolus graminis. Williams (174) found that most antagonists of Fusarium roseum were representatives of the genus Aspergillus or Penicillium and that their frequency was related to the specific crop or soil.

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Fungistasis: The inhibition of germination of fungal spores in soil is a wide-spread phenomenon attributed to diffusible toxic factors associated with biological activity in the soil (81). Fungistasis has been restored to sterilized soils by the addition of Fusarium roseum and other soil fungi (57, 179), bacteria (116), and actinomycetes (82, 83).

Lingappa and Lockwood (80) were unable to isolate antibiotics from the soil. However, they found that isolated lignicolous compounds liberated during the microbial degradation of organic materials were fungistatic and possibly responsible for the fungistasis of soils. They also demonstrated (81) that indirect methods employed to study fungistasis resulted in the production of antibiotics by microorganisms growing on the surface of the assay media rather than the result of free toxic materials in the soil.

Another hypothesis concerning the nature of the wide-spread soil fungistasis was based on the observation that certain organic amendments to the soil removed the fungistatic principle. This indicated the possible deficiency of nutrients required for germination. Additions of glucose (46), wheat germ, bran, molasses, orange juice, and green plant tissues were highly effective in removing fungistasis while wheat straw and wheat roots were ineffective (32). Root exudates were also effective in overcoming fungistasis (132, 133, 134, 179). Lingappa and Lockwood (81) found spores normally inhibited in soil would readily germinate in soil extracts.

Lysis: The disintegration of mycelium or other fungal parts, is commonly observed in studies of fungistasis (31, 82, 83, 84). Lytic phenomenon as reviewed by Weindling et al. (170) may be the result of enzymatic activity of soil organisms (2, 60, 61), or autolysis after death or disruption of the protoplasm from toxic materials (5, 27, 54, 124).

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The lytic properties of soil Actinomycetes (27, 31, 99, 100, 169, 171) and bacteria (44, 60, 61, 102, 112) have been reported. Lysis has been correlated with the reduction of Fusarium diseases (16, 102, 103).

Alexander and Mitchell (2) obtained bacteria lysing Fusarium and other fungi from infested soils. Chitin and laminarin amendments to soils greatly reduced the severity of Fusarium diseases and also induced increased enzymatic activity of the mycolytic organisms. Although chitinase, or combinations of chitinase, cellulase, and proteases failed to produce lysis, the fact that Pythium debaryanum, which contained no chitin in its cell walls, was not suppressed after chitin amendment may indicate an enzymatic factor in lysis.

Carter and Lockwood (27) reported the lysis of several fungi by soil Streptomyces which lysed both living and dead mycelium on peptone agar. Antibiotics and fungicides effective against Glomerella cingulata resulted in the lysis of living but not of dead mycelium.. Lockwood (84) reported that mycelium of many pathogenic fungi was destroyed when agar cultures were covered with field soil for one to two weeks. No differences were observed in the lysis of soil-inhabiting and soil-invading fungi; although individual organisms within these groups differed widely. Mycelium of nonsoil-borne fungi on agar media was more readily lysed than soil-inhabiting or soil-invading fungi. Chinn et al. (33) reported that soybean amendments to the soil stimulated the germination of Helminthosporium sativum. The germ tubes were then readily lysed in the absence of growing wheat plants. A similar mechanism for the biological control of Phymatotrichum root rot after organic soil amendments was observed by Mitchell and Alexander (102, 103).

Hyperparasitism: The parasitic existence of one organism on another, is a common relationship with many fungi. Bliss (12), Darley and Wilbur (41), Overman and Burgis (117), and Garrett (51)

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reported the selective stimulation of Trichoderma viride in soils after various fumigant treatments. Armillaria mellea was rapidly destroyed by the direct parasitic action of Trichoderma, after, but not before, carbon disulfide fumigation. Trichoderma killed Armillaria in sterilized but not in natural soils without fumigation. According to DeWolfe et al. (45) the application of wood shavings as a mulch to Phytophthora infested citrus soil resulted in the elimination of Phytophthora through the parasitic action of Trichoderma. Campbell (26) reported the breakdown of the mycelium of Helminthosporium sativum by Trichoderma viride whereas Phoma humicola and Epicoccum purpurascens produced internal mycelium in Helminthosporium. All three pathogens considerably reduced the pathogenicity of H. sativum.

Weindling (168, 169) studied the mode of parasitism of Trichoderma. He found that parasitism may be affected by the host and other factors. Trichoderma is generally lethal to both Rhizoctonia and Pythium, and is generally coiled around Rhizoctonia but seldom around Pythium. This relationship could be altered to one of compatibility or intense parasitism by varying environmental conditions. He suggested the application of Trichoderma to Rhizoctonia infested soil as a possible control measure. Chi (30) reported the coiling of Trichoderma hypha around Fusarium. Acrostalagmus, Aspergillus, Penicillium, Fusarium, Botrytis, and Verticillium were also able to directly parasitize Rhizoctonia in culture in a manner similar to Trichoderma (14, 168). Brown (15) attributed the resistance in Texas of watermelon susceptible to Phymatotrichum root rot in Arizona to the presence of Trichoderma which checked or killed the pathogen by direct attack. Butler (23) and Butler and King (24) observed two methods of attack when Rhizoctonia parasitized the mycelium of Phycomycetes. The first, included coiling around the host mycelium

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with the frequent penetration of infected hypha followed by an extensive internal mycelium. The second method of penetration was by a few infection hypha followed by limited or extensive internal mycelium. Complete destruction of the host was rare.

Warren (165) reported the parasitism of Rhizoctonia solani by Papulospora was effective in reducing the amount of black-root of sugar beets in pot tests. Papulospora rapidly coiled around the Rhizoctonia mycelium causing the host protoplasm to disintegrate. Huber and Andersen (64) reported the reduced severity of bean root rot on corn soils and the increased severity on barley ground. Bacterial necrosis of Fusarium was inversely related to root rot while crop sequence had little effect on the bacterial necrosis of Rhizoctonia (7). Necrosis of Fusarium was brought about by the parasitic action of a Xanthomonas sp. which was in intimate contact with the hypha of Fusarium and caused the agglutination of mycelial contents and the death of the Fusarium.

Predation: Reports on predacious fungi have been reviewed by Duddington (47). Although several experiments have suggested the use of predacious fungi for the biological control of plant pathogenic nematodes, much remains to be known of their physiology and ecology for optimum utilization (47, 90).

Host Response to Infection

Cicatrization is a relatively common phenomenon in limiting the penetration of fungal pathogens. Huber (63) showed that resistance to root rot could be induced with several plant growth regulators. The induced resistance was characterized by the formation of a wound periderm which limited the penetration of the pathogen. He assumed that the growth regulator activated a latent mechanism responsible for

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the physiological resistance and wound response. According to Bloch (13) the activated state of resting cells which results in increased metabolic activity, differentiation, and growth, occurs as a result of a non-specific degenerative response to wounding or invasion.

Conant (38) reported that tobacco plants susceptible to Thielaviopsis basicola were characterized by the slow initiation of pericycle formation, whereas rapid cell division protected the cortex from further penetration by the pathogen in resistant plants. Marshall (91) reported Fusarium oxysporum f. gladioli failed to penetrate gladiolus corms after wound periderm formation (approximately 5 days after cutting). He noted that periderm formation was enhanced when corms were stored for one week at 95° F. and 80 percent relative humidity immediately after digging.

Infection of sweet potatoes by Ceratocystis fimbriata (black rot) and other fungi was prevented by curing at 95° F. until a wound periderm developed (6 days) (76,77). This periderm was characterized by a heavily suberized layer of cork, a cork cambium, and a phelloderm (105). Fusarium oxysporum (a wilt organism) can, however, inhibit periderm formation (94). A similar protection from infection is present in white potatoes. Simonds and Johnson (138) found that most of the orthodihydric phenols stimulated the formation of a thick suberized layer. The stimulatory effect of these compounds was associated with their ability to serve as a substrate for tyrosinase. Politis (126) reported the stimulatory effect of chlorogenic acid on cicatrix formation. He found that pathogens, as well as wounds, induced the elaboration of chlorogenic acid and the subsequent formation of a protective periderm in sunflower and lettuce plants. Johnson and Schaal (69) reported the activation of the cork cambium by chlorogenic acid.

Periderm formation and the dedifferentiation of tissues were stimulated with various chemical and physical treatments (63,65).

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Jacobs (65) demonstrated that indol acetic acid was required for dedifferentiation of *Coleus* tissues around a wound by removing the apical leaves and buds of a wounded plant with the result that no dedifferentiation took place. If a lanolin paste was applied or if the leaves were not removed dedifferentiation readily took place. English and Hagen-Smit (49) isolated a natural wound hormone from beans (traumatic acid) which initiated cell division and periderm formation.

Host response to infection is also characterized by the production of fungistatic or fungitoxic compounds. Chlorogenic acid, in addition to stimulating cell division, is fungitoxic to Streptomyces scabies and is associated with resistance of potatoes to scab (69, 70, 75). Other naturally occurring phenols and quinones were also implicated. Phytoalexins (68, 107) are produced by resistant plants in response to infection and prevent the further growth of the pathogen in the diseased tissue. Muller (108) believes that local lesion resistance results from the ability of the host tissue to block the metabolic activities of the pathogen resulting in the formation and accumulation of phytoalexins around the infection site. Ipomeamaron (155) and tannins (113) also enter the disease syndrome as fungitoxic or fungistatic compounds. The polyphenol oxidases probably play a significant role in resistance in respect to the elaboration of these compounds (128, 157). Allen (3, 4), Sempio (137), Walker and Stahmann (161), Bloch (13), and Scheffer (131) reviewed the literature on host resistance and metabolic activity. Akai (1) presented a detailed review of the histology of defense in plants.

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Enzyme Histochemistry

Enzyme histochemistry is a relatively new biological technique which is discussed in detail by Pearse (125). Van Fleet (156), Surrey (148), and Jensen (67) discuss the application of histochemical procedures to botanical research. Although histochemical procedures afford an opportunity to demonstrate the in vivo activity of various enzymes and the location of various metabolic products involved in host response to infection, the techniques have seldom been adapted by plant pathologists in their study of host-parasite interactions. Atkinson and Shaw (8) demonstrated a relatively high concentration of acid phosphatase within and on the haustoria of Erysiphe graminis (powdery mildew) with diazo coupling and Gomori methods. They believed that this enzyme played an important part in the transfer of metabolites between host and parasite via phosphorylated intermediates.

Pearse (125) presents a more detailed review of the various techniques used to study enzyme systems and includes their theoretical implications. Some of these, including acid phosphatase, alkaline phosphatase, esterase, sulfatase, beta-glucosidase, peroxidase, and tyrosinase were of interest in this study.

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METHODS AND MATERIALS

Soil Microflora

Cropping Sequence

The Michigan State University Soil Science Department's crop rotation plots located on the Ferden Farm near Chesaning, Michigan, were used to investigate the influence of crop sequence on soil microfloral populations and root rot development. The soil type was Sims Clay Loam. The experimental plots consisted of seven crop rotations with five crops in each rotation. Each rotation was replicated four times with each sequence appearing every year. Each 28 x 90 foot plot was subdivided lengthwise to form four 7 x 90 foot sub-plots. Two of the sub-plots received a high application and two a low application of nitrogen at planting time. One of the high and one of the low nitrogen plots received an additional side dressing of nitrogen during the growing season. In these experiments the high nitrogen plot without side dressing was sampled. Five of the rotations with beans were selected for this study.

Table 1. The cropping sequences on the Ferden Farm rotation plots Chesaning, Michigan, and the plots sampled in the microfloral studies.

Rotation		Cropping Sequence			
I	Beets	Corn*	Beans*	Wheat	Sweet Clover
II	Wheat	Sweet Clover*	Beans*	Beets	Soybeans
III	Soybeans	Wheat*	Beans*	Beets	Corn
IV	Corn	Beets*	Beans*	Wheat	Alfalfa
V	Beets	Barley*	Beans*	Wheat	Corn

* Crop sampled

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The method of preparing the land and the type and rate of fertilizer applied to the plots are presented in Table 2. All fertilizers were applied immediately before or at the time of planting. The land preparation varied with the crop. The plots planted to sugar beets, barley, and wheat were fall plowed. The plots planted to corn, beans, and soybeans were spring flowed.

Table 2. The kind, rate, and time of application of fertilizers to the plots sampled on the Ferden Farm rotation plots, Chesaning, Michigan.

Crop	Fertilizer Applied	Rate of Application	Time of Application
Sugar Beets	5-20-10 + 1% Manganese + 1/4% Boron	400#/acre	Prior to and at time of planting
Corn	5-20-10	400#/acre	Planting time
Barley	5-20-10	200#/acre	Planting time
Wheat	5-20-10	200#/acre	Planting time
Soybeans	5-20-10	200#/acre	Planting time
Beans	5-20-10	200#/acre	Planting time

The bean yield data is presented with permission from the Department of Soil Science, and is based on the yield of the two center rows from each plot. The soil microflora in the bean plots and in the crop preceding beans in the rotation were sampled just prior to maturity of the crop with the plate-profile method (6) and the immersion-tube technique as modified by Mueller and Durrell (106). Both methods are described under sampling methods.

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Sampling Methods

Immersion-Tube Technique: This method consisted of placing specially prepared tubes containing agar into the soil for a period of five days. The soil microorganisms that grew into the agar were isolated and identified to genera. The immersion-tubes were made by drilling ten 3/16-inch countersunk holes approximately one inch apart in a spiral around a 15 ml tapered polypropylene centrifuge tube. The tubes were wrapped spirally with black plastic electricians tape (Scotch brand), filled to within 3/4-inch of the top with reconstituted corn meal agar (Difco), covered with plastic caps, and autoclaved at 15# pressure for 20 minutes (Figure 1).

A metal dibble the same size as the inside of the tubes was used to make a hole in the soil. This eliminated excessive pressure when placing the tubes in the soil, yet provided maximum contact between the tube and the soil. Prior to insertion in the soil, a sterile needle was used to punch a hole through the tape and tube perforation into the agar. This provided an entrance for growing soil microorganisms. After five days incubation in the soil, the tubes were removed and brought into the laboratory where the tape was unwound exposing one hole at a time. A flattened dissecting needle was used to transfer the agar and the invading organisms into a Petri dish containing corn meal agar medium (Difco) fortified with 5 gm dextrose per liter.

Plate-Profile Technique: This method consisted of burying an 8 x 12 x 1/2-inch autoclavable polypropylene plastic plate with 3/16-inch holes (3/8-inches deep) spaced at one-inch intervals both vertically and horizontally (total of 77 holes) perpendicular to the row (Figures 2 and 3).

The soil profile was prepared by driving a sharpened steel plate into the soil at right angles to the row. Holes were punched through

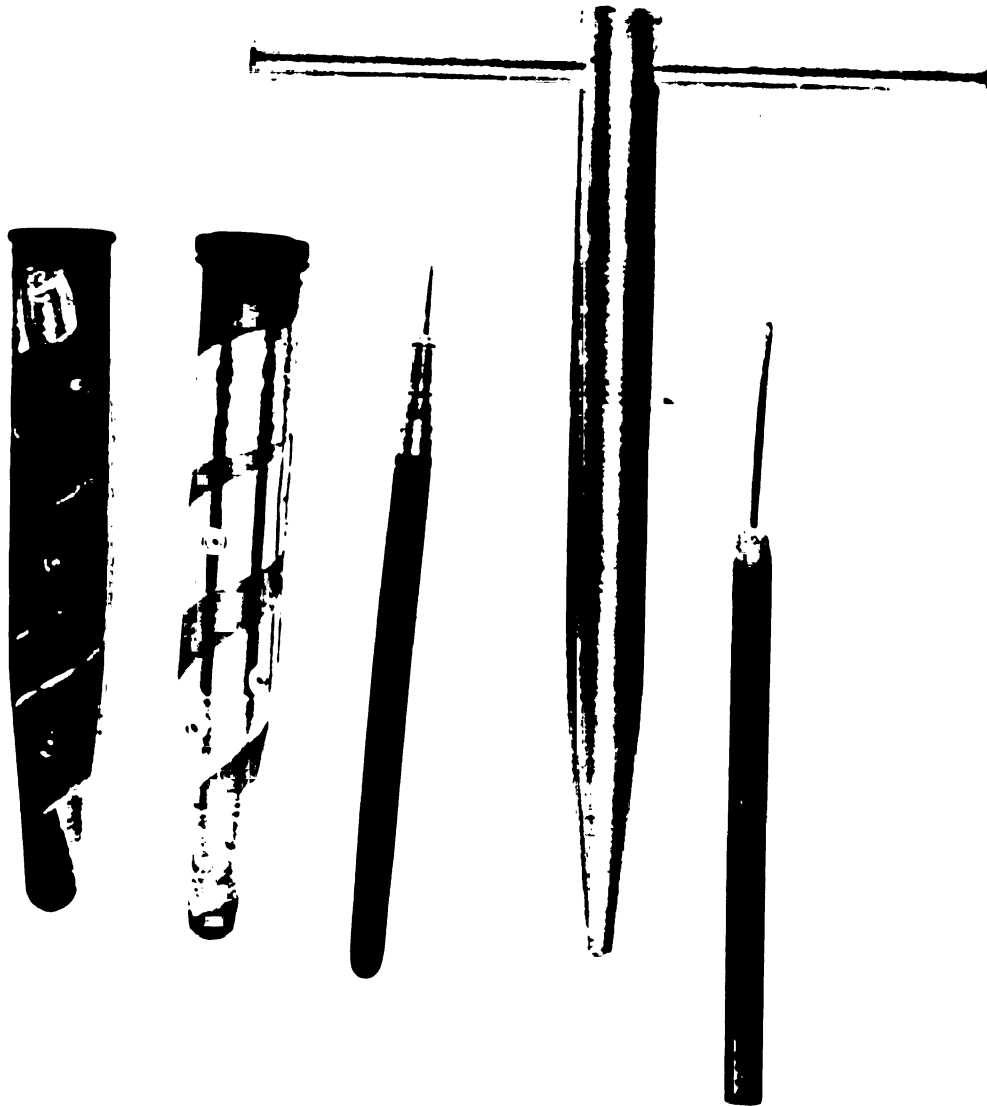
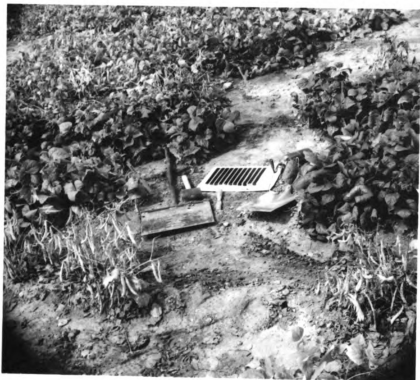


Figure 1. The equipment used for isolating soil microorganisms by the immersion-tube technique. From left to right: A polypropylene plastic centrifuge tube (16 x 120 mm) wrapped with electricians tape; a similar tube with holes, and capped; a needle in holder; soil dibble; a flattened dissecting needle for plating the invaded agar samples.

Figure 2. The equipment used for isolating soil microorganisms by the plate-profile technique. From left to right: Sharpened steel plate; rubber hammer; putty knife; plastic plate with plastic electricians tape over agar filled holes; propane torch; and square spade.

Figure 3. Plastic plate inserted against soil profile in bean row prior to covering. Plates remain buried for 5 days before removal.



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the plastic tape that covered the corn-meal-agar filled holes in the plate with a sterile needle prior to placing the plate against the soil profile. The plates were removed from the soil after five days and each agar plug was transferred to a Petri-plate containing corn meal agar (Difco) fortified with 5 gm dextrose per liter.

Pathogenicity Tests

All fungal isolations from the Ferden Farm were tested for pathogenicity in 1961 by placing a 6-day-old Sanilac bean seedling on each Petri-plate. Records on pathogenicity were made five days later.

Root Rot Index

Disease severity was determined by removing plants from the soil and scoring the roots on the degree of lesion development according to the following six classes: 0 (no lesions), 1 (small, local lesions), 2 (lesions coalescing), 3 (lesions coalesced and covering a considerable area of the tap root), 4 (severe, primary root system non-functional), 5 (very severe or dead, tap roots and most of the secondary roots non-functional). The root rot index for each treatment was calculated by multiplying the class value by the number of plants in each class, the products were summed, and then divided by the total number of plants to give a final value. The root rot indices were calculated when the plants were 10 weeks old by digging, washing, and indexing 100 plants from each replicate.

Analysis of Data

Microfloral data obtained using the plate-profile and immersion-tube methods were plotted to determine their distribution as compared to a normal distribution. The results indicated that it was not

necessary to transform the data in order to use analysis of variance techniques. Hence, standard analysis of variance techniques as outlined by Snedecor (141) were used in these experiments. Treatment means were compared by computing the difference between means for significance (D) if the "F" test was significant. "D" was computed as follows(141):

$$D = QS_{\bar{x}}$$

Where: Q = a factor from the table of Q (5 percent level)

$$S_{\bar{x}} = \frac{\sqrt{Msq}}{\sqrt{N}}$$

Histochemical Investigations

Varietal Material

The scarlet runner bean, Phaseolus coccineus (180) and N203, a Phaseolus vulgaris introduction from Mexico were selected as resistant materials. Sanilac, Red Mexican U.I. 34 and Dark Red Kidney (all P. vulgaris types) were the susceptible varieties used.

Induction of Resistance with Plant Growth Regulators

Sanilac, Red Mexican U.I. 34, and Dark Red Kidney beans were sprayed with 1 ml of a solution containing 25 ppm of sodium 2,4,5-trichlorophenoxypropionate 10 days after planting when the plants were in the simple leaf stage. "Tide" was used as a wetting agent. Seed from the treated plants were used to study induced resistance to Fusarium root rot.

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Plant Culture

A wire frame 8 x 12 x 12 inches was constructed on a sheet of plastic 8 x 12 x 1/2 inches. Black polyethylene film, ten mil in thickness, was used to cover the wire frame to form a box or "chamber." One-half inch slits were made at one-inch intervals (a total of 56 slits) through the top of the polyethylene film. Germinated seedlings were placed through the slits with the lower portion of the hypocotyl and the root in the chamber. A DeVilbiss atomizer was inserted through the plastic film near the bottom of the chamber and diagonally across it (Figures 4 and 5). The tip of the atomizer was at approximately a 45° angle. A nutrient solution was supplied to the plant roots through the atomizer from an 8-gallon carboy filled with Hoagland and Arnon's nutrient solution (101). This solution consisted of the following nutrients:

KH_2PO_4	0.001 M
KNO_3	0.005 M
$\text{Ca}(\text{NO}_3)_2$	0.005 M
MgSO_4	0.002 M

One ml of 0.5 percent ferric tartrate solution and one ml of the following micronutrient solution were also added:

H_3BO	2.5 g
$\text{MnCl}_2\cdot 4\text{H}_2\text{O}$	1.5 g
ZnCl_2	0.1 g
$\text{CuCl}_2\cdot 2\text{H}_2\text{O}$	0.05 g
MoO_3	0.05 g
H_2O	1000 ml

No observable deleterious effects or nutrient deficiencies were noticed on the plants during the course of these experiments.

Figure 4. The hydroponic growth chamber used in the laboratory to grow bean plants for histochemical studies. Plants were suspended through the plastic and a nutrient solution was atomized onto the roots.

Figure 5. The hydroponic growth chamber with plants showing root growth after a two-week growing period.



Inoculation and Wounding of Plants

Inoculum from a mildly pathogenic isolate of Fusarium solani f. phaseoli was obtained by growing the fungus on corn meal agar in Petri-plates. Strips of inoculum (agar and Fusarium) two millimeters wide and two inches long were placed on the hypocotyls of four plants of each variety. In addition, four plants of each variety were wounded mechanically by making three one and one-half inch-long longitudinal cuts of varying depth on each hypocotyl. The plants were inoculated or wounded two and one-half weeks after placing them in the chamber. Two plants of each variety were maintained as controls.

Histological and Histochemical Methods

The enzymatic reactions of specific tissues were of interest in this study since Fusarium solani f. phaseoli is primarily a cortical, rather than a vascular pathogen. Penetration is limited by the endodermis in susceptible plants, and meristematic and vascular tissues are not colonized (28, 36, 63).

The enzymatic activity of specific tissues of bean hypocotyls was determined by placing 8 to 10 freehand cross sections of infected or wounded areas of the hypocotyl in staining dishes containing the desired reagent(s) and observing the histochemical (color) reactions. This was done 30, 72, and 120 hours after wounding or inoculation.

Localization of acid phosphatase, alkaline phosphatase, esterase, beta-glucosidase, sulfatase, peroxidase, and tyrosinase, in the various tissues was determined. Suberin depositions were stained with Sudan IV. Diazotized salts of 2-benzylamino-2:5-dimethoxy aniline (salt #2), orthodianisidine (salt #6), and 5-chloro-o-toluidine (salt #9) were used as coupling agents. (These salts are hereafter referred to as diazo salts, number 2, 6, and 9 respectively.)

Phosphatases: The sulfide precipitation methods of Gomori (55), resulted in a heavy false precipitation of sulfide in all treatments and could not be used in these studies. Therefore, the diazo dye (coupling) techniques were used to detect the presence of alkaline and acid phosphatase (125).

Alkaline Phosphatase: The plant sections were immediately incubated for one hour at room temperature in media consisting of 10/mg of the stable diazo salt of numbers 2, 6, or 9 dissolved in 10 ml 0.1M tris buffer (pH 9.1) containing 10 mg sodium alpha naphthol phosphate or (naphthol-AS-MX-phosphate). Sections were transferred to fresh media every 15 minutes. After incubation, the sections were washed in running water for 2 minutes and then mounted on slides for observation. Control sections were incubated either in the absence of substrate or in the absence of the coupling reagent with all other factors similar.

The stabilized diazo salt of 4-benzoylamino-2:5-dimethoxy-aniline was purchased from Dajac Laboratories. All other stable diazotes were prepared in the cold room from the primary amines by dissolving 2 gm of the desired aryl amine in 2 N alcoholic HCl (1.5 ml concentrated HCl/15 ml ethanol). One gram of sodium nitrite and 10 ml ethanol were added slowly with rapid stirring for 10 minutes. The diazotized amine was then stabilized by adding 3.4 g naphthalene-1:5-disulfonic acid with vigorous stirring. The precipitate was filtered with suction and washed with 100 percent ethanol and then with ethyl ether. After drying, the stable diazotate was protected from light and high temperatures.

Acid Phosphatase: Sections were incubated three hours at room temperature in media made by dissolving 10 mg of the diazo salt of numbers 2, 6, or 9 in 10 ml acetate buffer at pH 5.0. Fifteen milligrams

of sodium alpha-naphthol phosphate (or naphthol-AS-MX-phosphate) were then added to the diazo solution. Incubated sections were washed in water and mounted on glass slides.

Esterase: Three methods were used to determine non-specific esterase activity. Two of the methods are dependent on simultaneous coupling with a diazo salt, and the third is dependent on the oxidation of indoxyl acetate to indigo blue. In the first method (alpha-naphthol-acetate) the sections were incubated at 22°C. for five to thirty minutes in 10 mg of alpha-naphthol acetate dissolved in 0.5 ml acetone to which 10 mg of the diazo salt of numbers 2, 6, or 9 was added in 10 ml. of 0.1 M phosphate buffer at pH 7.4. Sections were rinsed in distilled water and mounted in glycerine jelly.

The second method was similar to the first but involved about one hour incubation at room temperature in 10 mg naphthol-AS-D-acetate (dissolved in one ml acetone) to which 10 ml of 0.05 M phosphate buffer containing 1- mg of the diazo salt of number 2 or number 9 was added. The sections were rinsed in distilled water and mounted in glycerine jelly.

The third method (indoxyl acetate) consisted of incubating the sections in freshly prepared media comprised of 5 mg of indoxyl acetate dissolved in 2 ml of 0.1 M tris buffer at pH 7.0 to which one ml each of 0.05 M potassium ferrocyanide, 0.05 M potassium ferricyanide, and 0.1 M calcium chloride were added along with 5 ml of distilled water. The sections were washed and examined after incubation.

Sulfatase: Non-specific sulfatase activity was determined by the post coupling method of Surry (148). Sections were incubated for one to three hours at room temperature in 10 mg (0.005 M) 8-hydroxy-quinoline sulfate in 10 ml of 0.1 M phosphate buffer at pH 5.8. Sections were washed in distilled water and post coupled for 10 minutes

with diazo salt number 2 (10 mg in 10 ml 0.1 M tris buffer) at pH 9.1 since coupling is sometimes difficult at the acid pH for enzyme activity.

Beta-glucosidase: Beta-glucosidase activity was studied by simultaneously coupling the quinone cleaved from arbutin with the diazo salt of numbers 2, 6, or 9 at pH 7.4 as suggested by Surrey (148). The sections were incubated for 12 hours at room temperature in 10 mg of arbutin dissolved in 10 ml of 0.1 M tris buffer containing 10 mg diazo salt. Sections were then rinsed in distilled water and mounted in glycerine jelly.

Enzyme activity was also compared at pH 4.1 in 0.025 M phosphate buffer by post coupling at pH 9.0. No differences in localization were observed between the simultaneous and post coupled sections.

Tyrosinase: Sections were incubated for 24 hours at 37°C. in media consisting of 0.0056 M 3:4-dihydroxyphenylalanine (DOPA) in 0.1 M phosphate buffer at pH 7.4. The sections were transferred to fresh media every two hours. Incubated sections were washed and photographed.

Peroxidase: Sections were incubated two to five minutes in media (with stirring) consisting of saturated benzidine (35-40 mg benzidine dissolved in 100 ml water at 80°C., cooled, and filtered) to which one drop of 3 percent (20 volume) hydrogen peroxide per 2 ml was added immediately before use. Peroxidase activity was expressed as a blue or reddish brown precipitate. The reaction product is not stable and begins to fade after a few hours. Lignified tissues stained a light yellow in control sections without substrate.

Suberin: Suberized tissues were stained with Sudan IV by incubating sections for 10 minutes in 0.09 percent Sudan IV in 70 percent ethanol. Sections were washed rapidly in 50 percent ethanol and mounted.

EXPERIMENTAL RESULTS

The Distribution of Soil Microorganisms Isolated by the Plate-Profile and Immersion-Tube Techniques

In order to determine the methods for analyzing the data on the frequency of isolation and association of the microflora obtained by the plate-profile and immersion-tube method, comparisons were made (Figures 6, 7, and 8) of the distribution of data on a number of organisms. The histograms were compared to the patterns of distribution (Figure 9) as given by Lindquist (79), who reported on a study by D. W. Norton (115) on the effects of non-normality and heterogeneity of variance upon the distribution of "F." It was apparent from these comparisons that the patterns of distribution for the most frequently isolated micro-organisms were either normal, moderately, or markedly skewed, or J-shaped. Since the data in the Norton study (115) was analyzed without transformation, and it was found that moderately skewed distributions gave results comparable to normality, and that markedly skewed and J-shaped distributions gave a more critical actual value of significance than the apparent level obtained from the "F" table, it appeared unnecessary to transform the microfloral data prior to applying the analysis of variance as had been done by others (96, 97, 176, 177). Thus, Menon (96) and Menon and Williams (97) used the square root transformation to convert their data from a Poisson distribution. Williams and Schmitthenner (176) reported that many fungi commonly isolated from the soil by the dilution-plate method failed to fit any known distribution. They adopted the arc sine transformation to their data (176, 177).

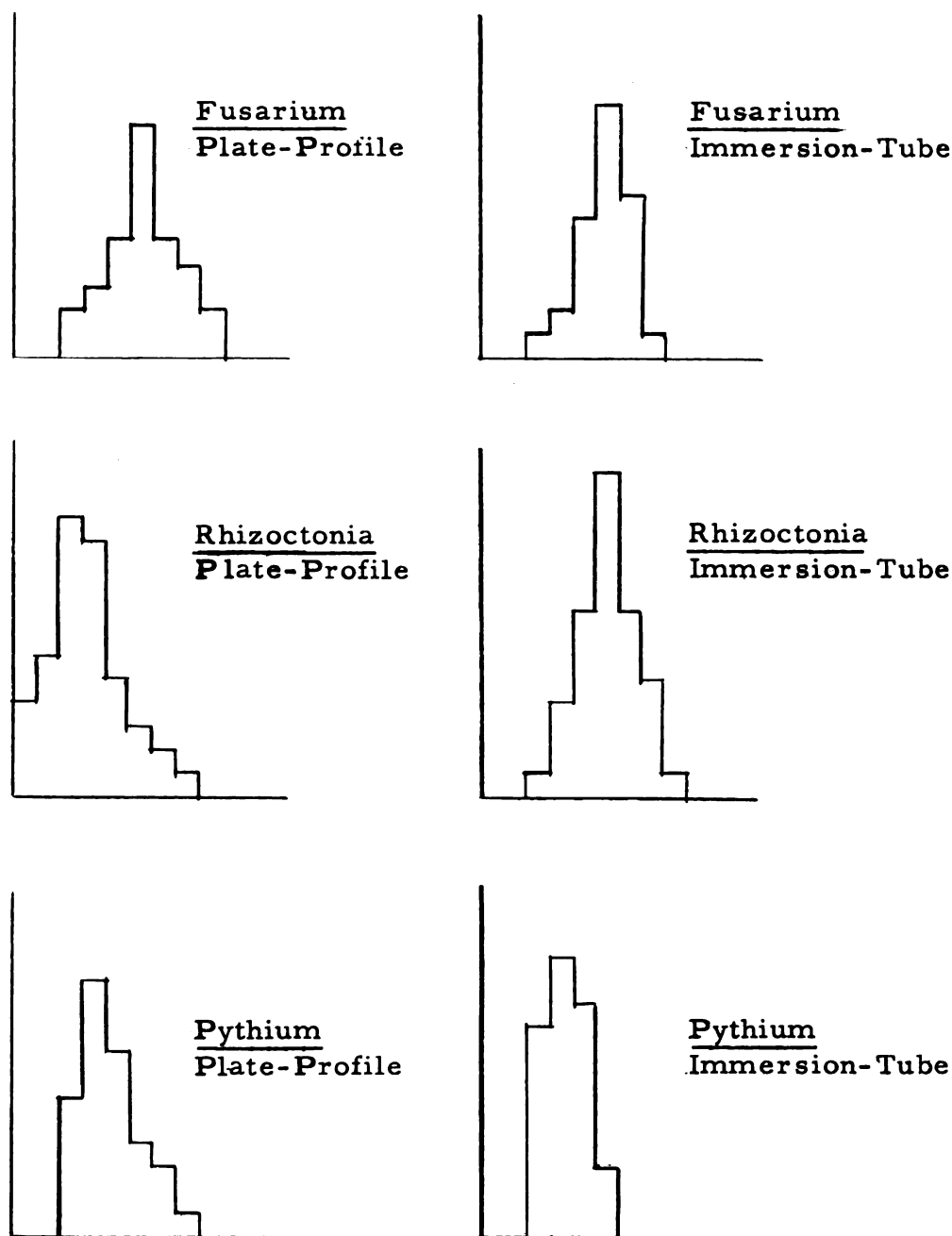


Figure 6. Histograms showing the distribution of the frequency of Fusarium, Rhizoctonia, and Pythium in 1961 by the plate-profile and the immersion tube techniques. Plate-profile results are based on total isolations from 40 plates and the immersion-tube results are based on total isolations from 200 tubes. Ordinate represents the number of samples; abscissa the number of isolates per sample.

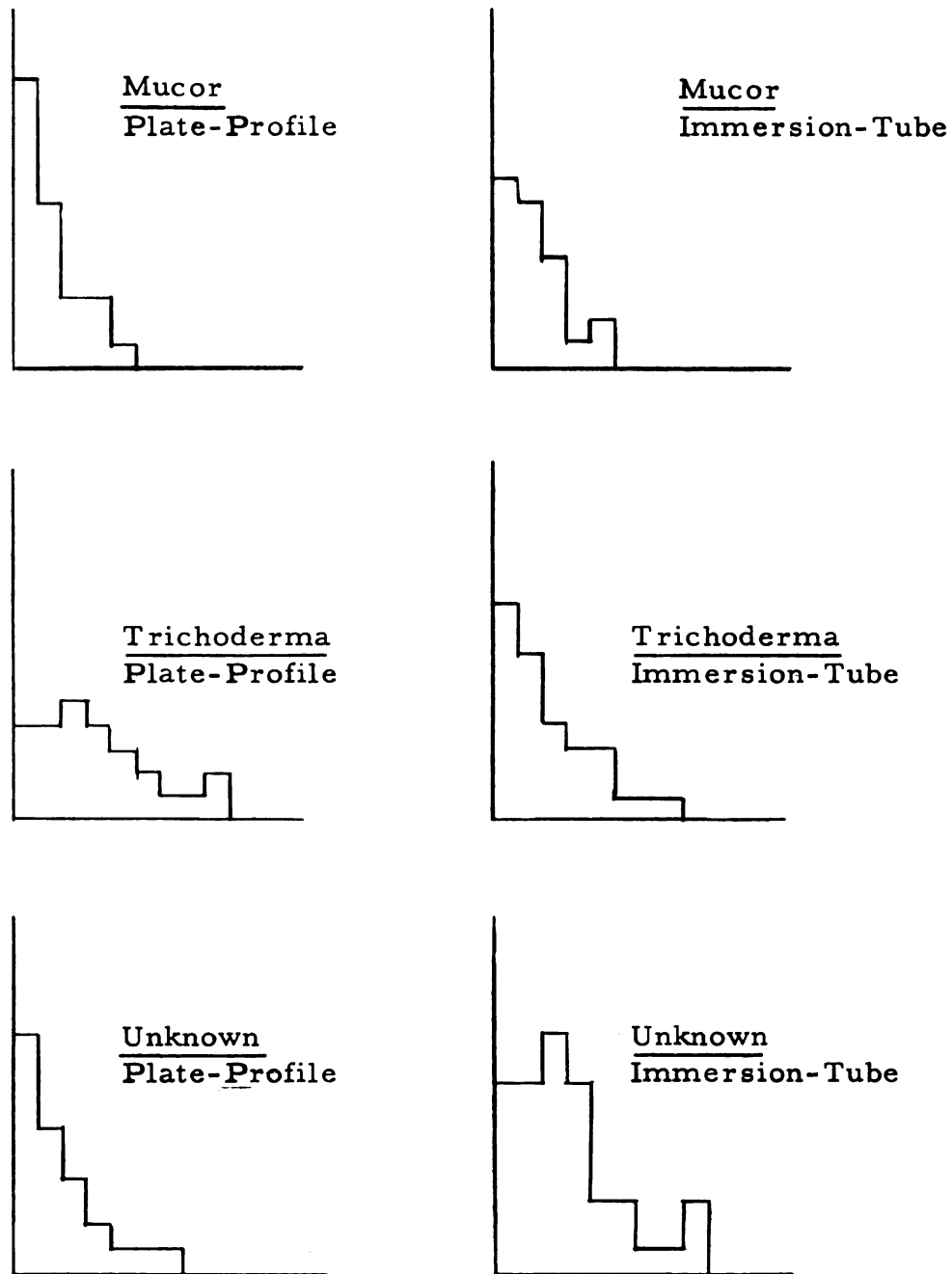


Figure 7. Histograms showing the distribution of the frequency of Mucor, Trichoderma, and unknown in 1961 by the plate-profile and the immersion-tube techniques. Plate-profile results are based on total isolations from 40 plates and the immersion-tube results are based on total isolations from 200 tubes. Ordinate represents the number of samples; abscissa the number of isolates per sample.

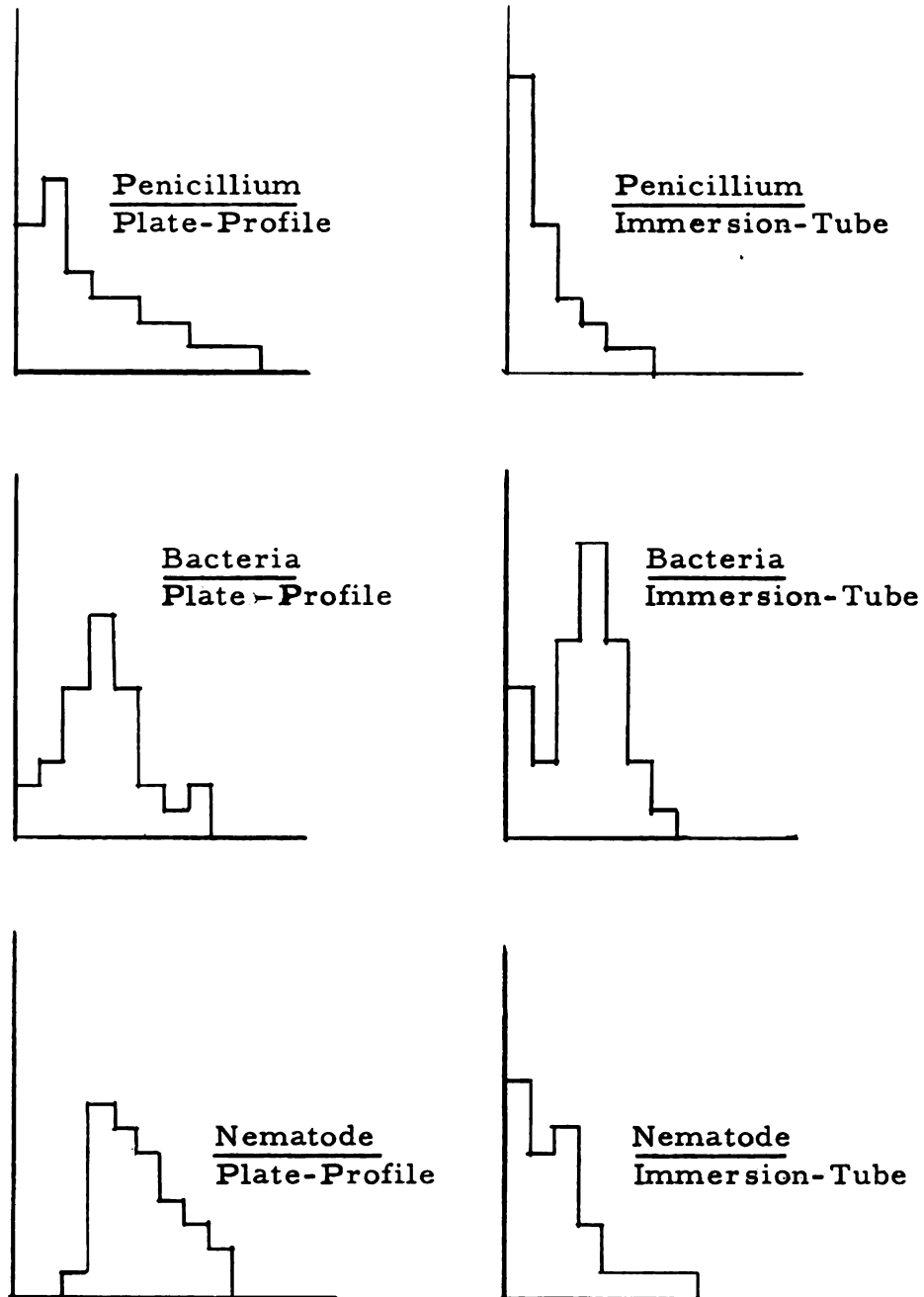


Figure 8. Histograms showing the distribution of the frequency of Penicillium, bacteria, and nematodes in 1961 by the plate-profile and the immersion-tube techniques. Plate-profile results are based on total isolations from 40 plates and the immersion-tube results are based on total isolations from 200 tubes. Ordinate represents the number of samples; abscissa the number of isolates per sample.

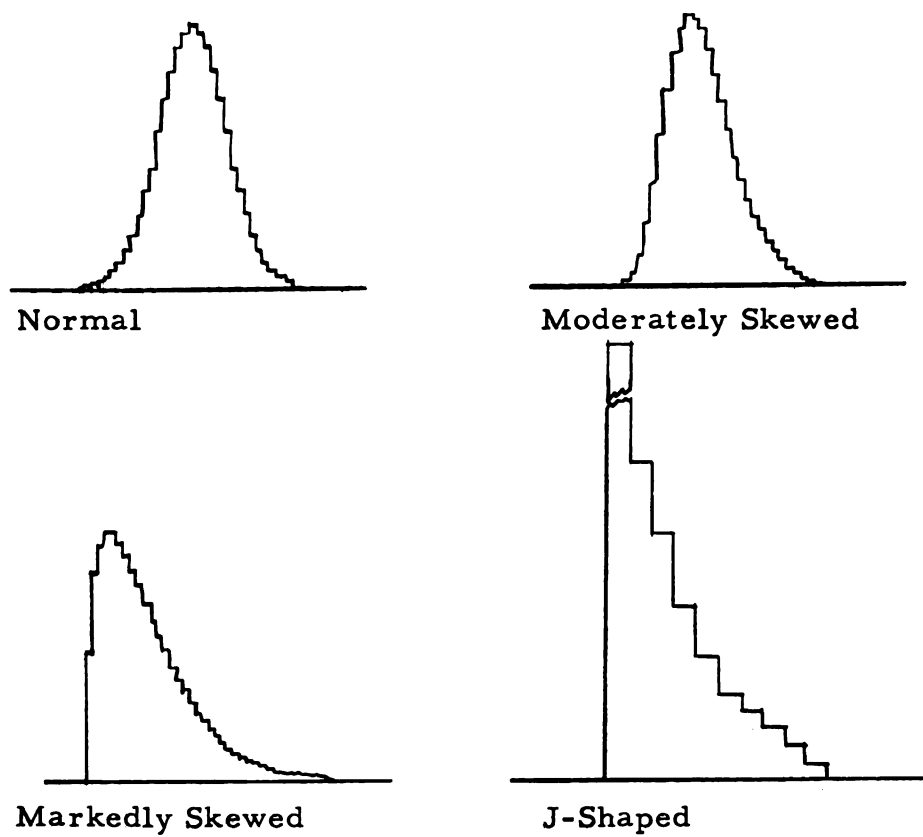


Figure 9. Histograms of the non-normal distribution of "F" values obtained by Norton (115).

Since root exudates are known to stimulate the germination of fungal spores, a moist sheet of sterile Whatman No. 1 filter paper was placed along the face of a plate prepared in the same manner as those placed in the soil. The plate and filter paper were placed in a plastic bag and incubated in a verticle position in the laboratory for five days after which the filter paper was removed and sprayed with ninhydrin reagent (0.2 percent in n-butanol) to test for amino acids or proteins which may have diffused through the pin-hole in the tape. A similar procedure was followed to test for carbohydrates with p-anisidine hydrochloride (3 percent in n-butanol) used as the test reagent. All of these tests were negative.

The "fungal profile" of the soil usually is made up of a composite of many soil microorganisms, some in association and others apparently, seldom occurring together. Microbial-associations and distributions occurring in a bean field following sugar beets are illustrated in Figures 10 and 11. In Figure 10 a one-inch square of agar containing the isolated organisms from one hole in the plate-profile was cut from the center of each Petri-plate. Each square was located in proximity to the location from where it was isolated on the plate-profile in order to reconstruct the "fungal profile." This represents a cross-section of the soil microflora as found in the "A" horizon of this soil.

The Effect of Cropping Sequence on the Severity of Bean Root Rot

The crop preceding beans in the rotation appeared to influence the prevalence and severity of Fusarium root rot (Table 3). Thus, root rot appeared least severe following corn and sugar beets in the rotation, with a higher incidence recorded following wheat and sweet clover, while it was the most severe after barley. Beans following successive years of beans always appeared to have more root rot than

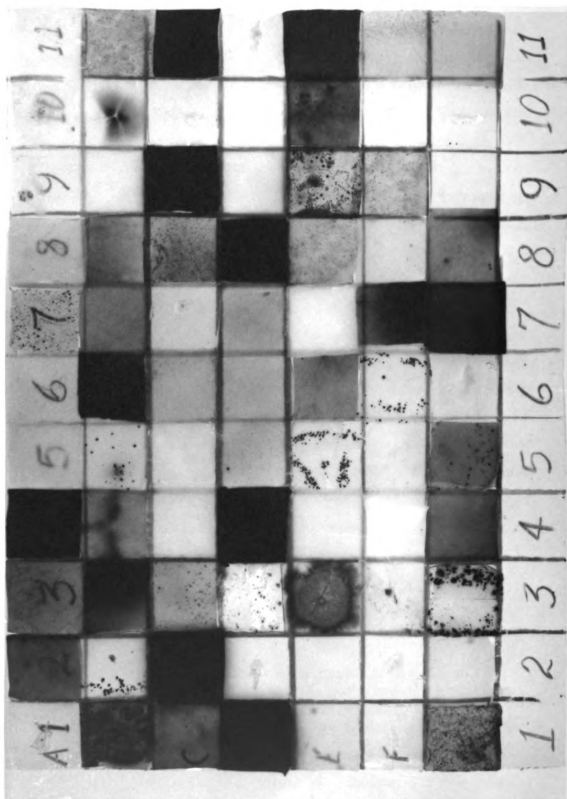


Figure 10. A fungal profile of a sugar beet root rhizosphere as reconstructed from the plate-profile.

	1	2	3	4	5	6	7	8	9	10	11
A	Bacteria	Fusaria Rhizoc (Incl. Bacteria	Fusaria Mucor Bacteria	Fusaria Rhizoc Alternar. Bacteria	Gliocladi Rhizoc Unknown Bacteria	Fusaria Rhizoc Bacteria	Chaetophoma	Fusaria Bacteria Nema	Penicillium Bacteria	Bacteria	Rhizoc
B	Fusaria Rhizoc (Incl.) Bacteria	Rhizoc Trichod-Bacteria	Fusaria (Hyper) Rhizoc (Incl.) Unknown	Fusaria Rhizoc (Incl.) Bacteria	Nematode Pythium Rhizoc Trichod-Bacteria	Gonytricum	Fusaria Nema	Fusaria (Incl.) Bacteria Nema	Fusaria Rhizoc Bacteria	Pythium Unknown Nema	Fusaria Bacteria
C	Fusaria Rhizoc (Incl.) Bacteria	Fusaria Rhizoc (Incl.) Bacteria	Fusaria Bacteria	Rhizoc Nema Bacteria	Phythium Rhizoc Bacteria	Fusaria Bacteria	Nema Bacteria	Fusaria Rhizoc (Incl.) Rhizopus Bacteria	Fusaria Rhizoc (Incl.) Nigrosp-Bacteria	Bacteria	Nigrosp-Fusidium Bacteria
D	Fusaria Rhizoc (Incl.) Pythium Bacteria	Bacteria	Fusaria Bacteria Trichod-	Fusaria Rhizoc (Incl.) Bacteria	Fusaria Bacteria	Fusaria Bacteria	Fusaria Bacteria	Fusaria (Incl.) Bacteria	Fusaria Rhizoc Bacteria	Pythium Rhizoc Bacteria	Bacteria
E	Fusaria Rhizoc Unknown Bacteria	Sterile	Fusaria (Hyper) Unknown	Fusaria Bacteria	Fusaria Trichod-Bacteria	Fusaria Rhizoc (Incl.) Bacteria	Fusaria Pythium Unknown Bacteria	Fusaria Bacteria	Fusaria Rhizoc (Incl.) Bacteria	Fusaria Rhizoc (Incl.) Nigrosp-	Fusaria Rhizoc
F	Bacteria	Bacteria	Fusaria Penicil-Unknown	Fusaria Bacteria	Fusaria Bacteria Trichod-	Fusaria Rhizoc (Incl.) Bacteria	Fusaria Rhizoc (Incl.) Bacteria Nema	Fusaria Bacteria	Fusaria Rhizoc	Bacteria	Fusaria Bacteria
G	Rhizopus	Fusaria Rhizoc Bacteria	Fusaria Rhizoc Trichod-Bacteria	Fusaria Rhizoc Bacteria	Fusaria Rhizoc Bacteria	Bacteria	Fusaria Rhizoc Bacteria	Fusaria Rhizoc Bacteria	Sterile	Bacteria	Fusaria Bacteria

Figure 11. A key to the reconstructed fungal profile presented in Figure 10.

Table 3. The yield of beans and the severity of root rot on the Ferden Farm rotation plots in 1960, 1961, 1962 when beans followed corn, wheat, sweet clover, beets, or barley in the rotation.

Preceding Crop	Year					
	1960		1961		1962	
	Root Rot Index	Yield (bu/a)	Root Rot Index	Yield (bu/a)	Root Rot Index	Yield (bu/a)
Corn	1.0	40.6	1.3	33.1	1.2	33.3
Beets	1.5	33.8	1.4	32.1	1.1	29.8
Wheat	2.0	38.6	1.4	35.6	1.4	32.1
Clover	2.0	41.1	1.4	32.6	1.4	29.8
Barley	2.8	25.6	1.9	22.8	1.4	30.6
Beans*	3.2	21.9	2.0	35.3	1.7	20.6
D**	0.22		0.08		0.17	

* A nearby plot with continuous beans.

** Difference between treatments for significance.

beans following other crops. The results from these experiments together with observations on beans throughout the bean area, demonstrated the importance of the previous crop in the expression of bean root rot.

Apparently other factors besides the previous crop influenced the amount of root rot since the amount of root rot varied from year to year (Table 3) in the same rotation. From the results obtained on the Ferden Farm plots it is evident that climatic factors may be responsible, but in what way is hard to determine. Present evidence indicates that cold, wet soils favor root rot.

The Frequency of Isolation of Soil Microorganism with the
Plate-Profile and Immersion-Tube Techniques

Fungi from 87 genera were isolated and identified to genera from the crop rotation plots. Those occurring 9 or more times during one isolation year are listed in Table 4. Bacteria and nematode isolations are also included. No attempt was made to identify the bacteria. Many of the nematodes were examined by the Nematologist, but only non-pathogenic types were identified. Most of the microorganisms were isolated in association with one or more other organisms. Fusarium was the most frequently isolated fungus accounting for over 40 percent of the isolates using the plate-profile method. Rhizoctonia was the next most frequently isolated fungus occurring in 16 to 18 percent of the plate-profile isolations. Large numbers of bacteria were isolated and generally associated with, and growing in close proximity to a fungus. Many nematodes were also isolated, especially with the plate-profile technique.

A few fungi which are not readily isolated using the dilution-plate method were frequently isolated with the plate-profile and immersion-tube techniques (Table 4). For example, Rhizoctonia and the non-sporulating, unidentified, group of fungi accounted for 20 to 25 percent of the isolates with the above techniques, compared to the plate-dilution techniques of less than one percent, as reported by Williams and Schmitthenner (176, 177). Some heavy sporulators were also isolated; however, their frequency was more consistent with the results of soil washing (166) or other modifications of the dilution-plate which reportedly increased the percentage of mycelial isolations. Those genera most frequently isolated by dilution techniques (166, 176, 177) such as Rhizopus and Chaetomium were conspicuous by their infrequent isolation (Table 4).

Table 4. The number of fungi, bacteria, and nematodes isolated from the Ferden Farm rotation plots using the plate-profile and immersion-tube techniques, and the comparative frequency of the fungi.

Microorganism	Year					
	1960		1961		1960	
	Frequency		Frequency		Percent	
	Plate	Tube	Plate	Tube	Plate	Tube
Total Fungi	3463	3786	6796	4852		
Alternaria	59	93	64	97	1.70	2.46
Aspergillus	3	3	19	4	.08	.08
Cephalosporium	0	12	0	5	.00	.35
Chaetophoma	9	1	4	4	.26	.03
Chalaropsis	23	4	8	11	.66	.11
Fusarium	1683	1518	2896	1719	48.59	40.10
Hormodendron	20	2	7	4	.58	.05
Mucor	95	141	996	1218	2.74	3.72
Nigrospora	10	7	1	2	.29	.19
Penicillium	202	98	94	52	5.83	2.59
Phoma	9	2	5	12	.26	.95
Pythium	114	266	777	500	3.29	7.03
Rhizoctonia	639	772	1079	764	18.45	20.40
Rhizopus	28	502	56	279	.81	13.26
Sepedonium	34	43	197	133	.98	1.14
Streptomyces	104	2	40	30	3.00	.05
Thielaviopsis	10	9	6	5	.29	.26
Trichoderma	180	255	183	150	5.20	6.74
Unknown	171	46	273	136	4.94	1.21
Bacteria	2144	1417	2834	1277		
Nematodes	255	15	1368	209		

The Effect of Cropping Sequence on the Isolation Frequency
of Microorganisms in the Soil

The fungi representing most of the more frequently isolated genera occurred in all the rotations so that differences between crop sequences were often a matter of differences in the frequency of the specific genera (Table 5). The differences between replications were low for the more frequent isolates. The growing crop generally exerted the greatest influence on the microbial population although the effects of the previous crop or year were often noticeable (Table 6). Many genera such as Verticillium, Thielaviopsis, and Botrytis were not isolated frequently enough to determine their activity in the soil or their response to cropping sequence.

There was a significant increase in the isolation frequency of Fusarium, Mucor, Sepedonium, Trichoderma, and bacteria after beans, and a corresponding decrease in the frequency of Alternaria, Penicillium, Streptomyces, and unknown fungi (Table 5). The isolation frequency of Alternaria, Fusarium, Gliocladium, Mucor, Penicillium, Pythium, Rhizopus, Sepedonium, Streptomyces, Trichoderma, bacteria, and nematodes were affected by the previous crop (Table 6). Gliocladium, Mucor, Fusarium, Pythium, Sepedonium, Streptomyces, bacteria, and nematodes were significantly affected by year.

The frequency of Rhizoctonia was not significantly influenced by the present crop, previous crop, year or method of isolation; and non-sporulating unknowns were only affected significantly by the present crop. Penicillium and bacteria were isolated in the lowest frequency under corn and in beans following corn, and most frequently from barley. Fusarium, Pythium, Mucor, Sepedonium and Trichoderma were isolated more frequently from beans than any other crop, while corn preceding beans resulted in the most significant reduction in the frequency of Fusarium, Mucor, Pythium, Rhizopus, and Sepedonium. Nematodes were

Table 5. The influence of cropping sequence on the isolation frequency of several microorganisms on the Ferden Farm rotation plots in 1960 and 1961 as measured by the plate-profile technique.

Organism	Year	Cropping sequence										D**
		Beet-corn-bean	Soys-wheat-bean	Wheat-clover-bean	Corn-beet-bean	Beet-barley-bean	Barley* Bean*	Beet* Bean*	Barley* Bean*	Beet* Bean*	Barley* Bean*	
Alternaria	60	6	1	7	2	23	4	0	0	15	1	7.56
	61	2	5	11	3	24	3	3	2	11	0	7.56
Chalaropsis	60	2	0	1	2	0	3	0	10	1	3	
	61	1	1	0	0	0	0	1	2	1	2	
Fusarium	60	139	180	101	199	130	237	166	188	131	212	23.00
	61	265	253	273	380	260	311	240	347	309	258	55.52
Gliocladium	60	4	3	7	7	36	4	1	4	4	3	5.24
	61	6	2	3	11	12	6	3	0	4	2	4.68
Mucor	60	8	10	6	15	7	12	11	18	4	4	5.84
	61	82	110	69	156	80	128	41	145	80	105	36.64
Penicillium	60	14	28	11	25	7	25	29	24	14	25	32.04
	61	3	4	15	9	9	6	6	5	31	6	5.84
Pythium	60	10	6	14	13	12	16	3	10	21	9	14.28
	61	98	66	89	89	63	64	102	88	72	46	11.48
Rhizoctonia	60	50	62	60	58	58	46	70	158	107	64	19.30
	61	83	91	86	122	92	103	95	64	108	141	
Rhizopus	60	5	2	2	1	7	4	1	0	2	4	
	61	8	11	0	5	2	7	3	1	5	14	
Sepedonium	60	14	1	4	1	7	2	1	0	4	0	
	61	0	20	9	37	8	32	9	40	16	26	11.48
Streptomyces	60	10	12	10	10	6	5	26	18	1	6	6.24
	61	9	2	5	3	10	3	0	4	3	2	11.20
Trichoderma	60	8	38	12	15	6	14	17	20	17	33	11.48
	61	4	38	15	41	2	23	1	10	16	33	10.76
Unknown	60	15	29	12	11	19	11	22	21	20	13	
	61	53	17	17	36	19	16	34	15	43	23	
Bacteria	60	209	230	186	214	192	233	231	219	200	231	
	61	284	235	269	391	223	331	200	347	299	255	14.61
Nematode	60	42	10	49	28	57	19	27	1	20	2	15.40
	61	133	75	128	183	128	172	144	145	199	140	19.46

*Crop sampled

**D: The difference between means for significance at the 5 percent level

Table 6. The effect of the present crop, previous crop, and year on the isolation frequency of the soil microorganisms on the Ferden Farm rotation plots.

Organism	Present crop	Previous crop	Year
Total Fungi	X		X
Alternaria	X		
Fusarium	X	X	X
Gliocladium	X		X
Mucor	X	X	X
Penicillium	X	X	X
Pythium	X	X	X
Rhizoctonia			
Rhizopus	X	X	
Sepedonium	X	X	X
Streptomyces	X	X	X
Trichoderma	X	X	
Unknown Fungi	X		
Bacterium	X	X	X
Nematode	X	X	X
Pathogenic Fusaria	X	X	

significantly reduced under beans compared to other crops. All of the organisms which occurred frequently except Rhizoctonia, were affected in one way or another by the rotation. Some of the fungi isolated but not included in the tables were: Absidia, Botrytis, Cephalosporium, Chaetomium, Chaetophoma, Chloridium, Cunninghamella, Helminthosporium, Hormiscium, Nigrospora, Phoma, Pullularia, Spicaria, Sporotrichum, Stemphylium, Tetracoccusporium, Thamnidium, Torula, Thielaviopsis, Verticillium, Gonytrichum, and Stachybotrys.

The Influence of Cropping Sequence on Microbial Associations

The effect of cropping sequence on microbial association was determined by sampling the rotation plots in two stages of the rotation in the same year and in two successive seasons. This enabled a comparison on the effects of the present crop, the previous crop, and the year to year difference which may be due to other environmental changes. The overall associations of the several more frequently isolated fungus genera, as well as bacteria and nematodes are summarized in Table 7. The specific associations which were significant statistically at the 5 percent level of "F" and which were definitely affected by cropping sequence are presented in Table 8.

Using frequency of association as a basis for compatibility, it appears that two groups can be obtained. The one group which would include Fusarium, Mucor, Rhizoctonia, Sepedonium, Trichoderma, bacteria, and nematodes appear highly compatible, whereas Streptomyces and Penicillium were much less compatible.

Most microbial associations were consistent from year to year. However, many were affected by the present crop and several by the previous crop (Table 8). Apparently the effect of the previous crop on

Table 7. The percentage associations of various microorganisms isolated from the Ferden Farm rotation plots.

	Alternaria	Aspergillus	Cephalosporium	Chaetomium	Chaetophoma	Chalaropsis	Fusarium	Gliocladium	Mucor	Nigrospora	Penicillium	Phoma	Pythium	Rhizoctonia	Rhizopus	Sepodonium	Streptomyces	Thielaviopsis	Trichoderma	Unknown	Bacterium	Nematode
Alternaria	/						1.1	10.0	6.0		2.1		1.7	1.7		1.0	4.9			1.5	1.5	2.1
Fusarium	42.6			60.0	75.0	61.0	/	55.0	96.5	60.0	52.0	36.5	75.0	79.0	87.0	82.0	15.3	55.0	62.0	27.0	73.0	85.0
Gliocladium	6.6						1.55	/	38.0				..8	.7	23.0	1.0	2.4		.54		1.3	1.2
Mucor	9.4	5.3	20.0	20.0	25.0		19.3		/		13.8	20.0	17.9	14.0	12.5	43.0	7.3		11.0	7.3	16.0	13.0
Penicillium	3.1					25.0	4.0		1.6		/		0.9	2.3	1.8				2.2	8.6	4.3	2.9
Pythium	20.0				50.0	25.0	4.3	12.0	14.3		4.5	20.0	/	15.0	4.5	8.1	4.9	83.0	11.0	4.9	13.0	17.0
Rhizoctonia	21.0	5.3	20.0	60.0	75.0	25.0	30.0	15.0	23.0	0.7	11.0	22.0	40.0	/	5.4	27.0	2.5	26.0	27.0	14.0	29.0	30.0
Rhizopus							1.5	27.0	0.7		1.0	40.0	0.9	.3	/	0.5	1.0		1.6	1.1		1.2
Sepodonium	3.0	20.0					3.9	4.1	8.4		2.1		2.1	3.2	18.0	/			33.0		3.8	4.5
Streptomyces	3.0	20.0					0.6	2.0	0.3			0.3	0.6				/		.28	1.1	3.6	0.2
Trichoderma							5.1	2.0	2.0		4.2	40.0	6.8	0.6	5.4	6.6	4.8	1.6	/	2.6	4.9	5.0
Unknown	6.3			20.0		12.0	2.7	10.5	2.0		14.0	3.0	3.4	3.7	5.4		7.3	50.0	3.8	/		61.0
Bacterium	60.8		86.0	60.0	50.0	64.0	81.0	79.0	75.0		65.0	40.0	77.0	85.0		70.0	65.0	78.0	64.0	59.0	/	76.0
Nematode	45.0					37.0	27.0	40.0	20.0		10.0	20.0	32.0	29.4	29.0	21.0	7.3	66.0	7.2	20.0	8.4	/

Table 8. The effects of the present crop and the previous crop on microbial associations in the Ferden Farm rotation plots.

Association		Present Crop	Previous Crop
Alternaria	w/ Bacterium		
Alternaria	w/ Unknown		
Bacteria	w/ Nematode		
Fusarium	w/ Alternaria		
Fusarium	w/ Bacterium	X	X
Fusarium	w/ Gliocladium	X	X
Fusarium	w/ Mucor	X	
Fusarium	w/ Necrosis	X	X
Fusarium	w/ Nematode	X	
Fusarium	w/ Penicillium	X	
Fusarium	w/ Pythium		
Fusarium	w/ Rhizopus		
Fusarium	w/ Rhizoctonia		
Fusarium	w/ Sepedonium	X	X
Fusarium	w/ Trichoderma		
Fusarium	w/ Unknown		
Gliocladium	w/ Bacterium		
Mucor	w/ Bacterium	X	
Mucor	w/ Nematode	X	X
Mucor	w/ Sepedonium		
Mucor	w/ Pythium		
Penicillium	w/ Bacterium	X	
Pythium	w/ Bacterium		
Pythium	w/ Nematode		
Rhizoctonia	w/ Pythium		
Rhizoctonia	w/ Mucor		
Rhizoctonia	w/ Sepedonium		X
Rhizoctonia	w/ Bacterium		
Rhizoctonia	w/ Nematode		
Rhizopus	w/ Bacterium		
Sepedonium	w/ Bacterium	X	X
Sepedonium	w/ Nematode	X	X
Streptomyces	w/ Nematode		
Trichoderma	w/ Bacterium	X	X
Trichoderma	w/ Nematode	X	X
Unknown	w/ Bacterium		
Unknown	w/ Nematode		

the microbial associations is a result of the residue materials in the soil. Of special interest in these investigations was the consistent association of the various microorganisms with Rhizoctonia and the lack of any effect, other than with Sepedonium, on the associations by the present or previous crop. On the other hand, when not associated with Rhizoctonia the organisms were individually affected by both the present and the previous crop.

Alterations in association brought about by cropping sequence may indicate an altered frequency, antagonism, or incompatibility.

Fusarium and Rhizoctonia, although apparently compatible with most of the soil microorganisms were actively parasitized (necrosed) by a species of Xanthomonas (7,64). This parasitic association was significantly altered with cropping sequence and was also correlated with root rot severity (Table 9).

Table 9. The effect of cropping sequence and year on microbial associations with Fusarium of possible importance in the biological control of Fusarium root rot.

Association	Affected by		
	Present crop	Previous crop	Year
Bacterial necrosis	x	x	x
Actinomycosis	-	-	x
Lysis	-	-	-

Specific relationships (associations related to the biological control of soil-borne pathogens) were observed in all rotations. Their frequency was influenced by the present and previous crop, and some associations were directly related to a reduction in the severity of root rot (64).

Antagonism and antibiosis could not be measured by the techniques

employed except through a negative association of two organisms. Non-association appeared to have been the result of the absence of one organism rather than an antagonistic or incompatible relationship.

Parasitic relationships observed included the lysis of Fusarium and other fungi, the parasitism of Rhizoctonia by another fungus, the parasitism of Fusarium by an Actinomycete, and bacterial necrosis of Fusarium and Rhizoctonia. Lysis of soil fungi was only rarely observed in this study. Dissolution of the mycelium occurred only after the lytic bacteria grew along the hypha for some time. Hyphal tips were not lysed (Figure 12).

Parasitism of Rhizoctonia was observed. An unknown fungus coiled around and frequently penetrated the mycelium of Rhizoctonia (Figure 13). This association was also only rarely observed and was not influenced by cropping sequence.

Nematode trapping (Figure 14) by Arthrobotrys and Dactylaria was also apparent, but no relationship was observed between the various rotations and the amount of predation.

Direct parasitism of Fusarium by an Actinomycete (actinomycosis) (Figure 15) was observed less frequently than bacterial necrosis but fairly consistently under all rotations. It was not possible to separately culture the Actinomycete in the absence of Fusarium on chitin and laboratory media recommended for the isolation of Actinomycetes. Growth of Fusarium was generally stopped within five days after inoculating with the Actinomycete.

Bacterial necrosis (Figure 16) was directly correlated with increased yields of beans and inversely related to root rot. The bacterium, a Xanthomonas species, was consistently isolated from the soil in association with Fusarium or Rhizoctonia as it grew along and in close proximity to their mycelium. Initially the fungus and bacterium appeared to be in a compatible relationship. The protoplasm of hyphae



Figure 12. The lysis of Fusarium by a soil bacterium. Lysis (A) of the mycelium occurred only after the bacterium (B) grew in close association with it for several days. Hyphal tips were not lysed.

Figure 13. The parasitism of Rhizoctonia by an unknown fungus which coiled around and frequently penetrated the host mycelium.

Figure 14. Nematodes being trapped by Arthrobotrys, a predacious fungus.

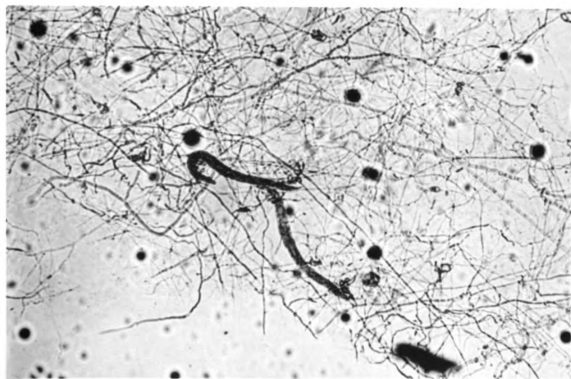
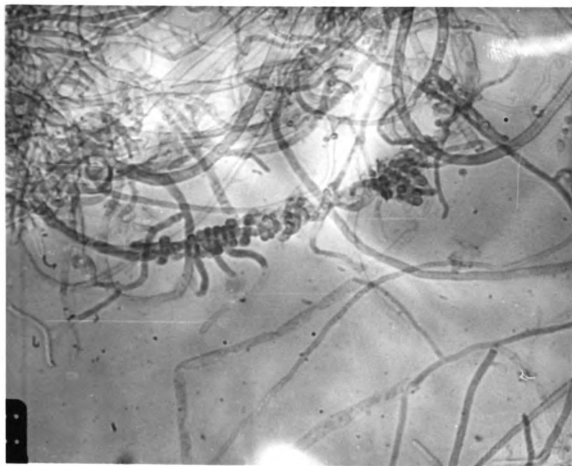
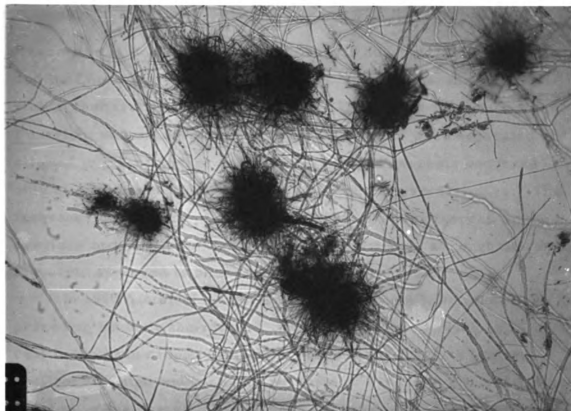


Figure 15. Parasitism of Fusarium hyphae by an Actinomycete (dark colonies).

Figure 16. Bacterial Necrosis of Fusarium resulting from the agglutination of the hyphal constituents.



lined with the Xanthomonas for two to four days became agglutinated and took on a deep red or purple color. Necrosed areas were non-viable when transferred to new media. Only slight inhibition of the Fusarium was observed when the two organisms were separated and allowed to grow together on Petri-plates, and necrosis occurred only after the bacterium had lined the hypha for several days. The frequency of isolation of necrosed Fusarium was influenced by the previous crop and year, while that of Rhizoctonia, although more frequent, appeared to be independent of the rotation.

In 1960 bacterial necrosis of Fusarium was low in the crops preceding beans in the rotation, but increased to as much as 27 percent in the bean crops (Table 10). When beans followed barley, root rot was high and bacterial necrosis of Fusarium and yield of beans was low. When beans followed corn the incidence of root rot was low, while the incidence of necrosis was high. The necrosis of Fusarium and the yield of beans were the highest after sweet clover. Bacterial necrosis was observed more frequently in all plots in 1961. However, the relationships of yield, root rot, and necrosis were the same.

The isolation frequency of Fusaria pathogenic on beans was also affected by cropping sequence with both the previous crop and the present crop altering the frequency. Beans following sugar beets had the highest frequency and corn the lowest frequency of pathogenic Fusaria. All the bean plots had a higher population of pathogenic Fusaria than any of the other crops preceding beans in the rotation.

Table 10. Bacterial necrosis of Fusarium, root rot severity and yield of beans under five cropping sequences in 1960.

Crop sequence	Bacterial necrosis of <u>Fusarium</u>		Root rot severity	Yield (bu/a)
	Preceding Crop (percent)	Beans (percent)		
Beets-corn-beans	5	22	1.0	41
Corn-beets-beans	10	20	1.5	34
Soybean-wheat-beans	5	18	2.0	39
Wheat-clover-beans	11	27	2.0	41
Beets-barley-beans	0	15	2.8	26

The Influence of Soil Temperature, Soil Moisture, and the Growing Bean Plant on the Microflora of a "Bean Soil"

It appeared that more critical information on the year to year variability of root rot due to environment could be obtained by a controlled experiment using soil temperature tanks in the greenhouse. Actual moisture levels dropped only three to six percent in the first 60 days in the pots covered with polyethylene film, and approximately 5 to 10 percent between adjustments of the moisture levels in the pots which were planted to beans.

A total of 2384 fungal "propagules" distributed among 55 genera of fungi in various associations with each other, nematodes, and bacteria were isolated during this study. Eighteen genera, occurring 5 or more times, accounted for 97 percent of all fungal isolations. An additional fifteen genera occurred two to four times and twenty genera occurred only once. Forty-six percent of all fungal isolates were Fusarium, 17 percent Rhizoctonia, 11 percent Pythium, 4 percent Rhizopus, 4 percent Thamnidium, 3.6 percent unknown fungi, 3 percent Trichoderma,

3 percent Mucor, and 2.5 percent Penicillium. Seventy-four nematodes and 1624 bacteria were also isolated in association with the fungi.

The microorganisms present in the "bean soil" responded in various ways to the different environmental conditions which were set up in this experiment (Table 11). Only Gliocladium responded to changes of all the environments. Fusarium, Gliocladium, and Streptomyces were increased in soil with beans as compared to fallow conditions. Bacteria, Rhizopus and Thamnidium were influenced only by moisture. Many of the fungi isolated were not influenced by either the soil temperature, moisture, or by the growing plant.

Table 11. The effects of soil temperature, soil moisture, and the growing bean plant on the microorganisms of a "bean soil."

Organism	Fallow		Bean		Bean-Fallow
	Temp.	Moist.	Temp.	Moist.	
Total Fungi	X	X	-	-	X
Fusarium	X	X	-	X	X
Fusarium (Necrosed)	-	-	-	-	-
Fusarium (Parasitized)	-	-	-	-	-
Fusarium with Bacterium	-	-	-	X	X
Fusarium with Mucor	-	X	-	X	-
Fusarium with Rhizoctonia	-	X	-	X	X
Fusarium with Thamnidium	X	X	-	-	-
Gliocladium	X	X	X	X	X
Mucor	-	-	-	X	-
Pythium	X	X	-	X	-
Rhizoctonia	-	X	X	X	X
Rhizoctonia (Necrosed)	-	X	-	-	X
Rhizopus	-	X	-	X	-
Streptomyces	X	X	-	-	X
Thamnidium	-	X	-	X	-
Trichoderma	-	-	-	-	-
Bacteria	-	X	-	X	-
Nematodes	X	-	X	-	-

The Histochemical Localization of Enzymes in Plants
Resistant and Susceptible to Fusarium Root Rot

Beans grew well in the hydroponic chamber, and root growth generally filled the chamber (Figures 4 and 5). No nutritional deficiencies were observed during the course of the experiments although there was a tendency for abnormal elongation because of low light intensity. Inoculated plants developed pronounced root rot lesions within three days and susceptible plants were severely stunted or killed within 14 days. Resistant plants of N203, Phaseolus coccineus, and plants with induced resistance showed pronounced lesion development, but the lesions failed to enlarge or coalesce. Root rot appeared much more severe under these growing conditions than on plants grown in artificially infested steamed soil in the greenhouse.

Susceptible plants (Red Mexican and Sanilac) inoculated with a highly virulent isolate of Fusarium solani f. phaseoli were killed within 3-5 days whereas N203 and Red Mexican and Sanilac induced resistant plants were not killed until the end of 10-14 days. Phaseolus coccineus and Dark Red Kidney induced resistant plants were severely stunted but still growing four weeks after inoculation. No deleterious effects from wounding were observed, and mechanically wounded plants grew normally.

The results of enzyme localization in the hypocotyl tissues of five varieties of beans are summarized in Table 12. No differences consistent with varietal resistance or susceptibility were observed, but tissues in all varieties resistant to penetration of Fusarium, i. e., the endodermis, phloem, cambium, and xylem, exhibited many differences from the senescent cortical and pith tissues which were very susceptible to invasion. Resistant tissues were characterized by an overall increase in enzymatic activity and increased activity of specific enzyme systems not commonly active in susceptible tissues.

Table 12. Enzyme activity and suberin deposition as determined by histochemical reactions of various tissues in the hypocotyl of resistant and susceptible bean varieties.*

Enzyme	Variety	Tissue					
		Cortex	Peri- cycle	Endo- dermis	Phleom bium	Cam- bium	Pith
Acid Phosphatase	Sanilac	M		M	H	VH	L
	Red Mexican	M		M	H	VH	L
	Dark Red Kidney	M		M	H	VH	L
	N203	M		M	H	VH	L
	P. Coccineus	M		M	H	VH	L
Alkaline Phosphatase	Sanilac	L		M	H	H	L
	Red Mexican	L		M	H	H	L
	Dark Red Kidney	L		M	H	H	L
	N203	L		M	H	H	L
	P. Coccineus	L		M	H	H	L
Esterase	Sanilac	VL	M	H	H	VH	VL
	Red Mexican	VL	M	H	H	VH	VL
	Dark Red Kidney	VL	M	H	H	VH	VL
	N203	VL	M	H	H	VH	VL
	P. Coccineus	VL	M	H	H	VH	VL
Beta- Glucosidase	Sanilac				VH	VH	
	Red Mexican				VH	VH	
	Dark Red Kidney				VH	VH	
	N203				VH	VH	
	P. Coccineus				VH	VH	
Sulfatase	Sanilac	L			H	H	L
	Red Mexican	L			H	H	L
	Dark Red Kidney	L			H	H	L'
	N203	L			H	H	L'
	P. Coccineus	L			H	H	L'
Peroxidase	Sanilac	VL	H	M	VH	VH	VH
	Red Mexican	VL	H	M	VH	VH	VH
	Dark Red Kidney	VL	H	M	VH	VH	VH
	N203	VL	H	M	VH	VH	VH
	P. Coccineus	VL	H	M	VH	VH	VH
Tyrosinase	Sanilac	VL		H	M	H	H
	Red Mexican	VL		H	M	H	H
	Dark Red Kidney	VL		H	M	H	H
	N203	VL		H	M	H	H
	P. Coccineus						
Sudan IV	Sanilac			H			M
	Red Mexican			H			M
	Dark Red Kidney			H			M
	N203			H			M
	P. Coccineus			H			M

*L-light reaction; M-moderate reaction; H-heavy reaction; V-very

No differences in enzyme localization were observed in susceptible and induced resistant plants. The localization of the various enzymes was as follows:

Phosphatase

Localization of alkaline phosphatase was patchy and variable when alpha naphthol phosphate was used as a substrate. Naphthol-AS-MX-Phosphate, however gave satisfactory results. Alkaline phosphatase activity was light in the cortex, xylem, and pith; moderate in the endodermis, and heavy in the phloem and cambium.

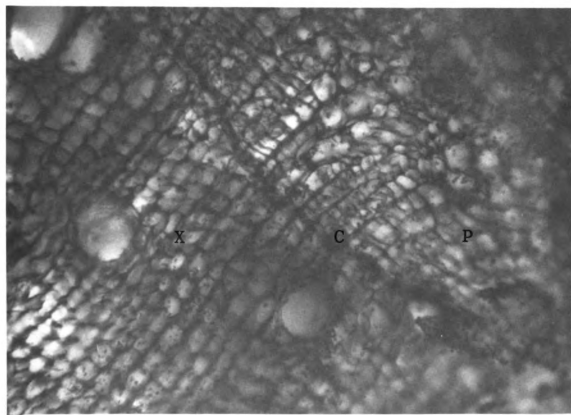
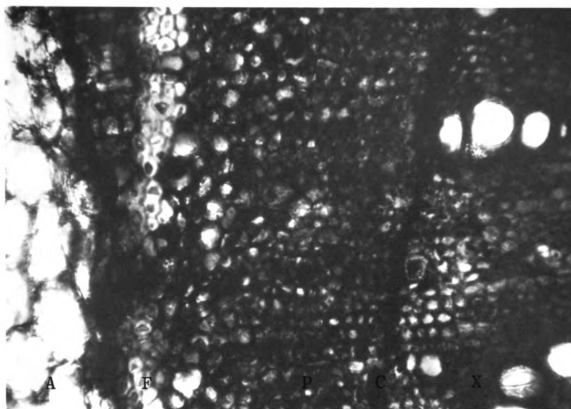
Acid phosphatase was localized satisfactorily when either alpha-naphthol phosphate or naphthyl-AS-MX-phosphate was used as a substrate for enzyme activity. Dye deposition was uniform and heavy and demonstrated a similar distribution and activity in bean hypocotyl tissues as alkaline phosphatase with the exception that moderate activity was demonstrated in the xylem.

Esterase

Esterase activity was demonstrated satisfactorily with all substrates. The cortex and pith had a very light precipitate when naphthol-AS-D acetate was used as a substrate. No activity in the cortex or pith was demonstrated when alpha-naphthol-acetate or Indoxyl acetate was used as the substrate. The pericycle tissues of Sanilac, Red Mexican, and Phaesolus coccineus were moderately active while those in Dark Red Kidney and N203 produced a heavy precipitate. The phloem and xylem of all varieties demonstrated high esterase activity with the cambium showing very high activity (Figure 17).

Figure 17. The localization of esterase activity in the bean hypocotyl. Areas of high enzyme activity (phloem (P) and xylem (X)) and very high activity (cambium (C)) show a heavy precipitate. The cortex (A) and phloem fibers (F) were negative.

Figure 18. The localization of peroxidase activity in the bean hypocotyl. The phloem (P), cambium (C), xylem (X) and endodermis tissues show high enzyme activity (black stain).



Beta-glucosidase

Beta-glucosidase activity was limited to the phloem and cambium where activity was very high. The cortex, endodermis, xylem, and pith showed no activity.

Sulfatase

Cortical and xylem tissues hydrolyzed 8-hydroxyquinoline sulfate slightly to yield a faint precipitate of the coupled quinol. No activity was observed in the endodermis or pith. Heavy deposition of the final product was observed in the phloem and cambium.

Peroxidase

The phloem, cambium, and xylem exhibited very high peroxidase activity (Figure 18). The pericycle had high activity and the endodermis moderate activity. Only very light activity was observed in the cortex and pith.

Tyrosinase

High tyrosinase activity was evident in the endodermis, cambium, and xylem by the heavy deposition of melanin. Moderate activity was localized in the phloem and very light activity could be observed in the cortex. Pith and pericycle tissues were negative (Figure 19).

Suberin Deposition

The epidermis (cuticle), endodermis, and xylem were the only tissue giving a positive result with Sudan IV stain.

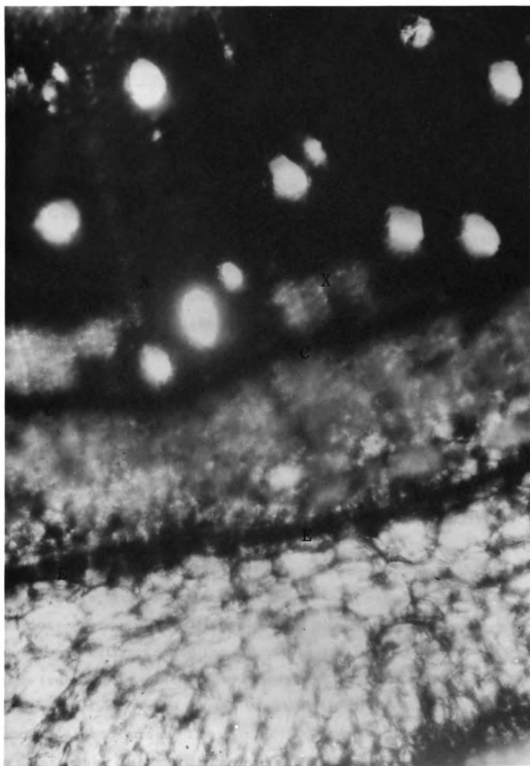


Figure 19. The localization of tyrosinase activity in the bean hypocotyl. High enzyme activity was present in the endodermis (E), cambium (C), and xylem (X) tissues (dark stain).

The Histochemical Study of Wound Response in Plants
Resistant and Susceptible to Fusarium Root Rot

No differences between wound response and infection were observed in these studies. Periderm formation was initiated in four to five days in N203, Phaseolus coccineus, and in induced resistant plants. Cell division was erratic and slow in Sanilac, Red Mexican, and Dark Red Kidney and was never observed to form an effective barrier to the continued invasion of the pathogen. P. coccineus demonstrated the most rapid wound response in all tests. Thirty hours after wounding, a heavy light-brown deposit was evident around the "infection" site in N203, Phaseolus coccineus, and the chemically induced resistant plants. This material coupled with the diazo couplers and was assumed to be partially comprised of quinones or phenols. Fungal penetration was limited to two to four cells in depth in the resistant varieties. The deposition of wound compounds was not observed in susceptible varieties until 72 hours after wounding and then to a lesser extent than had appeared in 30 hours with Phaseolus coccineus.

Several enzymes became active around the wound or the infection site. Of these, acid and alkaline phosphatase, esterase, beta-glucosidase, peroxidase, and tyrosinase showed high activity. Sulfatase became very active around the wound. Sudan IV stain for fatty compounds (suberin) was also heavy around the wounded areas. Wound compounds were observed to fill the vascular tissues adjacent to deep wounds in all varieties. The same deposition was observed in susceptible plants when Fusarium penetrated to the endodermis. No vascular penetration was observed. Deposition in the vascular tissues of all plants was obvious after 30 hours when it was stimulated by a deep cortical wound. Induced resistant plants responded similarly to

wounding and infection as N203 and Phaseolus coccineus. This response was specifically observed by the rapid deposition of wound compounds around the "infection" site followed by periderm formation in four to five days. Fungal penetration was limited to a few cells.

DISCUSSION

During the course of the investigations on bean root rot many items of special interest were observed. Some of these items included: (1) the need for an improved method for studying the ecology of the soil microflora, especially the actively growing fungi in the soil; (2) the active nature of many soil-borne fungi in cultivated soils; (3) the pronounced effect of the previous crop on the expression of bean root rot; (4) the pronounced effect of the present or growing crop on the soil microflora; (5) the intimate nature of microbial associations of possible significance in the biological control of soil-borne pathogens; (6) the similarity of the resistant mechanisms to root rot as possessed by N203, Phaseolus coccineus, and the growth regulator treated plants.

Application of the Plate-Profile Technique to Microfloral Studies

To study the effects of cropping sequence on the soil microflora it is important to use techniques which sample the actively growing organisms, rather than dormant spores which may have been produced in previous seasons. The dilution techniques generally used to study the soil microflora, fail to provide information relative to the mode of existence of the isolated organisms in the soil. Species which sporulate abundantly are more frequently isolated with these methods (71), and Hack (58) demonstrated that the dilution-plate method gave information relative to the spore population of fungi in the soil but not of mycelial, or active forms. Another problem with dilution methods is that many fungi, although active in the soil, occur in a spore population less than one in one thousand (1:1000) and are not isolated with

these techniques (122). When Watson (166) washed spores from the soil and plated the mycelial fragments, he obtained an isolation frequency different from "unwashed" soil. Furthermore, non-sporulating genera were more frequently isolated from the "washed soil." Warcup (162) also modified the dilution-plate method by plating soil directly in the hopes that spore masses would hold together and have less influence on the final results.

Since the dilution techniques were demonstrated to measure the spore population of the soil rather than the actively growing organisms, an attempt has been made to use more direct techniques to study the actively growing fungi. Techniques such as Thornton's screened immersion-plate (150) and Mueller and Durrell's modification (106) of Chester's immersion-tube technique may provide information on the actively growing fungi in the soil since the organisms isolated had to grow into the media. The frequency of isolation of the various microorganisms studied with these methods were shown to be predominantly non-sporing forms (150) and in general agreement with direct observations in the soil (163). Thornton's screened immersion-plate is difficult to prepare, however, and the isolation of organisms between holes was not always possible. The immersion-tube technique only sampled a small area so that a distribution of the organisms was not obtained.

The need for an improved method for studying the actively growing fungi in the soil was apparent. Hence, the plate-profile technique was developed to study actively growing organisms in the soil. By comparing the results from the plate-profile with those from the dilution-plate it was readily observed that many organisms frequently isolated by the plate-profile were only rarely isolated or not isolated at all by the dilution plate. For example: Penicillium accounted for less than 3 percent of the organisms isolated with the plate-profile

(Table 4) but 35 to 60 percent with the dilution-plate. Rhizoctonia, or non-sporulating fungi which were known to actively grow in the soil (52, 162), accounted for 20-25 percent of the isolates obtained with the plate-profile, while these fungi were not isolated with the dilution-plate method. This frequency was also consistent with the results obtained by Thornton (150) and similar to those of Watson (166). Since non-sporing fungi were frequently isolated, and since chromatograms demonstrated that no carbohydrate or proteinaceous compounds diffused through the tape or hole to stimulate the germination of resting spores, the plate-profile was considered to measure actively growing fungi in the soil. Because of this, the plate-profile technique can be considered an excellent method for studying the effects of cropping sequence on the soil microflora. Not only was a large area of the soil sampled, but actively growing organisms were isolated in their various associations and the data obtained was less variable than that obtained by other methods, and could be analyzed without prior transformation.

Activity of Fungi in the Soil

The mode of existence and survival of Fusarium and other fungi in the soil has been a controversial topic for many years. From the results of this study it was apparent that Fusarium species were the most active fungi in this soil. This was to be expected since Fusarium will grow actively in the soil in the presence of most plants (135). The active nature of Fusarium in the soil is also indicated by its compatibility (through association) with most of the soil-organisms isolated (Table 7). Williams (174) found that most antagonists of Fusarium roseum were representatives of the Aspergilli or Penicillia. The fact that Fusarium was compatible with Penicillium (Table 7) and that these antagonists were rarely active in this soil is an indication that the reported antagonism of Fusarium by Penicillium may not occur in soil.

Rhizoctonia was also a very actively growing fungus in the soil based on its isolation frequency and compatibility with other organisms. The studies of Thornton (150) and the direct observations and hyphal transfers of Warcup (163) in cultivated soils also verify the active nature of Rhizoctonia and non-sporulating (unknown) fungi in the soil. Mucor, Pythium, non-sporulating (unknown) fungi and Trichoderma were also actively growing in these soils based on their isolation frequency. Penicillium, Sepedonium, Streptomyces, and Alternaria were actively growing in the soil but their activity was dependent on various factors in addition to the growing crop. Aspergillus was rarely active in the soil. These results are in direct contrast to those reported by Williams and Schmitthenner (177). Their results showed that fungi which sporulate heavily such as Penicillium, Aspergillus, and Verticillium were isolated the most frequently from dilution-plates. This conflict is best explained by the difference in techniques used to study the soil microflora since the dilution-plate yields data relative to the spore population or potential frequency of organisms in the soil, but not necessarily their activity.

Studies on soil fungistasis have indicated that the inhibition of fungal growth and the germination of fungal spores, especially of soil-invading organisms may be a wide-spread phenomenon. Although fungistasis is associated with biological activity in the soil, isolation of the fungistatic principle has not been possible. Soil extracts generally stimulated fungal germination and growth (81) and fungistasis can be restored to sterilized soil with fungi, bacteria, or Actinomycetes and may be the result of released degradation products. Since organic amendments, root exudates, or a growing plant could readily overcome fungistasis then fungistasis is probably non-existent for soil-inhabitants in cultivated soils during the growth of the crop or the presence of its residue in the soil. For example, Fusarium solani f. phaseoli existed

in the form of chlamydospores in fallow soil (110) but readily germinated and grew as mycelium in the presence of growing plants or root exudates (88, 133, 179).

The existence of mycelium of several other soil fungi has been observed directly in the soil. Garrett (52) characterized Rhizoctonia as a soil-inhabitant, capable of prolonged mycelial growth through fallow soil. Warcup (163) readily isolated Basidiomycetes and some Ascomycetes from the soil as hyphae.

Studies of the rhizosphere, that portion of the soil that is adjacent to the root system of a plant and influenced by it, have also verified the active nature of soil organisms in the presence of a growing plant (71). Although no precise limits are generally given, the rhizosphere has often been thought of as limited to one or two millimeters around the root. The studies on scopoletin, a natural root exudate which diffuses for a considerable distance from the root (92), and alpha-methoxyphenylacetic acid (127), however, extend the rhizosphere to several inches around the root. Therefore, when the extensive root development of most agricultural plants is considered in addition to the diffusion and movement of root exudates in the soil, it seems probable that the entire "A" horizon of a cultivated soil could be considered as the rhizosphere.

It is now more apparent than originally thought, that cultivated soils may be characterized as the habitat for actively growing organisms in a complex of compatible and incompatible interactions, rather than limited to an area of dormant or dead spores and structures.

The Influence of Cultural Practices on Disease Severity

There has been some inconsistency regarding the effects of barley preceding beans in the rotation. In this investigation, bean root rot was consistently more severe after barley and less severe after corn.

A significant reduction in root rot was also observed when beans followed sugar beets, wheat, or sweet clover. These results were not in conformity with those of Snyder et al. (142) and Maier (86) who reported that barley residues significantly reduced the severity of root rot. The effect of nitrogen fertilizers may be responsible for the observed differences in root rot since Burke (17) reported that barley amendments reduced root rot when no commercial fertilizer was applied to the beans but that barley residues greatly increased root rot when nitrogen fertilizers were applied. The reduction in root rot after the addition of organic amendments has generally been attributed to a large carbon to nitrogen (C:N) ratio. The differences in root rot severity when beans followed corn, wheat, sugar beets, clover, and barley certainly suggest that the effects of the specific crop or its residue are not necessarily the result of an altered C:N ratio. The microflora established under each crop is undoubtedly of greater significance in the reduction of root rot than the C:N ratio.

Garrett (50) pointed out that adequate control of soil-borne pathogens may be accomplished through the reduction of the pathogen to a low level rather than by its complete elimination. Burke (17), however, reported that Fusarium solani f. phaseoli was resistant to competition or suppression by other microorganisms. Snyder et al. (142) reported the reduction in root rot with a specific cropping sequence even though the population of the pathogen increased. Maloy (88) speculated that the effects of crop rotations on the saprophytic growth of Fusarium may account for the observed reduction in severity of the disease without a reduction in the population of the pathogen. The effect of specific cropping sequences on the population of pathogenic Fusaria which were observed in this study may have been the result of the stimulation of non-pathogenic forms of the fungus as suggested by Maloy or the reduced pathogenicity of the virulent form through a specific microbial interaction such as necrosis.

Most likely other factors play an important role in the expression of bean root rot. Among these are the effects of climate on the soil microflora or the resistance of beans to infection. Year to year variation appeared to be due to climatological factors; however, the greenhouse studies on the effects of soil temperature and moisture on microfloral associations, isolation frequencies, and root rot development were generally, but not always, consistent with the observed year to year changes in the field. For example, Rhizoctonia populations were affected by soil temperature in the greenhouse studies, but no year to year effect was noticeable in the field. The greenhouse results with Trichoderma, on the other hand, were consistent with the field results in that no year to year effects were noticeable. In general, soil moisture appears to be the climatic factor having the greatest influence on the fungal and bacterial populations, whereas soil temperature was more important to nematode activity.

The Influence of Cropping Sequence on Soil Microorganisms

The most frequently isolated microorganisms were present under all cropping sequences, but their frequency was altered by the effects of the present and previous crops. The pronounced effect of the present crop on the soil microflora was possibly the result of root exudates and sloughed plant tissues which appeared to favor specific organisms, resulting in their altered frequency in the soil. The stimulatory effect of root exudates from bean on Fusarium chlamydospore germination as reported by Schröth and Snyder (133) could account for some of the increased frequency of Fusarium under beans in the rotation. However, exudates of most non-host crops also stimulated the germination of Fusarium solani f. phaseoli

chlamydospores in the soil; therefore, other conditions conducive for growth and activity in the soil rather than just germination must be postulated.

It was significant that the present crop had the most pronounced effect on the microflora, although the effects of the previous crop and year were also observed. It also appeared that the microflora established during the growth of the plant was partially maintained by the crop residues remaining after harvest. Since organisms affected by the present crop were also affected by the previous crop, this possibly could account for the low frequency of Pencillium and bacteria under corn and the very high frequency after corn, since both types of organisms are known to actively respond to organic soil amendments. Heavy spore production of Pencillium as a result of residue amendments in the fall may also account for the predominance of this fungus in the studies of Williams and Schmitthenner (177). This was probably also responsible for their conclusion that the previous crop had the greatest influence on the microflora.

Those organisms consistently isolated in high frequency, which were also compatible with other soil fungi, may be considered as inhabitants of these soils. Fusarium, Rhizoctonia, Mucor, Pythium, non-sporulating (unknown) fungi, and Trichoderma could, therefore, be considered indigenous organisms. It was significant to observe that Rhizoctonia was not affected by the present or previous crop. Other fungi isolated such as Aspergillus, Hormodendron, Nigrospora, Thielaviopsis, and Phoma may be soil inhabitants, but were generally inactive in the soil. Those organisms which occurred only rarely in these studies may have been dormant in the soil or were soil invaders not normally existing in the soil for prolonged periods.

The frequent isolation of Pythium in these studies without the occurrence of damping-off is probably an indication of the increased

resistance of the mature bean plant to this pathogen, although non-pathogenic species of Pythium may have been isolated. The majority of the microbial associations from the soil were consistent from year to year even though some were affected by the present or previous crop. Fusarium, Mucor, Rhizoctonia, Sepedonium, Trichoderma, bacteria, and nematodes were compatible with most other soil microorganisms, if the frequency of isolation together was used as the basis for compatibility; while Streptomyces and Penicillium were much less compatible with other soil microorganisms. The stability of some of the observed associations was apparent with organisms associated with Rhizoctonia. Although individual frequencies of the associated organisms varied from crop to crop, their association with Rhizoctonia was generally not affected.

The Effects of Cropping Sequence on the Biological Control of Soil-Borne Fungi

On the basis of this study it was difficult to attribute the reduction in root rot to the production in the soil of antibiotics or other compounds toxic to Fusarium. Williams (174) found that Penicillia and Aspergilli were the most frequent antagonists of Fusarium roseum. The Aspergilli rarely existed in an active state in these soils, and the Penicillia were lowest in corn and in beans after corn where root rot was also low. The Penicillia were the highest in barley which favored root rot development in the following bean crop. Streptomycetes, known antagonists of many soil fungi in the laboratory, also showed no correlation with disease severity in these studies.

Microbial interactions in the soil of possible significance in the control of soil-borne pathogens were observed as an intimate relationship between two organisms rather than the result of diffusible toxic

entities. Parasitic associations observed included the coiling of an unknown fungus around Rhizoctonia followed by frequent penetration into the host mycelium similar to the method reported by Butler (23); the parasitism of Fusarium by an Actinomycete (actinomycosis); lysis of Fusarium and other fungi; and bacterial necrosis of Fusarium by a Xanthomonas. Lysis of fungal mycelium was never observed as the result of diffusible toxic materials as reported by Carter and Lockwood (27) or Mitchell and Alexander (103), but resulted only after the prolonged intimate association of two organisms. Parasitism of Rhizoctonia, Actinomyces parasitism of Fusarium, and lysis of fungi were not affected by cropping sequence and therefore, not directly responsible for the reduction in root rot with specific cropping sequences. Actinomycetes are generally considered antagonistic or lytic to fungi; yet, neither of these interactions were observed very frequently in this study. The intimate association and direct parasitism of Fusarium by an Actinomycete was frequently observed. This was the first known report of an Actinomycete parasitic on a fungus.

Bacterial necrosis, also reported for the first time, was influenced by specific cropping sequences and directly related to the observed reduction in root rot when beans followed corn. Necrosis of Fusarium could account for the significant reduction in root rot without affecting the isolation frequency of Fusarium in the soil since hyphal tips were resistant to necrosis but coated with the bacterium. Bacterial necrosis is considered of major importance in the biological control of Fusarium and the increased bean yield in this soil.

Other factors contributing to the reduction of root rot were apparent. The population of Fusarium pathogenic to beans was affected by cropping sequence with the highest frequency occurring under beans and the lowest under corn. This may be the effect of cropping sequence on certain species or forms of Fusarium as reported by Maloy (88), or



the result of an association of pathogenic *Fusaria* with other soil microorganisms which rendered it non-pathogenic.

Characteristics of Resistance to Root Rot

Resistance to *Fusarium solani* f. *phaseoli*, either the type found in N203 and *Phaseolus coccineus* or the type induced by growth regulators (113), appears to be a non-specific wound response and apparently the same mechanism in each case. This response was characterized by high enzyme activity in the resistant tissues and around the infection site resulting in a rapid deposition of inhibitory compounds. These deposited compounds probably resulted from the enzymatic oxidation of phenols to quinones as suggested by Kiraly and Farkas (73) for the resistance of wheat to rust, or to some other inhibitory compound such as chlorogenic acid (69, 70, 75). Resistant plants formed a wound periderm in four to five days which limited further penetration of the *Fusarium*. The limitation of *Fusarium* by wound periderm formations was also reported for gladiolus (91) and potatoes (138). Conant (38) characterized tobacco plants resistant to *Thielaviopsis* by a rapid periderm formation. Rapid wound response involving the production of inhibitory compounds and a protective periderm may be characteristic of resistance to most of the soil-borne cortical pathogens.

Enzymes associated with resistant tissues and the resistant wound response included peroxidase, tyrosinase, and beta-glucosidase, all of which are known to be important in lignification. Since lignified tissues are not invaded by *Fusarium solani* f. *phaseoli* (28, 36) and lignicolous compounds were inhibitory to fungi (80), the process of lignification may also be a part of the resistance mechanism.

SUMMARY

The plate-profile technique provided a means for studying the actively growing organisms in the soil in their various interactions. Bacteria and nematodes as well as fungi were frequently isolated with this method. Data obtained with the plate-profile technique was much less variable than data obtained with other methods, and could be statistically analyzed without prior transformation even though only four replicates per treatment were used.

The studies on cropping sequence in general emphasized the significance of the present and previous crop on the soil microflora, and demonstrated that microbial interactions in the soil effective in reducing the severity of plant diseases are of an intimate nature, rather than a general or wide-spread nature which would leave areas in the soil void of actively growing fungi.

Corn preceding beans in the rotation resulted in the greatest reduction of root rot and in a significant increase in bean yields. The most severe root rot was observed when beans followed barley in the rotation.

Rhizoctonia, Fusarium, Pythium, Mucor, Trichoderma, and bacteria were the most active of the indigenous soil organisms and were generally compatible with most of the other organisms isolated. All of the frequently isolated organisms, except Rhizoctonia, were affected by cropping sequence.

Microbial associations, although consistent from year to year, were generally influenced by the cropping sequence. The various microbial associations of possible significance in the biological control of Fusarium included the parasitism of Fusarium by an

Actinomycete (not previously reported), bacterial necrosis (not previously reported), and lysis. Only bacterial necrosis was influenced by cropping sequence.

The reduced root rot when beans followed corn was directly related to the increased frequency of bacterial necrosis. Necrosed Fusaria were seldom isolated from barley plots, and root rot was the most severe in beans following barley. Sugar beets, wheat, and sweet clover had an intermediate effect on root rot.

Soil moisture appeared to have the most pronounced effect on the soil microflora, while nematodes were influenced the most by soil temperature.

The endodermis, phloem, cambium, and xylem, all resistant to Fusarium, were characterized enzymatically by an over-all increase in enzyme activity as well as the activation of specific enzymes which were not active in the senescent cortical or pith tissues which are susceptible to Fusarium. No differences consistent with varietal resistance or susceptibility were observed.

Resistance to Fusarium was induced in susceptible plants with plant growth regulators. No differences in enzyme localization were observed after treatment, but phosphatase, esterase, beta-glucosidase, peroxidase, and tyrosinase were activated around a wound or infection site. Suberin was also deposited in tissues adjacent to a wound.

Resistance appeared to be the result of the rapid deposition of inhibitory compounds in N203, in Phaseolus coccineus, and in the induced resistant plants followed by the formation of a wound periderm which delimited the infected area. Only erratic cell division occurred in susceptible plants, and the deposition of wound compounds was slow.

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