INFLUENCE OF *STREPTOMYCES* SPP. FROM DESERT SOILS UPON *RHIZOCTONIA* DAMPING OFF OF COTTON

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ABSTRACT

Five polyene producing Streptomyces spp., isolates from rhizosphere of desert plants, were tested for their ability to reduce damping off in cotton caused by a particularly virulent strain of Rhizoctonia. Tests using a sterile plastic growth sack technique in a walk-in, controlled environment unit showed that addition of 5 Streptomyces isolates singly to nonsterile soil two weeks prior to infestation with Rhizoctonia and planting of cotton seeds resulted in statistically significant control with 3 of the 5 isolates. Adding the 5 isolates together in one treatment did not result in a synergistic effect. Neither addition of certain nitrogen sources to soil nor changes in C:N ratio resulted in significant increase in control. Results of laboratory experiments utilizing the same nitrogen sources and C:N ratios show no significant increase in growth of or antibiotic production by the Streptomyces spp., perhaps explaining results of the previous experiments. Ability of cotton root exudates to support growth of and antibiotic production by the Streptomyces spp. was investigated and positive results obtained.

INTRODUCTION

Studies on biological control of soil-borne plant pathogens implicate the saprophytic microflora, particularly rhizosphere organisms. The favorable results of organic amendments in the control of soil-borne pathogens have for some time been generally attributed to changes in microbiological balance (50, 84, 110, 153). Experimental results have implicated antibiosis as at least playing an important role in the control mechanism (2, 63, 92, 95, 163). The production and persistence of antibiotics in soils has been demonstrated (45, 46, 47, 57, 80, 135). The presence of antibiotics in amended and unamended sterile soil (51, 52, 53) and in amended unsterilized soil (49, 160) has also been recognized. Some studies have shown the production of antibiotics and subsequent controls exerted by them in a plant rhizosphere (103), in unamended soil (102), and in amended soil under greenhouse conditions.

It has been proposed that an understanding of the activities and relationships of organism, saprophytic and parasitic, occurring in desert soils might make possible the utilization of this knowledge to develop, in cultivated soils, desirable micro-floral communities effective in the control of pathogens.

It has been suggested by Whaley and Boyle (155), that native plants found in virgin desert soil may be protected from prevalent soil borne pathogens by antibiotic producing saprophytes. Physiological studies of 10 Streptomyces spp. indicated strong inhibitory capabilities to prevalent root pathogens such as: Phymatotrichum omnivorum (Shear) Dugg., Rhizoctonia solani Kuhn, Verticillium alboatrum Reinke and Berth., Fusarium oxysporum f. lycopersici (Sacc.) Snyder and Hansen and Pythium spp. Observations indicate that while some of these parasites are often present in the rhizosphere of uncultivated desert plants they do not infect the desert flora. Antibiosis may be a factor in this control.

A large percentage of our land under cotton production is reclaimed desert which abounded with the type of flora that harbors the Streptomyces spp. isolated in this study. Chavez (26), using numerous methods, made extensive isolations of soil borne microflora from cotton fields of Southern Arizona. His findings indicate the presence of these same Streptomyces spp. that are found in desert soils, although in reduced numbers, as compared to those found in the root zones of desert plants. Their presence in cultivated soils that have been devoid of original vegetation for years indicates an ability of the Streptomyces organisms to exist under conditions other than those provided in the root zones of the desert flora.

The experiments described in this thesis were based on two assumptions: (a) some Streptomyces spp. may influence pathogenic fungal populations in the rhizospheres of uncultivated desert plants, and (b) these antibiotic producing saprophytes are able to survive conditions found in desert soils under cultivation.

An initial study was made to: (a) survey organisms associated with "damped off" cotton seedlings in Arizona, and (b) select a pathogen which would display easily detectable pathogenicity when added to soil in conjunction with the Streptomyces isolates. A particularly virulent strain of Rhizoctonia was chosen on the basis of this experiment.

An experiment was conducted in soil initially infested with Streptomyces spp. and later with Rhizoctonia to test effects of the Streptomyces on the growth and development of seedling cotton.

The effect of organic amendments consisting of specific carbon:nitrogen sources and ratios of said sources on antagonism by a specific Streptomyces sp. was also investigated.

Laboratory work was instigated to determine the ability of Streptomyces spp. to grow and produce antibiotic in exudate obtained from cotton seedlings.

LITERATURE REVIEW

The Damping Off Disease Problem

To obtain a satisfactory stand of seedlings has long been a major problem in the production of cotton. Many chemicals applied as seed treatments have been tested (8, 12, 106, 138, 157). Seed treatment is valuable, but is regarded as inadequate (6). Usually farmers plant an excess of seed or dust with protectants or both but quite often replanting is necessary. Reseeding costs the cotton producer time which in turn results in substantial losses at the gin because of low yields caused by a reduction in the growing season.

Seedling loss can usually be attributed to soil-borne fungal pathogens which attack the young plant in this relatively unprotected stage. Reports indicated that seedling disorders are caused by a complex of organisms (100, 150, 159).

Atkinson (9), in 1892, first described damping off of cotton, although the descriptive term, soreshin, had been in use since 1855 (43). Later Duggar (100) identified the causal organism as Rhizoctonia solani.

After testing of fungi associated with diseased cotton seedlings, Arndt (4, 5) reported Colletotrichum gossypii Southworth and Pythium ultimum Trow, and several

other fungi to be pathogenic to cotton seedlings. Lehman (73) showed C. gossypii and Fusarium spp. were the most common species of fungi attacking young cotton plants in North Carolina. Fusarium oxysporium f. vasinfectum (Atk.) Snyder and Hansen has been shown by Rosen (109) to be pathogenic to cotton seedlings. Fulton and Bollenbacher (38) implicate Aspergillus niger. Neal (87) reviews the organisms encountered by plant pathologists in their studies of seedling diseases of cotton. Those noted are: Glomerella gossypii Edg., Fusarium spp., Thielaviopsis basicola (Berk. & Brown.) Ferr., Pythium spp., Sclerotium rolfsii Sac., Sclerotium bataticola Taub. and Rhizoctonia solani (Kuehn).

Aspects of Rhizoctonia Damping Off

Rhizoctonia solani Kuehn (Pellicularia filamentosa (Pat.) (Rogers)) is one of the most important pathogens involved in cotton seedling disease in the southwest (4, 5, 25, 38, 104, 129, 136).

The fungus species, R. solani is made up of many strains differing in growth characteristics, pathogenicity, host range, morphology, utilization of nutrients, enzyme secretions, optimum temperatures, pH requirements and reaction to chemicals (39, 64, 72, 104, 129, 130, 131, 132).

In a review by Kernkamp et al. (64), physiologic specialization in R. solani was established based on cultural characteristics. Using bean as a host Leclerg (72) tried to identify races of R. solani, but concluded variability between experiments was too great to warrant a conclusion. Shatla (129) tested 3 R. solani isolates under greenhouse conditions against 28 varieties of cotton and was able to show physiologic specialization. Variable responses have been reported for sclerotial development by different strains to influential factors in the environment (15, 16, 64, 122).

Factors Affecting Parasitism of Rhizoctonia

Parasitism of Rhizoctonia solani has been found to be influenced by certain chemicals, soil pH, nitrogen source and concentration, CO_2 levels in soil, temperature, inorganic and organic amendments to the soil and various other environmental factors.

A great deal of work has been done concerning the saprophytic and parasitic capabilities of strains of R. solani (54, 64, 71, 82, 117, 118, 119, 137). Soil temperature plays an important role in the persistent parasitic ability displayed by various strains and on disease expression (7, 64, 107, 119, 127, 149). The severity of damage by the fungus may be influenced by low temperatures (107, 119) and equally by high temperatures. On cotton,

temperatures above 24 C favor severe soreshin development (7, 149), but temperatures below 24 C favor rapid pre-emergence infection and severe damping off (127, 149).

The effect of CO_2 concentrations on vertical distribution and saprophytic activity of Rhizoctonia has been reported by Durbin (35) and Davey and Papavizas (94). Usually a general decline in vegetative growth and pathogenicity were observed, with an increase in CO_2 levels, but in some strains an increase in pathogenicity was noticed.

Only one incidence of the sexual stage of R. solani in the soil (145) has been reported. Ordinarily little or no spore production by R. solani takes place in the soil so the burden of survival and dissemination rests upon the sclerotial stage. The production and persistence of sclerotia in soil is affected by environmental factors (15, 16, 64, 122) and in turn these factors influence the incidence and severity of the damping off disease caused by R. solani.

Effect of Organic and Inorganic Soil Amendments and Cropping Practices on Plant Pathogens

The literature concerning the use of soil amendments to control soil-borne pathogens is voluminous and reveals many successes and failures. Most results indicate a definite relationship, but the lack of consistent data indicates a complex problem exists with a vast number of variables. The literature up until 1960 is reviewed quite

thoroughly in two books; one by Garrett (41), and one by Parkinson and Waid (97). These discuss the ecology and biology of soil fungi. Wood and Tveit (158) have an excellent journal review up to 1955 of all experimental contributions to the biological control of plant pathogens.

Crop rotation has long been a recommendation for implementing control of soil-borne pathogens, but due to the ability of many organisms to survive in the soil by over-wintering spore forms, sclerotia or saprophytic stages, this treatment is not always successful. Even under long term conditions, crop rotation frequently fails to control them (77, 98, 126).

Some observations have been made on the effect of cropping sequence as opposed to arbitrary crop rotation. Maloy and Burkholder (77) found that wheat and alfalfa in the rotation scheme reduced Fusarium root rot of beans. Later, Maloy (78) showed that only when wheat rather than alfalfa preceded beans in the schedule was there a significant reduction in root rot. Some evidence for control utilizing a specific crop sequence has been reported for the control of Phytophthora (27) Rhizoctonia and Aphanomyces (1) Gibberella zea (68), Streptomyces (89, 110).

The mechanism involved in control of root diseases with crop rotations and soil amendments is difficult to interpret. There are a number of theories, all supported

by some experimental data, which attempt to explain this phenomenon.

Huber, Watson and Steiner (56), after a survey of all available information, felt that there were 5 relationships, some or all of which affect disease incidence when soil amendments are added to soil: (a) the form of nitrogen added to the soil may be a factor determining the severity of disease caused by several soil-borne fungi, primarily through an effect on pathogenicity of the causal organism or on host resistance; (b) that different pathogens react differently to a specific form of nitrogen; (c) that organic and chemical amendments may manifest their effects on disease by altering the rate of nitrification in the soil; (d) that disease control is independent of the C:N ratio, and (e) that pathogenicity is affected by microbial interactions which may be stimulated by soil amendments.

From all indications, nitrogen, its form, concentration and its use in conjunction with organic soil amendments appears to have a greater effect on soil-borne fungi than any other single element (64, 143). The addition of certain forms of nitrogen alone has been shown to produce marked effects on the severity of infections by root rot pathogens. A correlation has been shown between increased Fusarium root rot of bean and increasing quantities of ammonium nitrate (137, 152). The ammonium

cation has been shown to increase the disease incidence caused by Fusarium, Rhizoctonia, and Aphanomyces (31, 32, 44, 83, 121), while the nitrate form reduced field losses.

In conjunction with organic amendments, ammonium nitrate and Uramite (a urea formaldehyde product) reduced the severity of damage by Rhizoctonia when added with corn or oat residues (31). Barley straw and ammonium nitrogen reduced disease incidence in the case of Verticillium wilt (142, 156).

The role of the form of nitrogen in soil has been investigated with the aid of compounds which inhibit nitrification in the soils. Such compounds are 1,3-dichloropropene (Telone) or 2-chloro-6-(trichloromethyl) pyridine (N-serve). These chemicals have shown a pronounced effect on disease severity. The severity of bean root rot (Fusarium solani f. phaseoli) was increased in soils treated with N-serve and ammonium sulphate versus soil only treated with the ammonium sulphate (83). The increase may be attributed to prolonged presence of ammonia which previously mentioned work has shown to be conducive to increased disease expression.

It has been shown that disease expression of Rhizoctonia is enhanced by the addition of ammonium form of nitrogen (30, 121). It has also been reported that the severity of disease caused by Rhizoctonia can be increased

by soil fumigation with Telone (36) while Verticillium can be controlled by this chemical (70).

Complex organic amendments have been used on the assumption that the C:N ratio of the residue incorporated has a direct effect on the nutritional requirements of soil-borne pathogens and that varying ratios influence pathogenicity. It has been demonstrated that the C:N ratio in the soil is an important factor influencing survival of several soil-borne plant pathogens (22, 40). In each case, survival of the pathogen in soil was limited by the available nitrogen supply. A high C:N ratio was most desirable for reduced disease incidence. By changing the ability of required nutrients of the pathogens, certain crop rotations affected soil-borne root rots (120). Various nutrients and differing nutrient levels caused marked variation in snap bean stem rot due to R. solani (3).

In some experiments with F. solani f. phaseoli, which were conducted with constant initial concentrations of inoculum, increased soil-N enhanced the severity of the disease expression (143). These studies showed that the increase in disease incidence could be attributed to an increase in the rapidity with which spore germination to host penetration took place and pathogenisis was established. In other work with this pathogen, an increase in soil-N increased the aggressiveness of the superficial

thallus of the pathogen on the bean hypocotyl without significantly changing the inoculum concentration (152).

Supplemental N has been shown to nullify the beneficial action of high carbon to nitrogen residues against saprophytic activity and survival of R. solani in naturally and artificially infested soils (32, 93). In this case the reduction of amendment effectiveness by N was tentatively ascribed to increased saprophytic activity of the pathogen and to reduction of microbial competition for available N (32).

Microbial Interaction

The role of microbial interaction in the prevention of pathogenisis by soil-borne microflora is as complicated and misunderstood as are most other aspects of soil microbial ecology. The literature reviewed on this subject tends to postulate three possible mechanisms by which plant pathogenic microflora populations are reduced or prevented from parasitizing the host: (a) competition, (b) hyper-parasitism or lysis, and (c) antibiosis.

Competition as defined by Clements and Shelford (28) and Milne (86) is an active demand in excess of the immediate supply of material or condition (space, etc.) on the part of two or more organisms; it is the endeavor of two or more organisms to gain the same particular thing, or

to gain the measure each wants from the supply of a thing when that supply is not sufficient for both.

The ability of a pathogen to survive in the absence of a suitable host may often depend on its level of competitive saprophytic ability besides the production of a resistant dormant stage. Garrett (41) and Park (96) review this subject rather extensively. Pathogens with a very low competitive saprophytic ability are unable to make independent growth in a normal field soil, and after parasitism rely for survival on a phase of saprophytic growth in the dead host debris. These pathogens with the low competitive ability are classified as root inhabitants by Garrett (41). Helminthosporium sativum (22), Sclerotium cepivorum (128), and Verticillium albo-atrum (156) fall into this category. Pathogens relatively high in competitive saprophytic ability are able to maintain their populations in a manner comparable with that of non-pathogens. This group was described by Garrett as soil inhabitants. Fusarium solani (11), and Rhizoctonia solani (42) are included as members of this group.

Hyperparasitism refers to the phenomenon of a fungus parasitic to another. The relationship is one where there is a direct penetration of one organism (fungus in this case) by another. Several excellent papers on parasitism and predation have been published (20, 29, 34, 123, 151).

Two interesting hyper- or mycoparasites are Rhizoctonia solani and Penicillium vermiculatum. Butler (23) demonstrated with photomicrographs the parasitism of R. solani on Rhizopus stolonifer and Gelbertella persicaria. Siegle (134) reported the penetration of Ophiobolus graminis Sacc. by Didymella exitialis Sacc. He observed that infection pegs emerge from the appressoria and penetrate the host wall possibly with the aid of chitinase. Boosalis (17) demonstrated the pathogenicity of P. vermiculatum on R. solani.

The role of antibiosis in the control of soil-borne root diseases has been a controversial subject. The laboratory observations of antibiotic producing fungi, bacteria and actinomycetes are numerous. Brian (19) compiled a list of antibiotics and a three volume edition on actinomycetes and their antibiotics was written by Waksman (146, 147) and Waksman and Lechevalier (148). But the importance of antibiotic production in the soil has been questioned, e.g., Waksman (146, 147) and Brian (19). Brian in 1951 argues that (a) antibiotic production in soil may be insignificant because concentrations and types of nutrients are inadequate, (b) the known properties of many antibiotics suggest that they will be chemically inactivated or microbiologically decomposed in the soil, or physically adsorbed and thereby neutralized by soil colloids, and (c) organisms or strains known to produce

strong antibiotics are not obviously more successful in the struggle for existence, as judged by their frequency of distribution, than comparable species or strains not producing antibiotics. Garrett (41) disagreed in part with Brian but generally agreed on the whole. He felt at this time antibiotic production was probably only significant within close range of the producer and sufficient nutrient supply was available. He also feels that antibiotic production was greatly reduced as more colonization occurred and the nutrient supply was reduced.

These ideas seem to be somewhat out-moded in the light of recent work on determining the production and persistence of antibiotics in the soil by Siminoff and Gottlieb (135), Martin and Gottlieb (79), Jefferys (57), and Wright (160, 161). There appears little doubt now that detectable quantities of antibiotics can be produced in soil as well as in vitro. The addition of antibiotic producing organism to sterilized soil with and without organic amendments has yielded detectable concentrations of antibiotic substances (46, 51, 52, 53). Gregory et al. (49) and Wright (160) showed their presence in unsterilized soil amended with organic supplements.

Antibiotic production would be most effective in the rhizosphere or rhizoplane of the plant. In order for a root pathogen to become established on the root surface it must penetrate this area and become established. The

presence of antibiotics at the root surface might prevent this infection. Direct evidence for the presence of specific antibiotics in the rhizosphere has been difficult to obtain (61). Fusaric acid was detected in the rhizosphere of tomato plants inoculated with Fusarium lycopersici at the rate of 7.5 µg, 11.25 µg (61). Even though Fusaric acid has been described as a wilt toxin, it is known to also be an antibiotic. Evidence for antibiotic production by a Streptomyces sp. in unamended soil was demonstrated by Rangaswami and Ethiraj (102). Using a buried slide technique with Helminthosporium spores cemented to glass slides and then placed in unamended soil containing the Streptomyces sp., they were able to interpret the malformation of spore germ tubes as the result of antibiotic influence. These malformations were identical with those produced in sterilized soil in which the Streptomyces sp. was grown and in the cell free culture filtrate of the antagonist grown in dilute soil extract under submerged aerated conditions.

Using this same buried slide technique and the same organisms mentioned previously, Rangaswami and Vidyasekaran (103) indicated the production of an antibiotic substance in the corn rhizosphere by the Streptomyces organism. When added into sterile or unsterile soil in which corn plants were grown, the Streptomycete reached the rhizosphere and was present in sufficient numbers to inhibit spore

germination, to inhibit growth, and to malform and lyse germ tubes of Helminthosporium sativum.

The addition of Trichoderma viride or Penicillium frequentans to soil artificially infested with Pythium ultimum, one week prior to seeding with beets significantly reduced the damping off loss ordinarily attributed to the Pythium (74). Some enhancement of antagonism was observed when soil amendments such as KH_2PO_4 and urea were provided.

Plant Root Exudates

One of the major influences on soil-borne plant pathogenic fungi is the release or excretion of compounds from the root systems of higher flora. Hiltner (1904) first described the close relationship between plant roots and microorganisms. The first evidence of any release of chemical moieties from plant roots was provided by Knudsen (66). He observed the production of reducing sugars when peas and maize were grown in sterile glucose solutions. He theorized that the glucose was taken up by the plant and excreted as reduced saccharide compounds.

In considering root exudates one must consider the two ways chemicals may leave the plant. One obvious way might be by the degredation of sloughed off epidermal and cortical tissue especially in the vicinity of the root cap and the second might be by leakage or excretion of substances through semipermeable membranes and cell walls.

Quantitative data are lacking because of the difficulty of separating compounds produced under field conditions by the plant from those naturally occurring in the soil and produced by microorganisms.

The most widely used method of obtaining true root exudates without the influence of microorganisms is in an asceptic culture system, where seeds are disinfected and placed in known sterile medium. The medium may then be assayed by a number of techniques for excreted compounds.

The environmental conditions under which these tests are performed influenced the amount and type of exudates. Katznelson, Rouatt, and Payne (59, 60) demonstrated that temporary wilting of plants increased the release of amino acids in sand or soil. Even though wilting does not occur in the laboratory, this condition would be of considerable importance in the field. Rovira (113) demonstrated that high light intensity and temperature increased exudation in the first few weeks of growth of a number of plant species.

Microorganisms in the rhizosphere have a profound effect upon exudate production. Martin (81) found that culture filtrates of certain bacteria gave a three fold increase in the exudation of scopoletin without affecting root growth, whereas, other culture filtrates of other bacteria had no effect on exudation. Norman (88) observed that the antibiotics of the polypeptide and polymixin

groups increased the leakage of inorganic and organic materials from roots. These findings demonstrate the effect antibiotic producing microorganisms may have on the pathogenicity of some soil-borne pathogens. Rovira (113) also found that the presence of microorganisms in the rhizosphere of clover and tomato plants changed the amino acid content of the final exudates collected. He explained the effect was probably due to the utilization of certain amino acids and release of others by the microorganisms.

Rovira (115) indicates in a 1965 review that exudate patterns can be affected by microorganisms in three ways: (a) by altering the permeability of root cells, (b) by modifying the metabolism of the root, and (c) by modifying material released from the roots. If these assumptions are all correct the results of the use of asceptic culture to study root exudates might be misleading. Buxton's (24) work lends support to this statement and suggests caution in interpretation of results. His work showed that although exudates from peas susceptible to Fusarium oxysporium stimulated the fungus in pure culture, they also caused many rhizosphere isolates to antagonize the pathogen; thus there were two opposing systems operating in the rhizosphere of susceptible pea plants.

Root exudates have been shown to directly stimulate growth of soil-borne fungi. In the case of Rhizoctonia, Kerr (65) found that the fungus was attracted by the

presence of lettuce and radish. Surface sterilized seed were germinated in sterile cellophane sacks and buried in soil inoculated with Rhizoctonia solani. Intense colonization of the fungus was observed on the outer surface of the cellophane. A non-susceptible variety of tomato failed to stimulate the same profuse growth on the cellophane that was noticed with the susceptible lettuce and radish varieties.

Exudates may have a favorable as well as detrimental effect on germination of spores and growth of a fungus. Buxton (24) demonstrated a definite specificity with relation to Fusarium oxysporium f. pisi in the exudates of three pea varieties differing in susceptibility to this pathogen. Root exudates from the wilt susceptible plants stimulated conidial germination while exudates from the wilt resistant varieties inhibited germination. The effect was limited to spore germination because little or no effect was observed on the growth of the mycelium or germ tube once they were formed. Three pea varieties have differential susceptibility to three physiologic races of F. oxysporium f. pisi. The exudates from each variety were found to differentially reduce germination of spores from at least one Fusarium race.

Kommedahl (69) attempted to confirm these findings, using three different races of Fusarium than those used by Buxton (24). He was unable to show that resistance was

manifested in the exudates. Germination of the spores from each race was consistent in each of the aseptically produced exudate solutions collected from all three pea varieties.

Composition of Root Exudate

The three categories of organic compounds most extensively worked with in root exudates are amino acids, carbohydrates and B-vitamins. Other compounds such as nucleotides, flavonones, enzymes and organic acids have been detected (14, 15). It must be reiterated that there are inherent problems associated with the determination of the presence and quantity of these chemicals due to the wide range of techniques and conditions used in determinations.

The identification of at least 10 sugars plus an oligosaccharide has been reported for a wide range of plants. The two sugars most commonly found and occurring in the largest quantity are glucose and fructose. The sugars encountered are: (a) glucose--peas (111), soybean, barley, wheat (60), oats and mustard (14), oats (111); (b) fructose--peas and oats (111), sorghum and mustard (14); (c) sucrose--bean (125); (d) xylose--sorghum and mustard (14), wheat (115); (e) maltose--mustard (14), bean (125); (f) rhamnose--wheat (115); (g) arabinose, xylose and raffinose--white pine (115); and (h) oligosaccharide--wheat (115).

A survey of the literature concerned with detection of amino acids in root exudates reveals the identification of 23 amino acids found in 15 different plant species.

Rovira (111) in his work with pea and oat seedlings grown in sterile quartz sand found sixteen amino acids associated with the aseptically produced exudates. He found slight differences in the amino acid composition and concentration of exudates collected at two different ages for both plant types but in general the amino acids found in the peas and oats were: asparagine, aspartic, serine, glutamic, glutamine, homoserine, alpha-alanine, tryptophane, methionine or valine, leucine, phenylalanine, lysine, gamma amino-butyric, tyrosine, threonine, glycine and eleven other compounds which migrated under the two solvent systems used in his paper chromatogram determination. A comparison of the amino acids in pea- and oat-root exudate showed homoserine, threonine, alpha-alanine, glutamine, asparagine and serine to be dominant in the pea root exudate while in oat root exudate serine, lysine, and glycine were most abundant.

Employing microbiological assay techniques, Sulochana (140) was able to demonstrate the presence of seven to eleven amino acids. The range in amino acids found could be attributed to the fact that she used two different soil solutions in her asceptic culture techniques. Nutrient solution for the cotton seedlings was provided in

the form of a soil solution prepared by adding 3 gm of soil to 30 ml of water and autoclaving. Analysis was made both of soil solutions with plants and soil solutions without plants. Arginine, proline, lysine, serine, glycine, cystine and tyrosine were found in both the plant roots plus soil solution and the soil solution alone, but significantly larger quantities were present in the plant root plus soil solution treatment. Aspartic acid, threonine phenylalanine and leucine were found in the root exudates but not in control soil solutions.

Rovira (113) found that roots of Phalaris and tomato roots excreted larger amounts of amino acids than did those of clover. Also the balance differed between plants, e.g., glutamic acid was abundant in tomato and Phalaris root exudate but not in clover exudate while glycine was a major constituent of Phalaris exudate but not of the others.

The case for the production of growth factors or vitamins is strong. Ten vitamins have been identified in conjunction with root exudates. Bhuvaneswari and Sulochana (13) demonstrated the presence of biotin and thiamine in exudate from roots of rice, bean and clover by using the strain of Piricularia oryzae heterotrophic to thiamin and biotin. The fungus was observed to grow well in media in which plants had grown, but not in the controls. This

suggested root exudates contained thiamine or biotin or something of replacement value.

Rovira and Harris (114) were able to make B-vitamin determination on plant root excretions by using a test organism with essential growth-factor requirements. Their results showed the most active substance was biotin, although present in extremely small amounts; this was exuded in largest amounts by field peas, moderate amounts by lucerne, small amounts by white clover and tomato, and less by the grass Phalaris tuberosa. Pantothenate was next in order of activity, but only in field pea, lucerne, and tomato were the amounts significant. Very little niacin was found in the concentrate and the amount was significant only for field pea. Traces of riboflavin and thiamine were found in field pea exudate, but for the others this bio-assay was negative.

Sulochana (139) again using cotton plants grown for two weeks in two different soil solution nutrient solutions was able to demonstrate the presence of some B-vitamins in the control soil solutions as well as cotton root exudates. She used three strains of a diploid cotton and four strains of an amphidiploid cotton. Results varied between strain and soil types but generally she was able to demonstrate the presence of six B-vitamins in the root exudates; Biotin, thiamin, choline, inositol, pyridoxine and p-amino benzoic acid.

Organic acids also appear in exudates: (a) tartaric and oxalic acid in sorghum, mustard, and wheat (14, 115); (b) citric acid in mustard (14); (c) malic acid in mustard and wheat (14); (d) acetic, proprionic, butryic, valeric acids in wheat (115); and (e) citric, succinic, fumaric, glycolic acids in wheat (115).

Polyene Antibiotics

Approximately 40 antibiotics containing a conjugated polyene chromophore have been reported since 1950. They are classified into four subgroups, depending on the presence of four, five, six, or seven conjugated double bonds in the polyene moiety. The first chemical composition studies were made by Patrick (33). The fungi are the principal organisms affected because there is evidence that the polyenes bind to sterol components of fungal membranes (33). The fact that bacteria (33) do not contain sterols in their membranes and are not affected by polyenes enhances this theory.

Some polyene antibiotics are rimocidin, pimaricin, fungichromin, nystatin, tetrin, filipin and candicidin. These are all produced by Streptomyces spp. and are active against a broad range of fungi (33).

GENERAL MATERIALS AND METHODS

Selection of Streptomyces Isolates

The antibiotic producing Streptomyces isolates chosen for this work were 5 of 10 described and evaluated by Whaley (154); S-367, S-383, S-415, S-466, and S-467 (Figures 1, 2, 3, 4, 5). They were selected primarily on the basis of their performance in these studies. These organisms were stored on Gottlieb's tomato paste-oatmeal medium (48), at 5 C in a stock culture refrigerator. When it was necessary to obtain large quantities of material for inoculation or infestation, the organisms were grown in a liquid or on a solid medium from here on designated as M-14 (154). Medium 14 consists of: glucose, 2g; Bacto-yeast extract, 2g; soil extract, 100 ml (prepared by gently pouring 1 kilogram of field soil in 1 liter of tap water, filtering off the soil and bringing up to 1 liter volume with more tap water); tap water, 900 ml; microelement solution, 20 ml (prepared by dissolving $Fe(NO_3) \cdot 9H_2O$, 723.5 mg; $ZnSO_4 \cdot 7H_2O$, 439.8 mg; and $MnSO_4 \cdot 4H_2O$, 203.0 mg in glass distilled water acidified with sulfuric acid to yield a clear solution and made up to a volume of 1 liter. Each ml of solution contained 0.1 mg each of iron and zinc and 0.05 mg of manganese. All media were steam sterilized at 15 p.s.i. for 15 minutes.

Figure 1. Streptomyces isolate 367 on M-14 agar.

Figure 2. Streptomyces isolate 383 on M-14 agar.

Figure 3. Streptomyces isolate 415 on M-14 agar.

Figure 4. Streptomyces isolate 466 on M-14 agar.

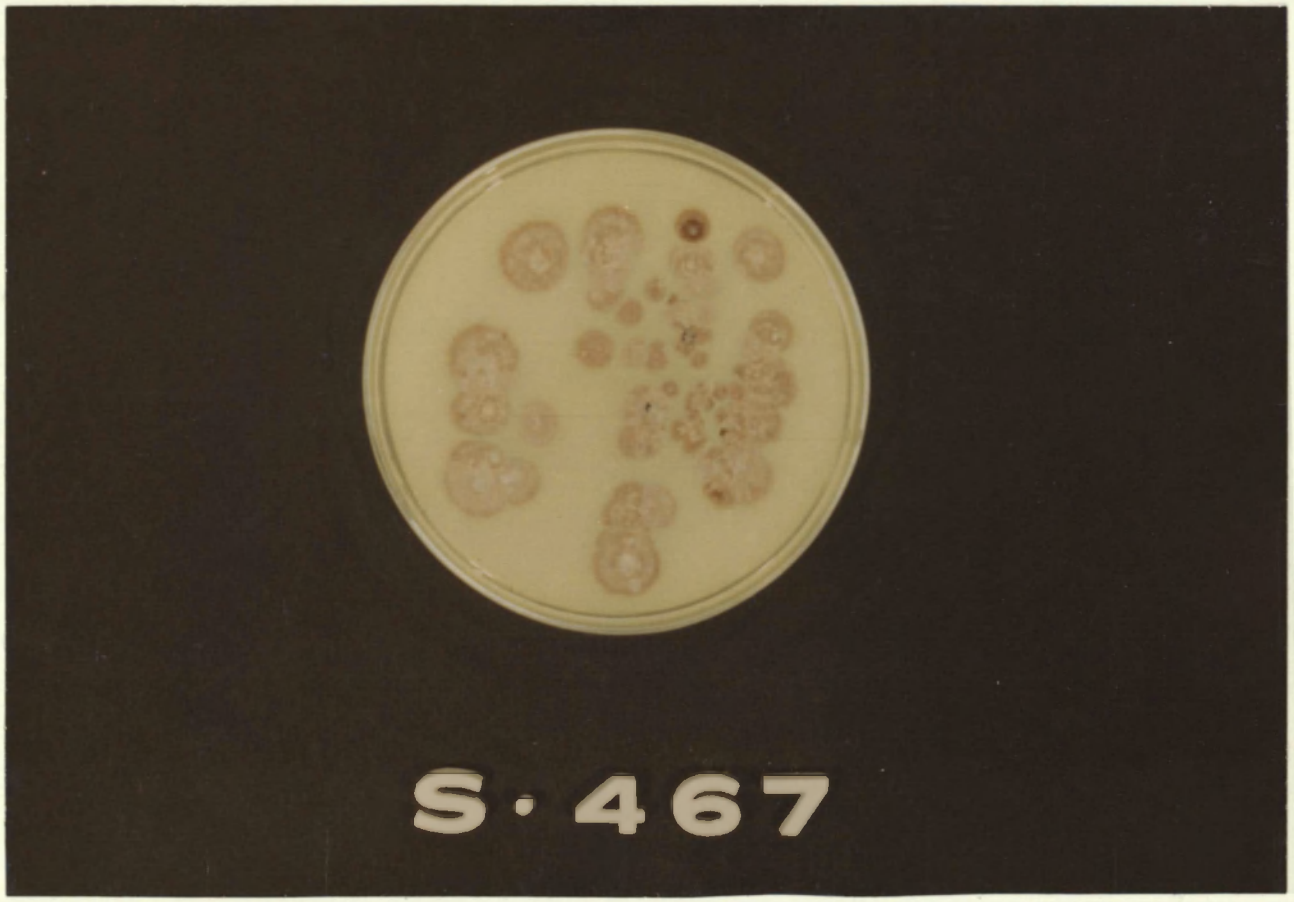

Figure 5. Streptomyces isolate 467 on M-14 agar.

Growth Chamber Experiments

An attempt was made to conduct all field related experiments under conditions that would parallel those conditions found in the field. Although no attempt is made to extrapolate the conditions found in the environmental control chamber herein described with those found under natural conditions, the implications and similarities are not thought to be too far removed.

Light intensity, humidity, air movement and temperature conditions can be controlled in the plant-growth chamber designed by Wright (162) (Figure 6). All construction and dimensional data may be found in this bulletin but a few details must be presented in this section to familiarize the reader.

The environmental control chamber consists of two separate insulated compartments. One section functions as a growth room and the other can best be described as an air "conditioning" chamber.

The growth room itself is approximately 12' x 8' x 8'. The lighting system consists of 54 power grove (double grove) fluorescent lamps and eighteen 75-watt incandescent lamps. The lamps are all on the ceiling and are divided into 3 equal modules which are linked to 3 separate time clocks, so that each module could be operated individually. In all work with this chamber the turn off and turn on times for each bay or module were staggered

Figure 6. Overall view of environmental control chamber facilities and experimental set up.

at 15-minute intervals to simulate sunrise and sunset lighting conditions. The light intensity prevailing when all illuminating modules were on was approximately 3500-3700 foot candles. The time clocks were programed for 12 hours of light and 12 hours of dark.

Air movement and distribution was provided by a squirrel cage type blower located in the air "conditioning" room immediately adjacent to the growth room. The fan produced a uniform flow of condition air at a velocity of 1/8 miles per hour, which was maintained 24 hours a day.

No humidity control was maintained due to the lack of data. Humidity present depended upon evaporation from the soil and leaf transporation.

The temperature of the growth room was regulated by electrically powered recording thermostatic control instruments designed for program control, using plastic cams cut to the desired temperature range. The temperature ranges simulated for all growth chamber experiments were those obtained from official thermograph readings taken from permanent records of the Safford Agricultural Experiment Station, Safford, Arizona (105). The data provided were those temperatures taken at a 4" depth for the month of April. To obtain desired soil temperatures it was only necessary to program the air temperatures in the chamber accordingly. The constant air movement in the chamber and

the use of special growing techniques resulted in minor differences between air and soil temperature.

The cams were cut to give the following average daily air temperatures and dirurnal fluctuations:

Daily High.....80 F Daily Low.....56 F	Ave. 67 F	1-3 Days
Daily High.....82 F Daily Low.....58 F	Ave. 69 F	4-6 Days
Daily High.....84 F Daily Low.....60 F	Ave. 71 F	7-10 Days
Daily High.....86 F Daily Low.....62 F	Ave. 73 F	11-14 Days

Temperatures tended to fluctuate 2 F once an hour because of the refrigeration units coming on and off.

All environmental control chamber experiments using infested soil were carried out using polyethylene plastic sacks (Figure 7) and specially designed flats (Figure 8). The 18 oz. sacks, sold under the brand name Whirl-Pak were purchased from the Nasco-Calstok Co., Modesto, California. They are pre-sterilized and because they are heat sealed, may be kept indefinitely. Tests by Kaufman and Williams (62) indicate their ready permeability to CO_2 and certain other gases. Water loss was determined to be less than 10% over a 6-week period. No significant reduction in fungal

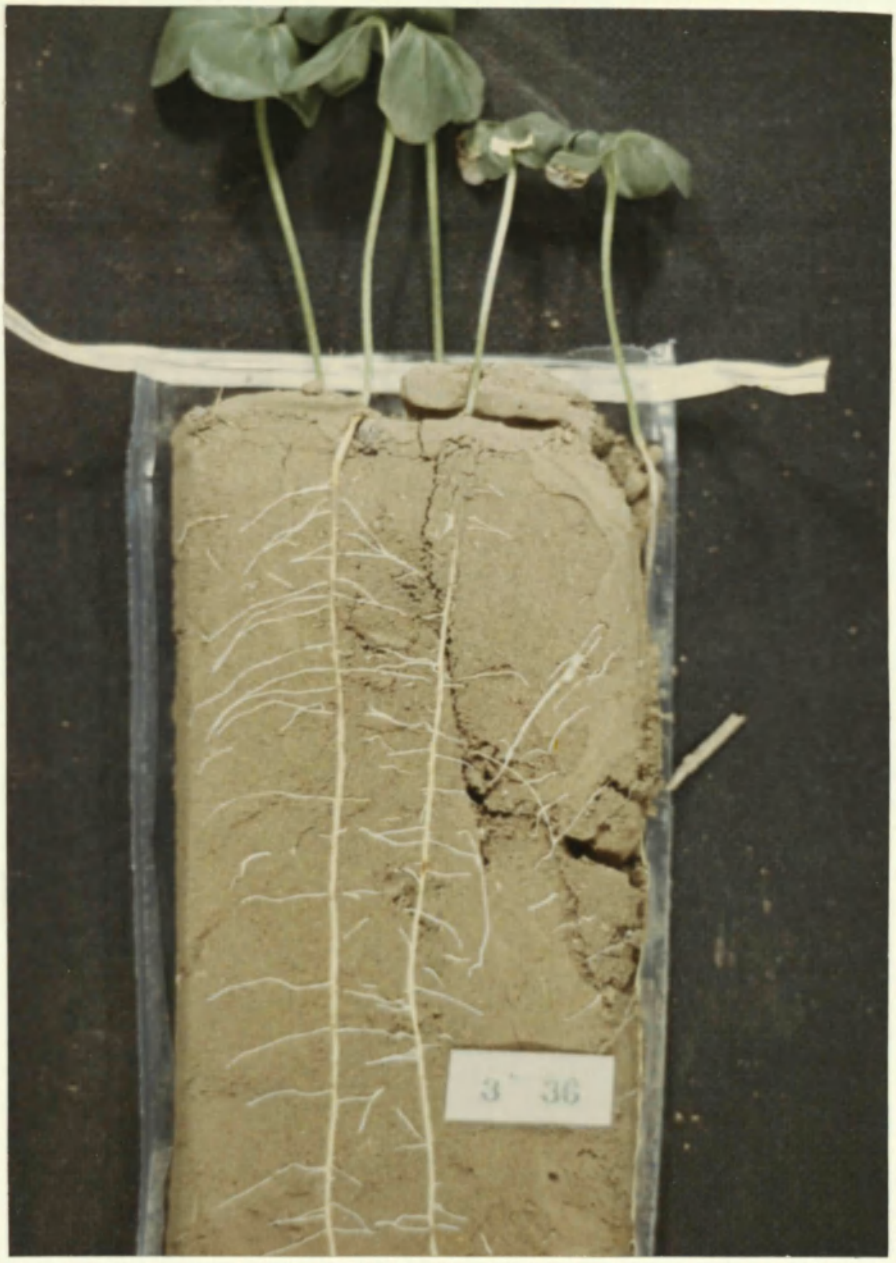

Figure 7. Close-up view of a plastic sack with plants and soil.

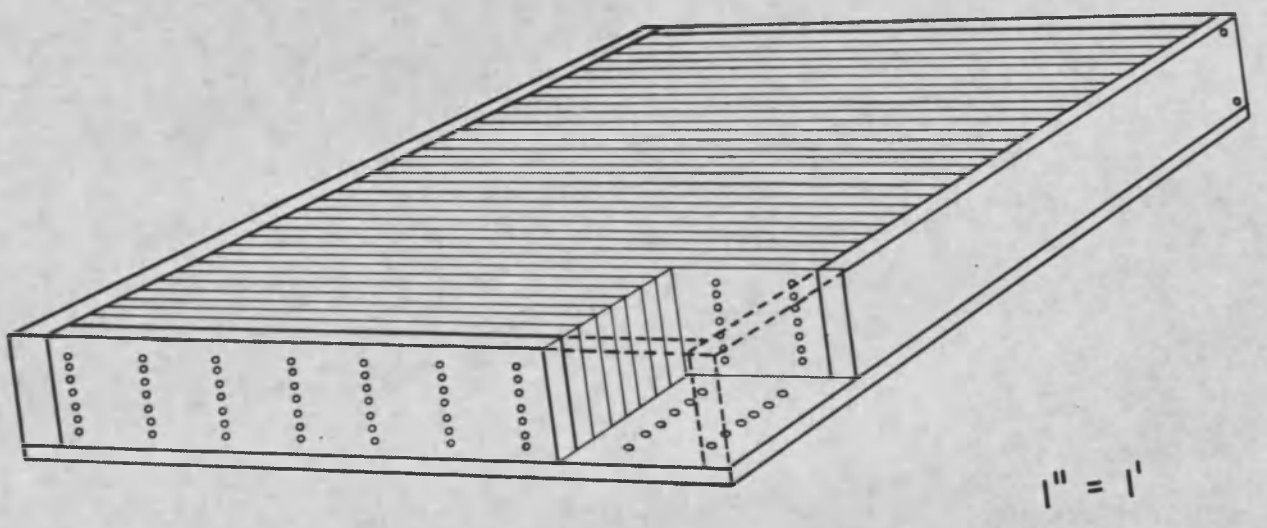

Figure 8. Scale drawing of flat used in all environmental control chamber experiments.

populations were observed between the polyethylene enclosed soils versus soils incubated in the open. The sterile sacks were only opened just prior to each test. At that time a plastic 12" irrigation tube was inserted into the sack and 400 gm of soil poured around it to fill the sack (Figure 9). The irrigation water as well as inoculum material in some cases were added through the irrigation tube. A funnel was placed at the mouth of the tube at the time of addition and as the liquid was poured through the funnel the tube was slowly withdrawn from the soil. The tubes and this method were used for three reasons: (a) it was a fast and efficient way to get even distribution of water and inoculum throughout the sack, (b) irrigation from below the soil surface tended to reduce the amount of "crusting" observed in heavy soils, and (c) the reduction in the amount of surface water would also help to reduce the growth of air contaminants.

Four flats were constructed to hold the soil filled polyethylene sacks. The perimeters of the flats were constructed of second grade Douglas fir, the floor of plywood and the dividers, which separate the growth sacks, of Masonite.

Each flat held 246 growth sacks but only 136 were used for each experiment to facilitate air flow. Air holes were drilled in the flat floor and sides to enhance air flow.

Figure 9. Close-up view of flat, sacks containing soil and plastic irrigation tubes prior to the introduction of irrigation water and inoculum.

The soil used in all experiments was obtained from a fallow wheat field on The University of Arizona Experimental Farm, Marana, Arizona. The soil texture was a clay loam with 44% sand, 32% silt, and 24% clay. Soil organic matter amounted to .77% with a subsequent carbon level of .45%. Kjeldahl nitrogen amounted to .063% and soil pH was 8.1. At the beginning of each experiment, once the soil was introduced into the sacks the moisture level was adjusted to 70% of field capacity.

All cotton used for the environmental control chamber experiments was undelinted Delta Pine Smooth Leaf obtained from a gin in Eloy, Arizona. Seed was delinted with concentrated sulfuric acid prior to their use in each experiment. This was also considered to be a good method of surface sterilization.

Plot Description and Statistical Methods

In all cases with growth chamber experiments the basic plot plan described herein was the same with the exception of type of treatment, number of treatments and the number of units found within a treatment.

Each treatment was replicated four times within the experiment. The ultimate germination and emergence of 160-192 plants was possible depending upon the particular experiment, thus giving a large population upon which to apply a statistical analysis.

All plots were randomized within the replications. All randomization was individually calculated for each replication so that none of the replications had the same arrangement. Each of the flats constituted a replication and were placed in the four corners of the chamber. The random arrangements were determined by printing numbers corresponding to predetermined treatment, on slips of paper and selecting these numbers at random from a container. As each number was selected it was assigned a flat space for that particular replication. The spaces were assigned on consecutive order so that the first number selected was placed in row 1, space 1, second number in row 1, space 2, etc.

Two types of measurements were taken: (a) a total count reading, which included only those plants in a treatment that had reached the secondary leaf stage prior to the termination of the experiment, and (b) a dry weight calculation based on these same plants. The secondary leaves were defined as those that immediately followed the cotyledons in the development of the cotton plant. The term "seedling" refers to the phase of growth and development of a cotton plant which occurs between the time the seed is introduced into irrigated soil and the appearance of the secondary leaves.

An analysis of variance and a computation of least significant difference was applied to all measurements.

Apparatus for the Collection of Root Exudates

Root exudates were collected in an apparatus originally designed by Quastel and Lees (101) and later modified by Audus (10), for the collection of soil perfusates. The system was further modified for the purpose of this work to accommodate cotton seedlings grown in asceptic culture (Figure 10).

The exudate collection unit works on the principle that water percolating over exposed root systems will wash away any chemical moieties exuded from them, and these washings may be collected in a reservoir directly beneath the growing chamber. The units are almost completely self-contained with the exception of sterile air which is used to circulate the liquid in the system.

The growing chambers for the seedlings were made from 11" valveless chromatography columns. A small plug of glass wool was inserted in the tapered end of the column and covered with approximately 250 g of 6 mm glass beads which filled the column about 2/3 full. A two-holed number 8 stopper sealed the top of the column and also provided for a liquid intake and an air outflow system. The lower tapered part of the column was inserted through a hole in a number 4 stopper which entered into and sealed the reservoir.

The reservoir in which the exudates were collected and which also supplied a source of liquid for percolating

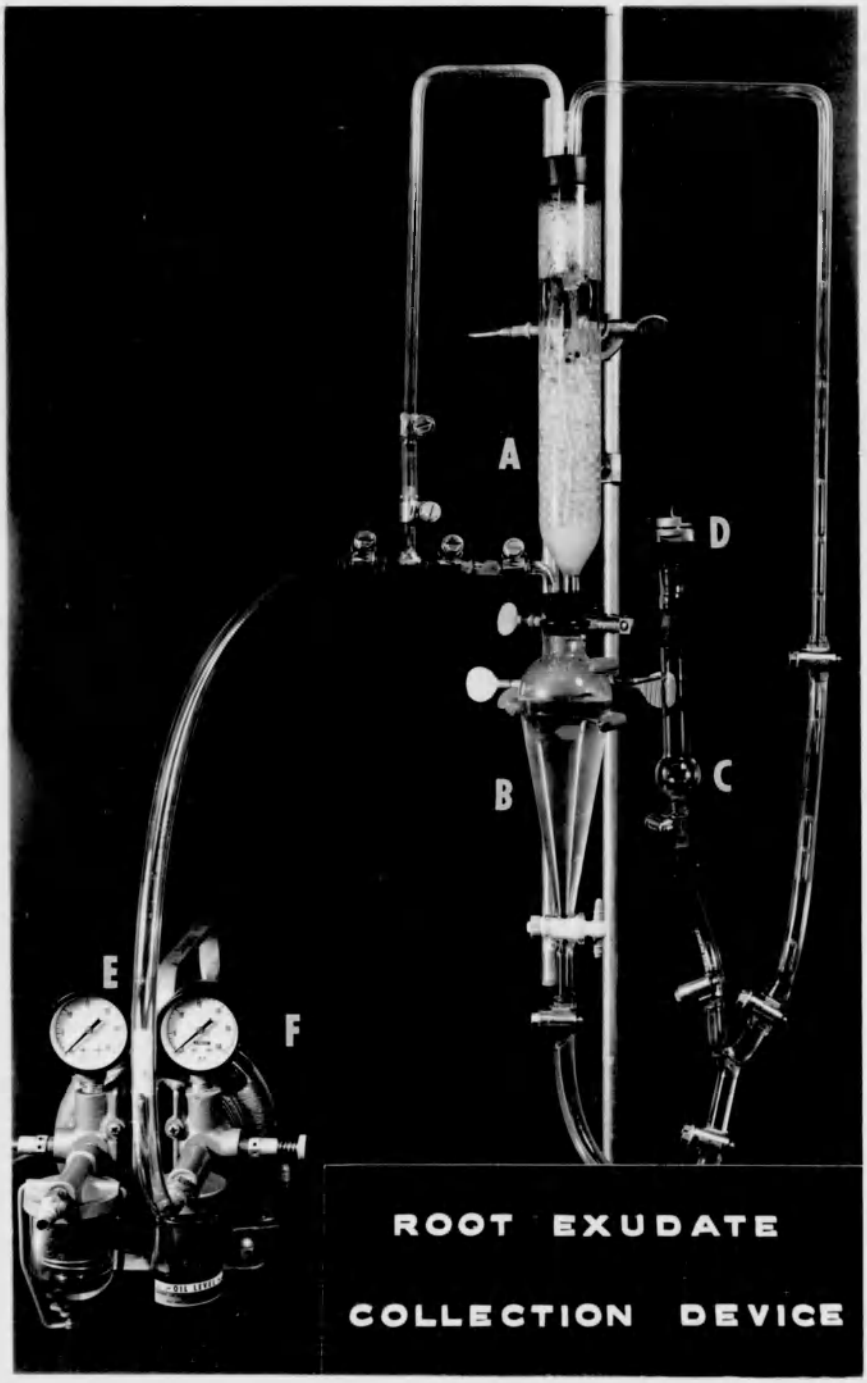

Figure 10. Modified soil perfusion apparatus used to collect cotton seedling root exudates. (A) Growing chamber. (B) Water and exudate reservoir. (C) Sampling tube. (D) Sweeny filter holder with .45 μ filter. (E) Cotton plug. (F) Vacuum pump.

was made of a 125 ml separatory funnel. The stopper which sealed the top of the funnel and provided an entrance for the exudates also was drilled and fitted with a glass tube to provide a pressure deficit when vacuum was applied. The funnel spout was in turn connected to plastic and glass tubing which was linked with the top of the growth chamber to provide a constant flow of liquid.

A drying tube was used as an air intake as well as sampling reservoir in this system. A Sweeny micro-syringe filter holder fitted with .45 millipore filter ensured a flow of sterile fresh air when a vacuum was created in the system. The filter, fitted to a number 2 stopper could be removed, under sterile conditions, for sampling at any time. All connection of strategic parts was made with rubber stoppers, 3/8 inch Tygon tubing and 8 mm glass tubing. All connections were secured with ordinary 1/4 inch hose clamps. Vacuum was provided by a 1/6 H.P. vacuum and pressure pump.

The units were supported by a ring stand and support bar set up so the complete unit could be removed and autoclaved.

All systems were autoclaved for 30 min. at 121 C prior to the introduction of seeds and liquid.

The apparatus described was set up in the laboratory under light and temperatures prevailing there. Light was provided by fluorescent tubes and the temperatures

ranged from 20-25 C. All systems were run approximately 4 hours per day.

SURVEY OF ORGANISMS ASSOCIATED WITH THE DAMPING OFF OF COTTON

Materials and Methods

A survey was made of the organisms found to be associated with damping off symptoms of field cotton. Infected plant material and associated soil were collected from seven of the major cotton growing areas of Southern Arizona. This entailed visiting or receiving mailed samples from a number of fields in the Yuma, Marana, Phoenix, Florence, Eloy, Willcox and Kansas Settlement areas. In instances where soil was collected for dilution series, the infected plants plus surrounding soil were removed with the aid of a 1" Hoffer soil probe. Upon removal the samples were placed in polyethylene bags and sealed with nursery ties. If the plants and soil could not be processed immediately upon returning to the laboratory, they were placed in a refrigerator at 5 C.

Two methods were selected to isolate organisms associated with infected seedlings: (a) a standard soil dilution described by Johnston et al. (58) utilizing the soil removed by the probe, and (b) the plating out of surface sterilized plant material.

Procedure number one included removal of the rhizosphere soil by dipping the roots in sterile distilled

water and combining the sediment with the loosely attached soil previously removed. Three modifications in the technique as described by Johnston et al. (58) were undertaken to improve the results:

1. A combustion or deflagration spoon was substituted for the pipette normally used. This is similar to the apparatus described by Menzies (85). This dipper design, superior to the pipetting method, allowed for a quick, neat transfer with very little surface upon which to leave a liquid film containing organisms.
2. The soil water suspension was allowed to agitate on a reciprocal shaker for 30 minutes at approximately 100 excursions per minute. This type of agitation was used in place of the Burrell wrist action shaker suggested.
3. The dilutions were added to media which had been solidified for 2-3 days.

Paharia and Kommedahl (90) found that this method resulted in 2-3 times as many fungal colonies per gram of oven dried soil as compared to the standard soil dilution plate methods which consisted of adding the sample as the cooled agar was poured.

Each dilution was performed upon a 2.5 g aliquot of soil removed from the combined soil of five plants. A total of 50 soil dilutions was made on samples taken from

an average of seven locations per area mentioned previously. In most instances this constituted seven different, widespread, separately owned and managed fields. It was felt this type of collection procedure would give an excellent cross-section of all types of cultural practices which might influence microbial populations.

Procedure number two used for the isolations of the soil-borne organisms was the plating out of diseased plant material. This was carried out in much the same manner as described by Riker and Riker (108). The cotton plants collected were all selected from any stage prior to germination up to the appearance of the secondary leaves.

The individual seedlings were freed of all soil particles in the fashion mentioned in the first isolation method, with the roots finally being deposited in sterile distilled water. The portion of the plant consisting of the cotyledons and the part of the hypocotyl protruding above ground level was removed and discarded. The rest of the hypocotyl including the root system was saved and either one of two different surface sterilents, M_gCl_2 1:1000 and .53% sodium hypochlorite were applied at 1, 2, 3, 4 or 5 minutes each.

Three plants were immersed per time span per chemical treatment so that a minimum of 36 plants was required to test the effectiveness of the time intervals and disinfectant. This treatment plan was devised prior

to collecting plant samples from the field so that a minimum of 36 plants was selected from an area approximately 3 ft.^{2} This selection of closely oriented plants was used to assure a representative sample.

After surface sterilization, the hypocotyl parts were rinsed in sterile distilled water for 30 seconds and then placed on a fresh paper towel for sectioning. The plant material was chopped into 1 mm sections with a sterile scalpel and transferred to the medium with sterile forceps.

All soil dilutions and surface sterilized plant tissue were plated out in plastic plates containing 5 different media.

Potato Sucrose Agar: Two hundred fifty g of washed but unpeeled White Rose potatoes were boiled in 1 liter of tap water on a hot plate for 1 hour and the liquid filtered through a single layer of muslin. The volume was then brought up to 1 liter with more tap water and 15 g of sucrose and 20 g of Difco agar added to complete the formula.

Glucose Yeast Extract: Glucose, 10.0 g; yeast extract, 2.0 g; microelement solution, 2.0 ml; agar, 20.0 g; and distilled water, 1 liter.

RB-M2: Glucose, 10.0 g; yeast extract, 0.5 g; peptone, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; K_2HPO_4, 0.5 g; KH_2PO_4,

0.5 g; rose bengal, 0.5 g; chloromycetin, 0.03 g; agar, 20 g; and distilled water, 1 liter (144).

V-8 Dextrose Yeast Extract Agar: V-8 juice, 200 ml; glucose, 2.0 g; yeast extract, 2.0 g; $CaCO_3$, 1.0 g; PCNB, 0.5 g of active ingredient; nystatin, 0.03 g; chloromycetin, 0.1 g; chlorotetracycline, 0.002 g; oxgall, 1.0 g; agar, 20.0 g; and distilled water, 800 ml (91).

Czapeks Medium: Sucrose, 30.0 g; $NaNO_3$, 3.0 g; K_2HPO_4, 1.0 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCL, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; agar, 20.0 g; and distilled water, 1 liter.

All plates were incubated at 24 C. Inspections were made daily from 24 hours after inoculation until the plates became desiccated. Fungal colonies were removed as they appeared and transferred to fresh plates of the same medium on which they were discovered. To obtain pure culture, 3-4 sub-cultures were made consecutively from the original transfer. Once pure cultures were obtained they were placed on glucose yeast extract agar slants in screw cap tubes and stored at 5 C in a stock culture refrigerator.

Results and Discussion

A total of 150 different fungal isolates were found to be associated with seedling field cotton showing symptoms of damping off. The bulk of the isolations made were Phycomycetes, Fusarium, Acrostalagmus and Rhizoctonia.

Twenty-four isolates of Rhizoctonia were secured, with at least one coming from each area visited.

Only two isolates of Thielaviopsis were obtained, one from the Phoenix area and one from Willcox. Two other isolates were secured from New Mexico. These had been isolated from infected cotton seedlings.

Fusarium, Acrostalagmus, and some Phycomycetes were obtained from almost every field visited.

Species of other genera isolated in moderate numbers were Aspergillus, Penicillium, Cephalosporium, Helminthosporium, Curvularia, and Trichoderma.

This survey was made in the spring of 1966, and the organisms isolated were thought to be somewhat representative of important soil-borne fungi found in southern Arizona associated with damped off cotton seedlings. Notable exception to this was the fact that no Phymotrichum omnivorum, Verticillium sp., or Pythium spp. were identified from all the isolates collected. It is not significant that Phymotrichum was not detected, because there are no reports of its being associated with seedling disease of cotton and there are no publications concerning isolation from soil dilutions. It is surprising that no Verticillium or Pythium were identified, because both have been isolated quite consistently from damped off cotton by Boyle (18) in previous years. It is difficult to explain the difference in observations because similar techniques

and media were used in the isolations. The answer may have been in the author's inability to make positive identification. None of the Phycomycetes could be identified to the genus Pythium.

Little work has been done with Thielaviopsis as a cotton seedling pathogen in Arizona, although some work by Maier and Staffeldt (76) in New Mexico indicates its importance. Damage to mature cotton by this organism in Arizona was reported by Presley (99) as early as 1942. It was thought that because New Mexico and California both report Thielaviopsis causing serious problems, the fungus might be more important in Arizona than realized.

All Fusarium isolates seemed to fall into two morphological groups: those that produced only microconidia and those that produced an abundance of macroconidia and chlamydospores. No attempt was made to classify to species or forma. These characteristics of the members of the two morphological groups were consistent on both potato sucrose and glucose yeast extract.

The Rhizoctonia isolates obtained varied widely in appearance when grown on potato sucrose agar. The variations appeared to be due in part to where and when sclerotia were formed on the agar. In some cases there was indiscriminate production which usually took place early in colonization of the agar. In others, a single ring of sclerotia was produced at varying distances from the

initial inoculum, while some only produced sclerotia when it appeared that a majority of nutrients had been utilized and the agar was becoming desiccated. No attempt was made to correlate these characteristics with pathogenicity.

It is a common practice in Arizona to use PCNB (pentachloronitrobenzene) for treatment of cotton seeds to prevent damping off. Some work by Elsaid and Sinclair (37) indicates that some isolates of Rhizoctonia show more tolerance to this fungicide than others and in plate culture will actually induce sclerotial production. They showed that the fungus could obtain greater levels of tolerance if exposed to gradual increases in concentrations of this chemical. This ability of some isolates to adapt to increasing concentrations of PCNB may lead to the selection of a strain of Rhizoctonia which is extremely pathogenic and because of the increased sclerotial formation be able to survive adverse conditions which might ordinarily reduce populations.

Acrostalagmus, Helminthosporium, Cephalosporium, Aspergillus, Penicillium and Trichoderma were all generally isolated from a number of areas and showed no preference to geographical location.

The type of damage observed on most diseased seedlings did not vary noticeably from plant to plant or area to area, so that no one particular symptom could be attributed to any one organism.

The main diagnostic symptom of damping-off caused by Rhizoctonia has been considered to be the presence of sunken lesions on the hypocotyl at the soil line and descriptively termed "sore shin." In this survey, this sore shin effect was observed only occasionally and Rhizoctonia was often isolated from plant material with no such symptoms. The type of damage most often observed was a general shrivelling and discoloration of the whole root system, with subsequent reduction in the number of secondary roots and eventual death of the plant.

EVALUATION OF ISOLATES FOR PATHOGENICITY

Materials and Methods

Thirteen fungal isolates were selected for evaluation of pathogenicity under conditions described in the general materials and methods. Eleven isolates were selected from the pathogen survey and two were acquired from New Mexico. The isolates used and pertinent information concerning them are listed in Table 1.

Inoculum to be used in pathogenicity tests was prepared by growing the isolates in 150 ml of potato sucrose broth in 250 ml Erlenmeyer flasks on a reciprocating shaker at 80 excursions per minute for two weeks at 20-25 C. Inoculation of the cultures was accomplished by transferring, asceptically to the culture flasks, plugs cut with a number 8 cork borer from potato sucrose agar cultures of the isolate.

After two weeks the fungal mats were separated from the culture broth by filtering through muslin, and rinsing with sterile distilled water. The fungus mat was homogenized with sterile distilled water in a Waring blender at low speed for 30 seconds, and the homogenate brought to a volume of 1200 ml with sterile distilled water. One hundred twenty milliliters of this suspension was introduced into each soil filled sack via the plastic irrigation

Table 1. Information concerning fungal isolates used in the pathogenicity evaluation experiment.

Isolate	Record No.	Isolated from	Misc. information
Rhizoctonia isol.	R-8	Yuma, Ariz. Snyder Ranch	Isolated from plant material on glucose yeast extract agar.
Rhizoctonia isol.	R-10	Marana, Ariz. Pl. Path. Dept. plots	Isolated from plant material on potato sucrose agar.
Rhizoctonia isol.	R-11	Tucson, Ariz. Sewage disposal plant's farm	Isolated from plant material on potato sucrose agar.
Rhizoctonia isol.	R-14	Phoenix, Ariz. Roswell & Rowel Ranch	Isolated from plant material on potato sucrose agar.
Rhizoctonia isol.	R-21	Stewart, Ariz.	Isolated from plant material on V-8 juice yeast extract agar.
Thielaviopsis sp.	T-1	New Mexico John Booth	----------
Thielaviopsis sp.	T-2	New Mexico John Booth	----------
Thielaviopsis sp.	T-4	Stewart, Ariz.	Isolated from plant material on glucose yeast extract agar.

Table 1.--Continued

Fusarium sp.	84	Stewart, Ariz.	Isolated from plant material on glucose yeast extract agar. Microconidia mainly produced on GYEA. Few macroconidia are straight with indistinct septa.
Fusarium sp.	59	Phoenix, Ariz. Sutton Ranch	Isolated from plant material on potato sucrose agar. Majority of spores macroconidia with prominent chlamydospore formation.
Helminthosporium sp.	124	Marana, Ariz. Pl. Path. Dept. test plots	Isolated from plant material.
Aspergillus sp.	89	Stewart, Ariz.	Isolated from plant material.
Acrostalagmus sp.	66	Marana, Ariz. Pl. Path. Dept. test plots	Isolated from plant material on potato sucrose agar.

tubes. One flask of fungus suspension constituted the inoculum for one replication. The dry weight of fungal material used per replication-treatment ranged from 1.5 g in the Thielaviopsis cultures to 2.5 g in the Rhizoctonia cultures.

After adding inoculum to the soil in the sacks, four freshly delinted cotton seeds were deposited on the soil surface in each sack, and just covered with soil. The experiment was then allowed to run for 14 days.

A total of 14 treatments replicated four times was included in this experiment representing 13 fungal isolates and one uninfested control. Forty growth sacks were used per treatment, or 10 sacks per replication. A sum of 160 plants per treatment was possible if 100% germination was achieved.

Results and Discussion

The results of the pathogenicity tests are presented in the form of raw data and pertinent statistical values in Tables 2 and 3, and in a graphical presentation in Figure 11.

Only three isolates were responsible for any significant damping off in this experiment, and they were Rhizoctonia isolates R-14, R-8, and R-21. These three

Table 2. Results of pathogenicity evaluation in mean and total numbers of cotton plants reaching the secondary leaf stage.

Treatments	Total number of plants reaching secondary leaf stage in each replication				Treatment totals	Treatment means
	Rep. 1	Rep. 2	Rep. 3	Rep. 4		
Uninfested control	16	15	15	19	65	16.25[a]
Thielaviopsis (T-1)	18	13	16	15	62	15.50
Thielaviopsis (T-2)	16	15	17	13	61	15.25
Thielaviopsis (T-4)	19	18	18	20	75	18.75
Rhizoctonia (R-8)	4	1	2	0	7	1.75
Rhizoctonia (R-10)	20	16	16	9	61	15.25
Rhizoctonia (R-11)	15	14	13	15	57	14.25
Rhizoctonia (R-14)	3	2	15	19	39	9.75
Rhizoctonia (R-21)	0	0	0	0	0	0.00
Fusarium (59)	15	17	17	12	61	15.25
Fusarium (84)	20	16	16	15	67	16.75
Acrostalagmus (66)	17	19	17	18	72	17.75
Aspergillus (89)	17	15	16	13	61	15.25
Helminthosporium (124)	19	15	17	17	68	17.00
Replication totals	174	176	195	185		
Replication means	12.13	12.50	13.92	13.21		

Replication F value--.799

Analysis of variance--

Treatment F value--14.14

Least significant difference--4.32--at the 5% level.

[a]Mean number of plants based on the total number of plants in each treatment divided by the number of replications.

Table 3. Results of pathogenicity evaluation in mean and total dry weights (in grams) of cotton plants reaching the secondary leaf stage.

Treatments	Dry weight of plants reaching secondary leaf stage in each replication				Treatment totals	Treatment means
	Rep. 1	Rep. 2	Rep. 3	Rep. 4		
Uninfested control	4.08[a]	5.92	4.20	4.30	18.50	4.63[b]
Thielaviopsis (T-1)	4.11	4.04	4.61	3.14	15.90	3.98
Thielaviopsis (T-2)	3.06	3.02	4.04	2.39	12.51	3.13
Thielaviopsis (T-4)	3.80	4.21	4.64	3.06	15.71	3.93
Rhizoctonia (R-8)	.61	.62	.49	.14	1.86	.47
Rhizoctonia (R-10)	3.15	4.90	3.42	1.58	13.05	3.27
Rhizoctonia (R-11)	3.31	3.62	2.99	2.02	11.94	2.99
Rhizoctonia (R-14)	1.05	.45	3.01	3.33	7.84	1.97
Rhizoctonia (R-21)	.08	.06	.12	.11	.37	.09
Fusarium (59)	3.72	2.74	3.70	2.44	12.60	3.15
Fusarium (84)	4.25	3.64	3.12	2.76	13.77	3.44
Acrostalagmus (66)	2.78	5.25	3.92	5.80	17.75	4.44
Aspergillus (89)	3.75	3.07	3.21	2.70	12.73	3.18
Helminthosporium (124)	4.38	2.92	3.26	2.65	13.21	3.30
Replication totals	44.73	36.42	44.46	42.13		
Replication means	3.19	2.60	3.18	3.00		

Analysis of variance--Replication F value--3.52; Treatment F value--12.11

Least significant difference--1.134--5% level.

[a]Dry weight in grams of total number of plants reaching secondary leaf stage.

[b]Mean dry weight based on the total dry weight for the treatment divided by the number of replications.

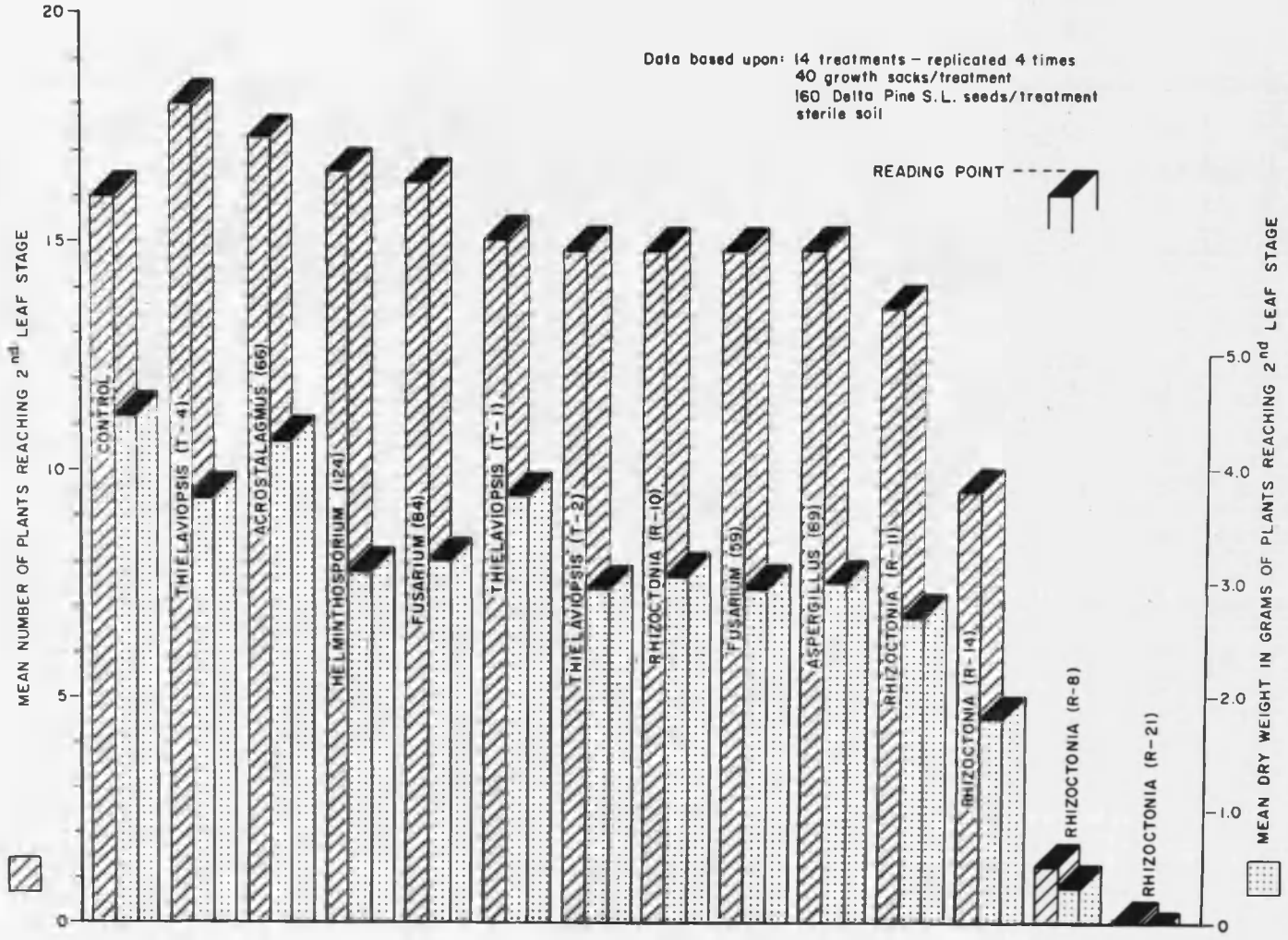

Figure 11. Effect of 13 fungal isolates secured from "damped-off" field grown cotton on the growth and development of cotton seedlings under controlled environmental conditions.

treatments showed a significantly lower number of plants than the uninfested controls. The same difference held true for the dry weight measurements.

This evaluation was undertaken for two reasons: (a) to select a pathogen which was particularly pathogenic and would guarantee consistent results when cotton was grown in soil containing it, and (b) to determine whether or not Rhizoctonia is the main damping off organism in southern Arizona.

The analysis of variance for the total number measurements indicated no significant difference among replications at the 5% level, while a significant difference among treatments was observed at the .5% level. A computation of least significant difference indicated that there was no significant difference between the mean number of plants found in the uninfested control and the mean number observed in treatments with Thielaviopsis isolates T-1, T-2 and T-4; Rhizoctonia isolates, R-10 and R-11; Fusarium isolates, 59 and 84; Acrostalagmus isolate 66; Helminthosporium isolate 124; and Aspergillus isolate 89. Rhizoctonia isolates R-8, R-14 and R-21 were all significantly different from the control treatment. In comparison of these three, R-14 was significantly different from R-8 and R-21, but the latter two were not different from each other.

The analysis of variance for the dry weight measurements of those plants reaching the secondary leaf stage, indicated no significant differences among replications at the 2.5% level while treatments were significantly different at the .5% level.

Computation of least significant difference indicated somewhat different relationships than observed in the total count measurements. No significant difference was observed between the uninfested control and Thielaviopsis, isolates T-1 and T-4; Acrostalagmus, isolate 66 and Fusarium, isolate 84, but there were significant differences between the control and each of the others. The effects shown by Rhizoctonia, isolates R-10 and R-11; Helminthosporium, isolate 124; Thielaviopsis, isolate T-2; Fusarium, isolate 59; and Aspergillus, isolate 89 were considered statistically to be the same, but again not the same as the control. The influence of Rhizoctonia isolates, R-8, R-14 and R-21 on dry weight was significantly different from the control as well as different from the group just mentioned, which showed only a moderate amount of damage.

It is observed that there are only two major statistical levels of damage shown by the total number counts, but three levels appear in the dry weight measurements. This may indicate damage of the type which is insufficient to kill the plant or reduce vigor but may

prove damaging in the long term growth and development of the plant.

Only three isolates showed any significant damping off damage and these were all of Rhizoctonia. Rhizoctonia isolate, R-21, from the Willcox area, was responsible for the highest percentage of damage. It is interesting to note that this organism was highly pathogenic under environmental conditions probably somewhat different from those found in a Willcox field situation. Rhizoctonia isolate, R-8, was responsible for damage not significantly different from that caused by R-21. This R-8 came from Yuma, Arizona, where environmental conditions differ from those under which it was tested. Some explanation may lie with the variety of cotton, and its tolerance or susceptibility to these isolates.

Isolate R-14 showed a high degree of pathogenicity, but the damage was significantly less from that found in the R-21 and R-8 isolates. The R-14 isolate came from the Phoenix area, which plants a number of varieties, Stoneville 7A, Hopicala, and Delta Pine.

The other two isolates, R-10 and R-11, came from Marana and Tucson, respectively. The environmental conditions simulated in the chamber could probably be considered closer to those found in the natural habitat of these two isolates than found in the surroundings from which the more pathogenic cultures were obtained.

The three Thielaviopsis isolates T-1, T-2, and T-4 did not show the damping off capabilities indicated by Maier and Staffeldt of New Mexico (76). Isolates T-1 and T-2 were isolated from New Mexico soils. Environmental conditions under which the test was conducted may not have been favorable to growth and/or infection by these isolates. Marked reductions in dry weight occurred when compared with the total number count. Root examinations revealed a lack of secondary roots and a blackening of the tap root, but reduction in total numbers of plants was not significantly different from that found in the uninfested control.

The response from the Fusarium, Helminthosporium, Aspergillus, and Acrostalagmus was not marked except for the root discoloration and the possible reduction of the dry weight versus total numbers in the Helminthosporium and Fusarium, isolate 84 treatments.

It was impossible to associate a specific organism with a particular type of damage, with the possible exception of isolates R-8 and R-21. Some discoloration of the root systems was present in all treatments. The roots of plants in the controls were sometimes darkened but not on the scale of the infested treatments. High inoculum potential and lack of competition with other organisms could account for the damage observed.

Rhizoctonia isolates R-8 and R-21 gave such severe hypocotyl damage that they could probably be separated from the others on the basis of overall symptoms. Both isolates very rarely allowed cotton plants to reach the expanded cotyledon stage, and the presence of marked necrosis was noted.

All organisms incorporated into the soil in this study were re-isolated, upon termination of the experiment, from plant material or from soil dilutions.

EVALUATION OF *STREPTOMYCES* ISOLATES FOR CONTROL OF DAMPING OFF

Materials and Methods

Five *Streptomyces* isolates, S-367, S-383, S-415, S-466 and S-467, were tested separately and in combination for their ability to control *Rhizoctonia* "damping off" of cotton seedlings.

This test was conducted under the same light, temperature, soil water and general growth chamber conditions indicated in the general materials and methods.

All *Streptomyces* isolates were tested against *Rhizoctonia* isolate R-21, because of its high pathogenicity rating in the previous experiment.

The *Streptomyces* isolates were cultured in 150 ml of M-14 broth in 250 ml Erlenmeyer flasks with cotton stoppers covered with aluminum foil at 20-25 C on a reciprocal shaker at approximately 100 excursions per minute, under alternating light and dark conditions. All isolates were cultured for a period of two weeks.

The *Rhizoctonia* isolate, R-21, was cultured in the same way except that potato sucrose broth was used as a growth medium.

In preparation for soil infestation with the *Streptomyces* isolates, the broth was separated from the

fungal mat by filtration through two layers of muslin. The mat was rinsed with sterile distilled water and lightly macerated with a stainless steel spatula. The macerated mat was then incorporated into soil with the spatula. One flask of each isolate constituted the treatment for the soil in one replication. In the treatment where all isolates were combined, five flasks constituted a replication treatment. The Streptomycetes were added to soil one week prior to the introduction of Rhizoctonia.

The Rhizoctonia cultures were prepared in much the same manner except the mats were homogenized in a Waring blender after filtration and washing. In this case the mycelial mat from one culture constituted a replication treatment. The Rhizoctonia homogenate and irrigation water were added together.

In this experiment six seeds of the Delta Pine Smooth Leaf variety were placed in the soil of each sack of unsterilized soil. The plot design was set up so that there were 14 treatments, replicated four times. A total of 32 sacks constituted a treatment. All results were subjected to an analysis of variance and least significant difference.

Results and Discussion

The results of this evaluation are presented in the form of raw data and pertinent statistical values in

Tables 4 and 5, and in a graphical presentation in Figure 12.

Data were collected on the total number of plants reaching the secondary leaf stage and the subsequent dry weight of them, as in the previous experiment.

The analysis of variance for both total count and dry weight measurements indicates no significant differences in the over-all replication results. The treatment F values for both types of data showed a significant difference among treatments.

The computation of least significant difference for the replication means of the total number observations gives some indication that some Streptomyces spp. are capable of influencing Rhizoctonia damping off, at least under the conditions prevailing in this test. Three treatments consisting of Streptomyces isolates and Rhizoctonia were not significantly different from the untreated control. Two isolates, S-467 and S-383 and the combination of all five isolates were apparently able to reduce the amount of damping off of the seedling cotton. The mean number of plants reaching the secondary leaf stage in these treatments was significantly different from the number reaching this stage in the Rhizoctonia control. Two other Streptomyces isolates, S-466 and S-367, gave mean counts which were significantly different from the untreated control, but were also significantly different from those found in

Table 4. Results of the study evaluating 5 Streptomyces isolates for the control of Rhizoctonia (R-21) damping off in mean and total numbers of cotton plants reaching the secondary leaf stage.

Treatments	Total number of plants reaching secondary leaf stage in each replication				Treatment total	Treatment mean
	Rep. 1	Rep. 2	Rep. 3	Rep. 4		
Uninfested control	36	36	30	39	142	35.50[a]
R-21	15	15	13	9	52	13.00
S-466	36	33	36	36	141	35.25
S-466 + R-21	32	35	28	11	106	26.50
S-467	36	38	36	38	148	37.00
S-467 + R-21	28	43	24	23	118	29.50
S-383	24	39	37	27	127	31.75
S-383 + R-21	39	29	24	17	109	27.75
S-415	26	26	18	19	89	22.25
S-415 + R-21	12	23	14	13	62	15.50
S-367	26	25	27	27	105	26.25
S-367 + R-21	26	28	28	12	94	23.50
All isolates	25	27	28	26	106	26.50
All isolates + R-21	31	27	28	25	111	27.75
Replication totals	393	424	322	371		
Replication means	28.07	30.28	23.00	26.50		

Analysis of variance--Replication F value--4.936
Treatment F value--7.330
Least significant difference--7.43--5% table

[a]Mean number of plants based on the total number of plants in the treatment divided by the number of replications.

Table 5. Results of the study evaluating 5 Streptomyces isolates for the control of Rhizoctonia (R-21) damping off in mean and total dry weights (in grams) of cotton plants reaching the secondary leaf stage.

Treatments	Dry weight (in grams) of plants reaching the secondary leaf stage in each replication				Treatment total	Treatment mean
	Rep. 1	Rep. 2	Rep. 3	Rep. 4		
Uninfested control	4.80[a]	4.17	3.02	4.93	16.92	4.23[b]
R-21	1.71	2.54	.94	.78	5.97	1.49
S-466	3.73	3.78	3.86	4.19	15.59	3.90
S-466 + R-21	4.02	4.11	3.33	1.39	12.86	3.21
S-467	4.42	5.09	3.95	5.28	18.75	4.69
S-467 + R-21	3.45	4.65	2.18	2.76	13.04	3.26
S-383	2.78	4.68	4.38	5.00	16.85	4.21
S-383 + R-21	4.87	3.42	2.31	2.86	13.45	3.36
S-415	3.83	3.38	2.09	2.86	12.16	3.04
S-415 + R-21	2.18	1.76	1.76	1.37	7.08	1.78
S-367	3.16	3.45	3.06	3.96	13.62	3.40
S-367 + R-21	3.20	2.69	3.18	1.76	10.83	2.71
All isolates	3.16	3.01	2.64	3.45	12.27	3.07
All isolates + R-21	3.87	1.84	2.80	2.55	11.03	2.76
Replication totals	49.20	48.59	39.47	43.15		
Replication means	3.51	3.47	2.82	3.08		

Analysis of variance--Replication F value--2.718; Treatment F value--5.619

Least significant difference--1.084--5% table

[a]Dry weight in grams of total number of plants reaching secondary leaf stage.

[b]Mean dry weight based on the total dry weight for the treatment divided by the number of replications.

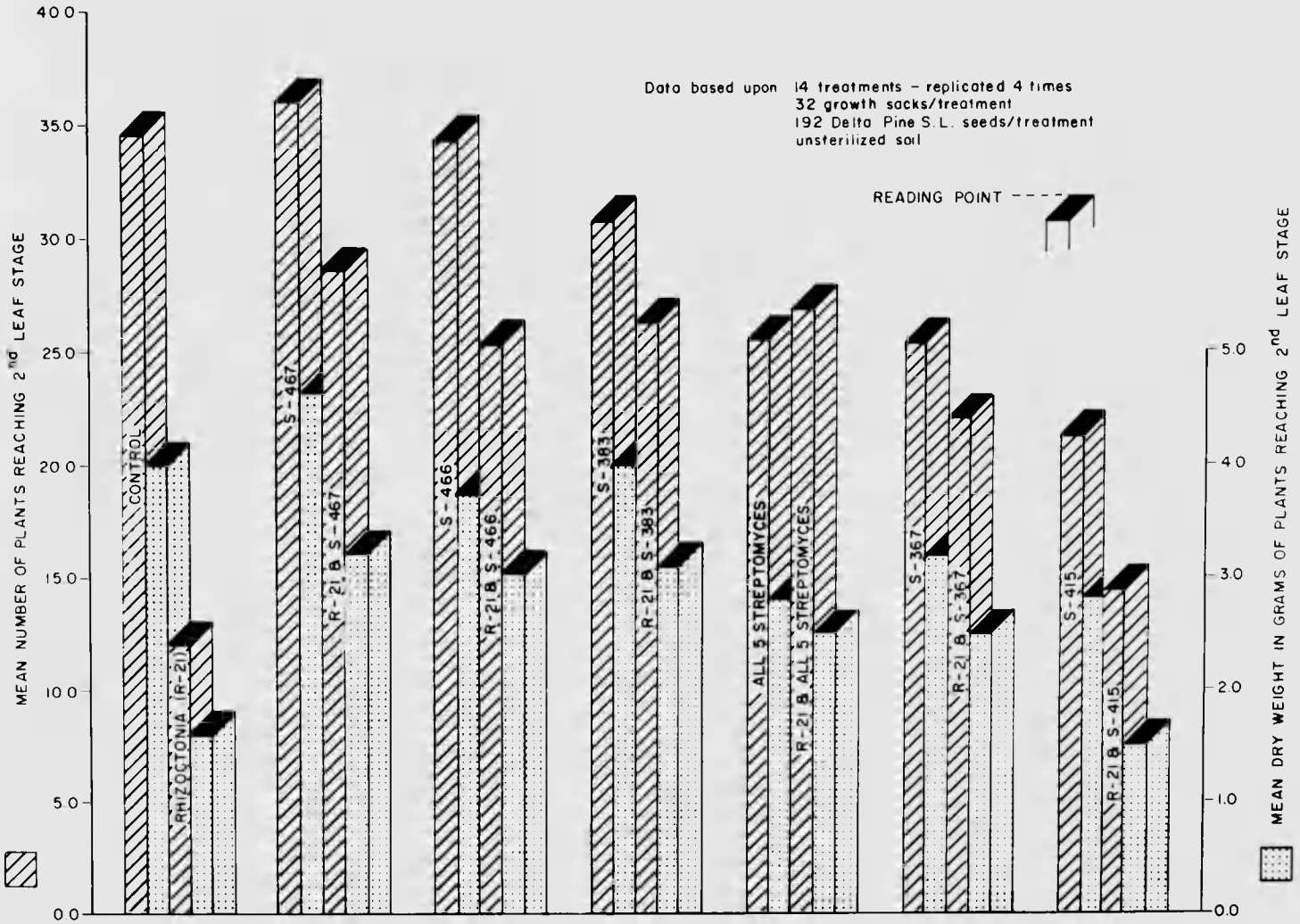

Figure 12. Influence of 5 antibiotic producing Streptomyces spp. in combination and separately, on Rhizoctonia (R-21) "damping off" of cotton seedlings under controlled envioronmental conditions.

Rhizoctonia (R-21) treated control. Only one isolate, S-415, gave no indication of influencing the amount of damping off. The treatment was significantly different from the untreated control, but not different from the Rhizoctonia control.

Three Streptomyces isolates, when present in soil uninfested with Rhizoctonia, showed no significant difference in mean number of plants from that found in the untreated control. Isolates, S-466, S-467 and S-383 were statistically the same as the uninfested control. Two isolates, S-367 and S-415 and the combination treatment were significantly different from both the untreated control and the R-21 treated control. No treatments without R-21 gave results similar to what was observed in the R-21 control.

The results of the dry weight evaluation corresponded closely to the total count data. Those treatments that were not significantly different from the untreated control in the total number count were also not significantly different from the control in the dry weight measurements, with one exception: the S-367 alone treatment was significantly different from both the untreated control and the R-21 control in the total number count, but the dry weight analysis indicated that results of treatment were not different from the untreated control, yet different from the R-21 control.

The antibiotic production and subsequent inhibition of Rhizoctonia mycelial growth in vitro by the five Streptomyces isolates used in this test has been reported. Preliminary greenhouse tests by Boyle (18) indicated some control of Rhizoctonia could be obtained when these same isolates were incorporated into the soil.

The work described here was an attempt to verify this initial observation, under conditions which would more nearly approximate a field situation. This hopefully will lend support to the use of antagonists for biological control of soil-borne pathogens.

The difficulties and criticisms arising from the use of antagonists in soil infestations are indicated in the literature review. In some instances an organism will show biological control, but when it is transferred to an area which deviates somewhat from the original habitat this ability is nullified. It is felt that since these Streptomyces isolates are known to be present in uncultivated as well as cultivated soils of this geographical area, that any technique which would increase population or influence antibiotic production might be of some practical value in the control of soil-borne fungal pathogens.

Two isolates, S-467 and S-383, and a combination of all five isolates were apparently very effective in reducing damping off of seedling cotton caused by Rhizoctonia isolate R-21. Actually, the treatment using five

isolates had about four times the amount of inoculum incorporated than did the treatments with individual Streptomyces isolates. No synergistic effect was observed, in that the control shown was not any greater than that found in any of the other treatments. However, the combination treatment without R-21 showed a marked reduction in number of plants and dry weight. This may have been due to phytotoxicity of the antibiotics. This same reduction in plant numbers and dry weight was observed in the controls treated only with S-367 and S-415. It would appear that if there were no difect influence on the growth and development of the cotton, there should be no significant differences among Streptomyces treated controls.

All organisms incorporated into the soil in this study were re-isolated upon termination of the experiment, from plant material or from soil dilutions.

CARBON:NITROGEN STUDY

Materials and Methods

This study was set up to test, under simulated field conditions, laboratory results, which indicates that certain nitrogen sources increase the excretion of antibiotics. Whaley and Boyle (155) observed that certain nitrogen sources influence the excretion of antibiotic from or retention in the mycelium of certain Streptomyces spp. For instance in some cases all antibiotic was retained in the cells when the organism was grown in a glucose-aspartic acid liquid medium, but the same isolates were found to excrete all antibiotic when grown in a glucose-ammonium tartrate liquid culture. It was also found that C:N ratio had a very limited influence on antibiotic production by the Streptomyces spp. studied but a marked effect on growth of Rhizoctonia.

An experiment was planned to test in soil the effect of nitrogen form and carbon:nitrogen ratio, on the antagonistic capabilities of Streptomyces isolate 383, towards Rhizoctonia (R-21). Response to the treatments was measured by the number of damped off plants in each treatment.

Three chemicals were used in the soil amendment study: (a) ammonium tartrate, (b) tartaric acid, and (c)

ammonium carbonate. These were used in combination and separately to give carbon:nitrogen ratios of 50:1, 20:1, 2:1, and 1:2, all of which were calculated on a gram molecular weight basis. Analysis of soil showed only trace amounts of nitrate and ammonium. These two nitrogen forms were in such small quantities that they were ignored and because of the difficulties in figuring available carbon, this was also deleted from the calculations.

In all carbon:nitrogen treatments the primary nitrogen source was ammonium tartrate, which was added in the quantity calculated to give 100 lbs. of actual nitrogen per acre, with an acre equal to 2 million pounds of soil. The amounts calculated to treat 2 million pounds of soil with desired ratios were reduced proportionately to treat 25 lbs. of soil which was the quantity used per treatment.

The 50:1 treatment consisted of the addition of 3.77 g of ammonium tartrate (15% N and 26% C) and 97.0 g of tartaric acid (31% C) to 25 lbs. of soil. To obtain a 20:1 ratio, 3.77 g of ammonium tartrate and 36.48 g tartaric acid, was added to 25 lbs. of soil. The 2:1 ratio consisted of a treatment with 3.77 g ammonium tartrate alone. The 1:2 ratio required the use of a compound which would contribute more nitrogen than carbon. Ammonium carbonate (24.5% N and 10% C) was selected to be used in conjunction with ammonium tartrate. In this treatment ammonium tartrate was added at the rate of 3.77 g and the

ammonium carbonate at the rate of 38.7 g per 25 lbs. of soil.

To incorporate the carbon and nitrogen sources into the soil, the chemicals in powder form were first mixed with small quantities of soil and this initial quantity mixed with a slightly larger amount of soil and so on, until all 25 lbs. was treated.

Streptomyces isolate 383 cultures were prepared and added to the sterile soil, with chemicals previously added, as described. The Rhizoctonia inoculum was introduced one week later.

The plot design for this experiment contained 18 treatments replicated four times, with 28 polyethylene growth sacks utilized per treatment. Each sack contained six seeds to give a possible 168 plants per treatment if germination were 100%.

Results and Discussion

The results of the carbon:nitrogen study are presented in the form of raw data and pertinent statistical values in Table 6, and in a graphical presentation in Figure 13. The carbon:nitrogen treatments did not influence Streptomyces isolate 383 to control Rhizoctonia damping off. There was significant control observed in the unamended S-383 + R-21 treatment.

Table 6. Results of the carbon:nitrogen study in mean and total numbers of cotton plants reaching the secondary leaf stage.

C:N ratio treatment	Organism treatment	Total number of plants reaching secondary leaf stage in each replication				Treatment totals	Treatment means
		Rep. 1	Rep. 2	Rep. 3	Rep. 4		
50:1	S-383 + R-21	3	9	11	16	39	9.75[a]
	S-383	34	34	23	32	123	30.75
	R-21	0	0	0	0	0	0.00
	Uninfest. Cont.	38	40	26	22	126	31.50
20:1	S-383 + R-21	5	8	6	3	22	8.0
	S-383	37	37	21	28	123	30.75
	R-21	0	2	2	3	7	1.75
	Uninfest. Cont.	38	39	36	33	146	36.50
1:2	S-383 + R-21	2	7	7	9	25	6.25
	S-383	33	33	28	33	127	31.75
	R-21	0	3	2	2	7	1.75
	Uninfest. Cont.	34	22	29	25	110	27.50
2:1	S-383 + R-21	3	6	7	7	23	5.75
	S-383	32	36	33	30	131	32.75
	R-21	2	12	12	8	34	8.50
	Uninfest. Cont.	37	33	35	14	119	29.75
Unamended control	Uninfest. Cont.	38	36	40	33	147	37.00
	S-383 + R-21	25	30	37	29	121	30.50
Replication totals		361	387	355	327		
Replication means		19.77	21.50	19.16	18.16		

Table 6.--Continued

Analysis of variance--	Replication F value--.764 Treatment F value--3.173
Least significant difference--10.13--5% table	

[a]Mean number of plants based on the total number of plants in each treatment divided by the number of replications.

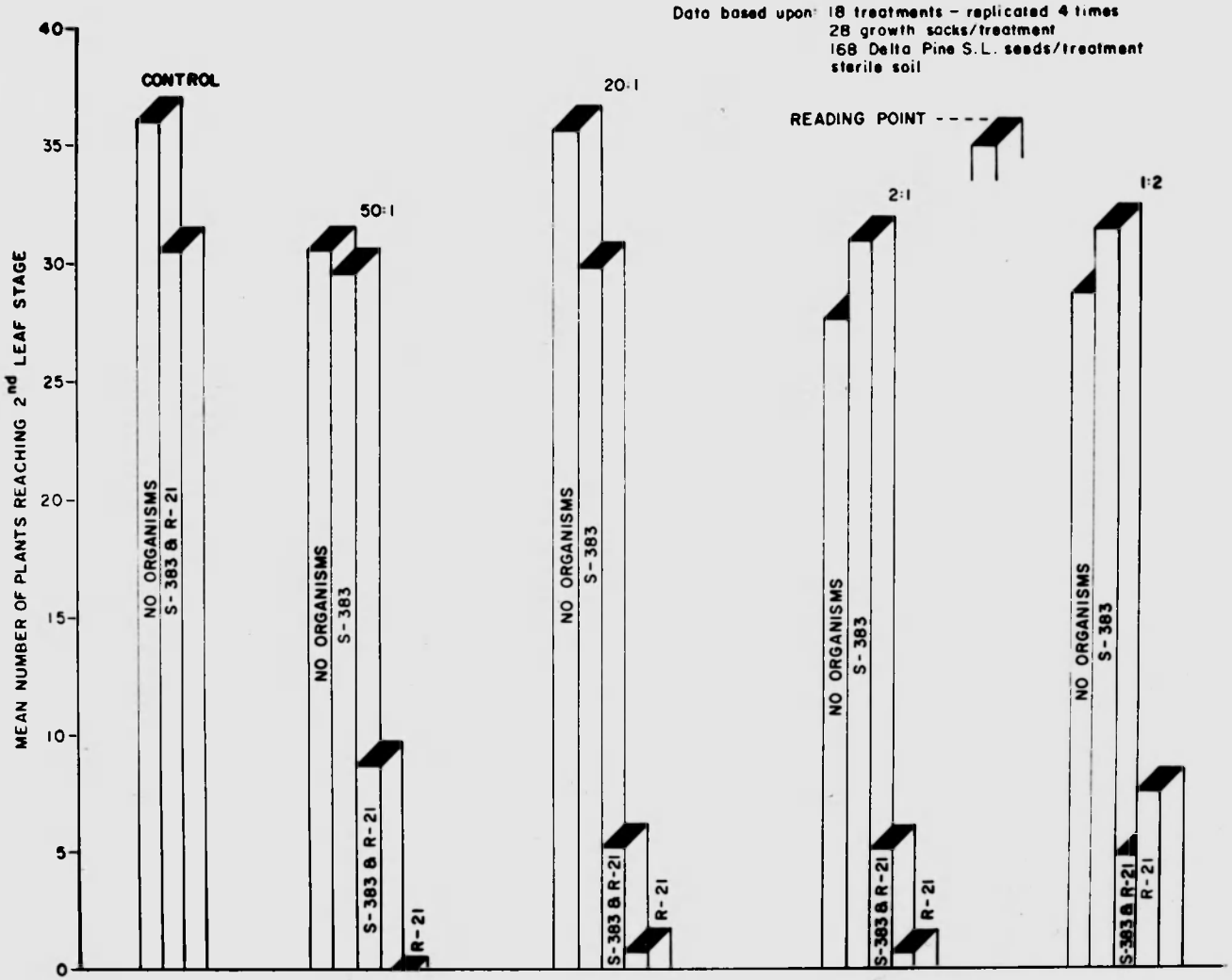

Figure 13. Influence of soil C:N ratios (ammonium tartrate, tartaric acid, and ammonium carbonate) on cotton development, pathogenicity of Rhizoctonia (R-21), and antagonistic capabilities of S-383.

Only one type of measurement was taken and that was a total number count of those plants reaching the secondary leaf stage. The results of this measurement were subjected to an analysis of variance and a least significant difference computation. No significant difference was noted at the 5% level among replications while significant differences were noted among treatments at the .5% level.

In all carbon:nitrogen amended treatments, no control was evident in any of the S-383 + R-21 infested soil. This meant that there was no significant difference in total number of plants reaching the secondary leaf stage between this type of amended treatment and the amended R-21 alone treatments.

In all carbon:nitrogen treatments, all amended treatments containing S-383 alone or no organisms were significantly different from those containing S-383 + R-21 or R-21 alone.

The two unamended controls consisting of an uninfested and a S-383 + R-21 treatment were not significantly different from each other and the two together were not significantly different from all the uninfested and S-383 amended treatments.

It is of interest that the unamended control of S-383 + R-21 had a significantly larger number of plants than found in any of the carbon:nitrogen amended treatments infested with S-383 + R-21. The mean number of plants in

the S-383 + R-21 unamended control corresponded quite closely to the results of the same type of treatment in the previous test which evaluated five Streptomyces isolates for their ability to control Rhizoctonia damping off. The two experiments differed in that the carbon:nitrogen study was carried out in sterile soil while the other was in unsterile soil. This could support the previous evidence that the antibiotic produced by S-383 is not inactivated in soil under field conditions.

Why the results did not support previous laboratory findings is not clear. It may be that when these chemicals are introduced into such a complicated medium as soil there is a loss or masking of properties ordinarily observed on the comparatively simple medium used in the laboratory. The chemicals used may have influenced the aggressiveness of the Rhizoctonia to overcome the inhibitory qualities of the Streptomyces isolate 383. There is some evidence to indicate that disease expression by Rhizoctonia may be increased by the ammonium form (30, 36, 70). The fact that sterile soil was used in the experiment to reduce nitrification and keep the ammonium form around for the benefit of the Streptomyces isolate may have actually enhanced the pathogenicity of the Rhizoctonia.

All organisms incorporated into the soil in this study were re-isolated upon termination of the experiment, from plant material or from soil dilutions.

ROOT EXUDATE STUDY

Materials and Methods

The root exudate collection apparatus was sealed and autoclaved for 30 minutes at 121 C. The cotton seeds of the Delta Pine Smooth Leaf variety were delinted with concentrated sulfuric acid and rinsed in sterile distilled water. Two hundred milliliters of double distilled water was autoclaved for introduction into the system.

When each unit had cooled after steam sterilization, the upper portion of the growth chamber was flammed and unstoppered. Four of the previously delinted seeds and 200 ml of the water were quickly added and the system resealed. Vacuum was then applied and the system allowed to equilibrate. The collection period for one run was limited to five days with four hours of percolation taking place each day.

Upon completion of the collection period each unit was bled of its liquid contents and the liquid checked for contamination by streaking potato sucrose and nutrient agar plates. The exudates obtained from each unit were kept separate until experimental procedure required them to be lumped together. Upon removal from the units, all exudates were filter sterilized using Kreuger filter holders with .45 micron filter pads. The filtrates were then lyphilized

to a dry powder and stored in the freezer compartment of a refrigerator.

To check for the presence of amino acids, the glass beads in the growth tube were removed and placed on a large piece of chromatography paper. The beads were placed parallel to one edge of the sheet, washed with 30 ml of .1N HCl, and allowed to air dry. After drying, the beads were removed and the paper subjected to descending chromatography using a butanol:acetic acid:water ratio of 3:1:1. After eight hours the paper was removed from this tank, sprayed with a cupric nitrate ninhydrin mixture and were inspected under an ultraviolet light.

To study the effects of cotton root exudates on growth and antibiotic production of Streptomyces isolate S-383, the organism was grown in liquid culture consisting entirely of root exudates and sterile double distilled water. For comparison of antibiotic activity and growth, four other media were used: (a) sterile double distilled water; (b) M-14; (c) soil extract, prepared by gently boiling 1 Kg of the same soil used in growth chamber experiments, in 1000 ml of tap water and then filtering through No. 4 Whatman filter paper; the filtrate was brought up to 1000 ml volume with more tap water; and (d) a semi-synthetic basal medium utilizing glucose, 10 g; ammonium tartrate, 2.76 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; biotin, 5.0 mg; thiamine, 100 mg; microelement solution, 2 ml;

Sorensen's phosphate buffer, 100 ml; distilled water, 898 ml. The exudate medium was prepared by dissolving the lyphilized material obtained from 32 aseptically grown cotton plants in 1000 ml of sterile double distilled water and filter sterilizing it.

All media were filter sterilized and separated into five 200 ml aliquots, each of which was placed in sterile, cotton plugged 500 ml Erlenmeyer flasks. The media were inoculated with suspension of S-383, prepared by placing chunks of agar taken from an agar plate culture of the organism in 300 ml of sterile double distilled water and agitating the suspension on a reciprocal shaker at approximately 80 excursions per minute for 30 minutes. Ten milliliters of the suspension were added to each 200 ml portion of each medium. All flasks were incubated on a reciprocal shaker at 80 excursions per minute, for two weeks at 20-25 C, under alternating light and dark conditions.

Upon completion of the incubation period, all flasks were placed in a refrigerator at 5 C for storage. In preparation for bioassay most cellular material was separated from the medium by filtering through two layers of muslin placed over a Buchner funnel with vacuum. The filtrate was treated with 50 ml of acetone and then reduced to 10-15 ml in vacuo. The cellular material was homogenized by hand in a glass tissue homogenizer in the presence

of 50 ml of acetone, reduced to dryness in vacuo and then suspended in sterile distilled water. All extracts were stored at 5 C in a refrigerator.

To bioassay the cellular extract and concentrated culture filtrate for antibiotic activity, bioassay discs (12.7 mm) were soaked in the liquid and placed on M-14 agar in petri plates along with a small plug of Rhizoctonia (R-21). Measurements of activity were based on the size of the inhibition zone present around the bioassay discs.

Results and Discussion

The growth of Streptomyces isolate 383 was observed in the flasks of exudate medium and the other media with the exception of the sterile distilled water. Bioassay of concentrates indicated antibiotic production by Streptomyces isolate 383 in the exudate medium and all other media with the exception of sterile distilled water (Figure 14). Ninhydrin positive and fluorescent spots were noted on all chromatograms of root exudates.

The presence of ninhydrin and ultraviolet positive spots indicates the presence of organic compounds in the root exudates. Attempts were made to identify the spots, but due to the crude methods used, the separation of compounds was not adequate to facilitate identification.

The growth of Streptomyces isolate 383 in the root exudate medium was not as great as that in the M-14 culture

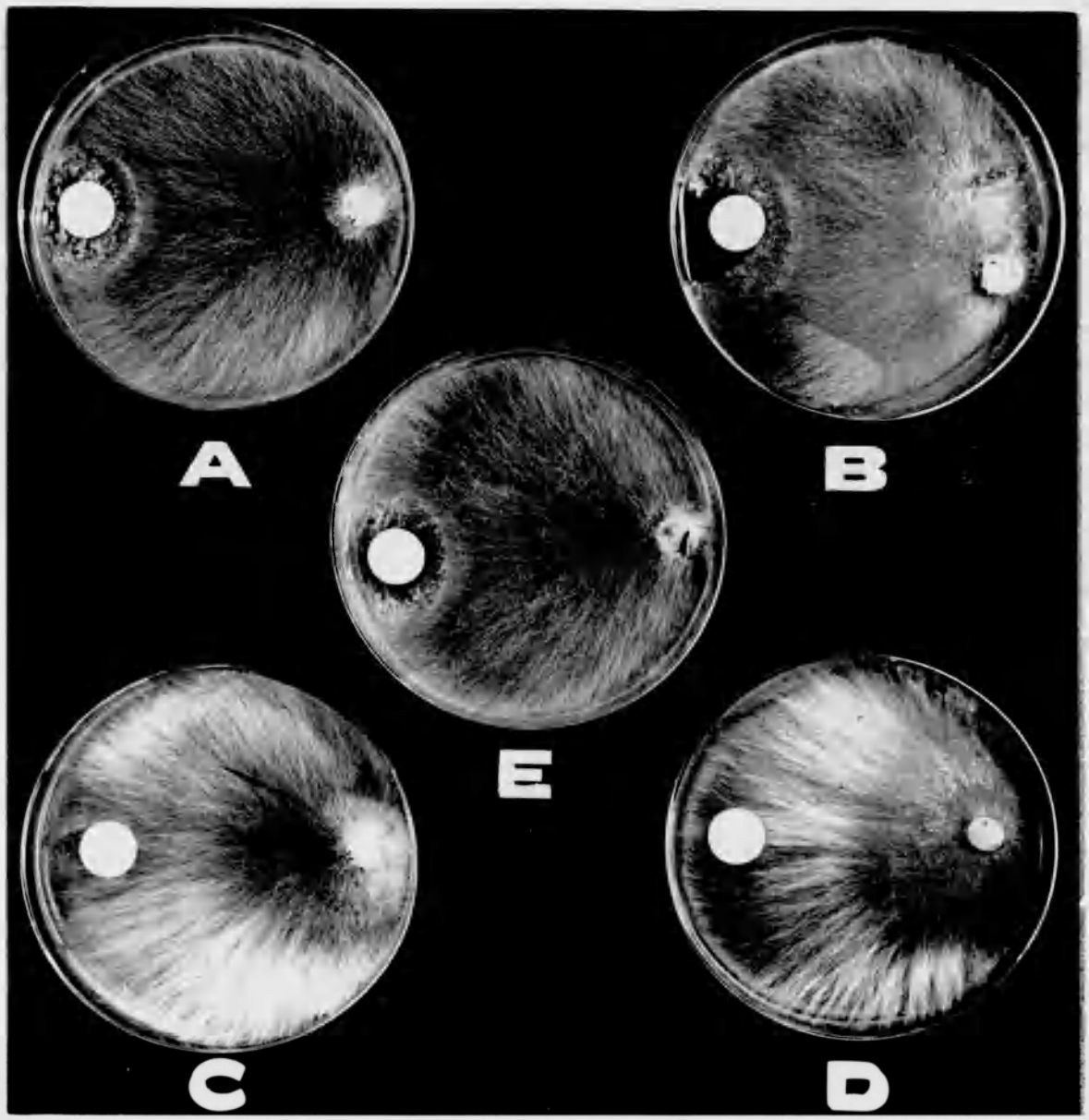

Figure 14. Illustrates the antibiotic activity of the concentrated filtrate from 5 media broths inoculated with S-383. (A) M-14. (B) Semi-synthetic basal medium with ammonium tartrate as a nitrogen source. (C) Soil extract. (D) Double distilled water. (E) Cotton root exudate.

yet somewhat greater than in the ammonium tartrate semi-synthetic basal medium. The soil extract medium gave growth comparable to that in the semi-synthetic basal medium and no growth was observed in the sterile distilled water treatment.

Antibiotic production was noted in all flasks except the sterile distilled water treatment, through use of bioassay discs soaked in cellular and filtrate concentrates. The concentration from flask to flask and treatment to treatment varied as indicated by differences in the size of inhibition zones. This was not as important though as the determination of whether or not cotton root exudates will support growth and antibiotic production of Streptomyces isolate 383.

There are a number of environmental situations which have been shown to influence the quantity and composition of root exudates. Examples of this are noted in the literature review.

CONCLUSIONS

Observations made in the pathogen survey and pathogenicity evaluation study indicate that no one fungal pathogen attacking seedling cotton can be identified solely on the basis of symptomatology.

Under the conditions prevailing in the pathogenicity evaluation, Rhizoctonia is the primary pathogen associated with the damping off of seedling cotton in Arizona, but pathogenicity varies from isolate to isolate.

Some seedling damage may be attributed to Thielaviopsis, Fusarium, Helminthosporium and possibly Aspergillus if in all cases the inoculum potential is high. Under the conditions of this test these organisms were not capable of causing any terminal infections during the seedling stage.

Under the environmental conditions and methods used in the experiment to determine the ability of five Streptomyces isolates to control Rhizoctonia damping off, it is evident that this seedling disease may be controlled through the introduction of large quantities of certain Streptomyces isolates to sterile and unsterile soil. The incorporation of Streptomyces isolates 467 and 383 and a combination of all five isolates gave significant control of Rhizoctonia (R-21). Isolates S-466 and S-367 when added

individually gave moderate control. Isolate S-415 when added alone gave no control.

Streptomyces isolates 367 and 415 are in themselves capable of adversely affecting cotton seedlings when added in large quantities.

No synergistic effect was obtained when all five isolates were added to soil together.

Under the conditions prevailing in the carbon: nitrogen study the addition of ammonium tartrate, tartaric acid and ammonium carbonate in the form of carbon:nitrogen ratios, to soils infested with Rhizoctonia (R-21) and Streptomyces isolate 383, did not influence the S-383 to control Rhizoctonia damping off. In fact significantly less control was evident when soil was amended with varying C:N ratios than when it was left unamended.

Streptomyces isolate 383 is capable of utilizing chemicals found in cotton seedling root and seed exudates for growth and antibiotic production, at least under the conditions described. This would indicate the ability of the isolate to establish itself and maintain its existence in close association with the root.

SUMMARY

A survey of organisms associated with seedling disease of cotton in Arizona was made by sampling or collecting from seven major cotton growing areas in Arizona. Isolations were made by soil dilutions and from damped off seedlings. The majority of isolates obtained were Rhizoctonia, Fusarium, Acrostalagmus, Helminthosporium, Penicillium, Curoularia, Trichodermia, Thielaviopsis, Aspergillus and unidentified Phycomycetes.

Eleven isolates were evaluated for pathogenicity under simulated field conditions created with the aid of an environmental control chamber. Cotton plants were grown in sterile soil infested with each isolate and observations were made, and number and dry weight of plants reaching the secondary leaf stage. Under the conditions of this test, Rhizoctonia was found to be the organism primarily responsible for seedling death of cotton, and variations occurred among Rhizoctonia isolates. Observations indicated other organisms were responsible for some damage if inoculum potential were high, and that it was almost impossible to associate a particular type of damage with a specific organism.

Five antibiotic producing Streptomyces isolates were tested, under the same environmental conditions as

were present in the first experiment, for their ability to control damping off of seedling cotton caused by Rhizoctonia (R-21). Each isolate was tested individually and in a combination treatment in soil infested with Rhizoctonia (R-21) and planted to cotton. Control was measured by the number and dry weight of plants reaching the secondary leaf stage. Results indicated that two Streptomyces isolates, 467 and 383, and a combination of all S isolates together gave significant control. Two isolates, S-466 and S-367, gave moderate control while isolate S-415 gave no control. Some damage to the seedlings could be attributed to isolate S-367 and S-415, and the combination treatment.

In earlier studies it had been found that certain nitrogen sources increased the production of and excretion by certain Streptomyces spp. and that C:N ratio using a favorable nitrogen source did not influence antibiotic production but did sharply influence growth of Rhizoctonia. An experiment was designed to test these results under conditions simulating those in the field. Ammonium tartrate, tartaric acid and ammonium carbonate were incorporated into soil at various carbon nitrogen ratios, in conjunction with Streptomyces isolate 383 and Rhizoctonia (R-21). The test was conducted under the same environmental conditions as the previous two tests. The results indicated a reduction in control.

A medium made from cotton root exudates, collected in a modified soil perfusion apparatus, was tested for its ability to support growth and antibiotic production of S-383. For comparisons several media known to favor antibiotic production by the organism were used. The purpose of this experiment was to test the possibility that S-383 might be able to establish itself and produce antibiotics in the rhizosphere of the cotton, thus controlling Rhizoctonia damping off. Results of this test were positive.

LITERATURE CITED

1. Afansiev, M. M., and H. E. Morris. 1950. The effect of preceding crops and nutrients on the growth and seedling diseases of sugar meets in Montana. Phytopathology 40:963. (abstr.)

2. Alexander, M., and R. Mitchell. 1962. Lysis of soil fungi in relation to biological disease control. p. 60. (abstr.) VIII. Intern. Congr. Microbiol., Montreal.

3. Anderson, E. J. 1939. Effect of nutrient variations on host and parasite in the Rhizoctonia stem rot disease of bean. Phytopathology 29:1. (abstr.)

4. Arndt, C. H. 1935. The etiology of damping off of cotton seedlings. Phytopathology 25:968-969. (abstr.)

5. Arndt, C. H. 1943. Pythium ultimum and the damping off of cotton seedlings. Phytopathology 33:607-611.

6. Arndt, C. H. 1953. Evaluation of fungicides as protectants of cotton seedlings from infection by Rhizoctonia solani. Plant Dis. Reptr. 37:397-400.

7. Arndt, C. H. 1957. Temperature as a factor in the infection of cotton seedlings by ten pathogens. Plant Dis. Reptr. Supp. 246.

8. Ashour, W. A. 1957. Effect of water treatment, seed treatment and date of sowing on emergence and damping off of cotton seeds. Damping off disease of cotton. I. Studies on the casual organisms and their pathogenicity. Ann. of Agr. Sci. Cairo. 1:161-176, 1956; 2:81-94, 1957. In Rev. of Appl. Mycol. 37:721.

9. Atkinson, G. F. 1892. Some diseases of cotton. Alabama Polytechnol. Inst. Agr. Exp. Sta. Bull. 41. p. 30-39.

10. Audus, L. J. 1946. A new soil perfusion apparatus. Nature 158:419.

11. Bakshi, B. K. 1954. Wilt of Shisham (Dalbergia sisoa Roh.) due to Fusarium solani f. sensui. Sunder and Hansen. Nature 174:278-279.

12. Bazan de Seguro (Consuelo). 1961. Control de la chupadero fungosa del algodonero. Compatibilidad de fungicidas e insecticidas en al tratimiento de la semilla de algod. Bol. Tech. Soc. Nac. Agron, Lima. 13. 1-11, 1960. In Rev. of Appl. Mycol. 41:33.

13. Bhuvaneswari, K., and C. B. Sulochana. 1955. Assay of root exudates. Current Science (India) 24: 376-377.

14. Bhuvaneswari, K., and N. S. Subba-Rao. 1957. Root exudates in relation to the rhizosphere effect. Proc. Indian Acad. Sci. 45:299-301.

15. Blair, I. D. 1943. Behaviour of the fungus Rhizoctonia solani Kuehn in the soil. Ann. Appl. Biol. 30:118-127.

16. Boosalis, M. G. 1950. Studies on the parasitism of Rhizoctonia solani Kuehn on soybeans. Phytopathology 40:820-831.

17. Boosalis, M. G. 1956. Effect of soil temperature and green manure amendment of unsterilized soil on parasitism of Rhizoctonia solani by Penicillium vermiculatum and Trichoderma sp. Phytopathology 46:473-477.

18. Boyle. 1966. Professor of Plant Pathology, Dept. of Plant Pathology, University of Arizona, Tucson (Pers. communication).

19. Brian, P. W. 1951. Antibiotics produced by fungi. Bot. Rev. 17:357-430.

20. Brian, P. W. 1960. Presidential address: Griseofulvin. Trans. Brit. Mycol. Soc. 43:1-3.

21. Butler, F. C. 1953. Saprophytic behavior of some cereal root-rot fungi. I. Ann. Appl. Biol. 40: 284-297.

22. Butler, F. C. 1953. Saprophytic behavior of some cereal root-rot fungi. III. Saprophytic survival in wheat straw buried in soil. Ann. Appl. Biol. 40:305-311.

23. Butler, E. E. 1957. Rhizoctonia solani as a parasite of fungi. Mycologia 49:354-373.

24. Buxton, E. W. 1960. Effects of pea root exudates on the antagonism of some rhizosphere microorganisms towards Fusarium oxysporium f. pisi. J. Gen. Microbiol. 22:678-689.

25. Camp, A. F. 1928. Cotton investigation. Fla. Univ. Agr. Exp. Sta. Bull. 199. p. 35.

26. Chavez, Henry B. 1966. Extension assistant in Plant Pathology, Dept. of Plant Pathology, University of Arizona, Tucson (Pers. communication).

27. Clayton, E. E. 1944. Control of flue cured tobacco root disease by crop rotation. U. S. Dep. Agr. Farm Bull. 1952. p. 12.

28. Clements, F. E., and V. E. Shelford. 1939. Bioecology. John Wiley & Sons, New York. 425p.

29. Darpoux, H. 1960. Biological interference with epidemics, p. 521-565. In J. G. Horsefall and A. D. Dimond (ed.) Plant pathology, an advanced treatise. Vol. III. Academic Press, New York and London.

30. Davy, C. B., and G. C. Papavizas. 1959. Effect of organic soil amendments on the Rhizoctonia disease of snap beans. Agron. J. 51:493-496.

31. Davy, C. B., and G. C. Papavizas. 1960. Effect of dry mature plant materials and nitrogen on Rhizoctonia solani in soil. Phytopathology 50: 522-525.

32. Davy, C. B., and G. C. Papavizas. 1963. Saprophytic activity of Rhizoctonia as affected by the carbon-nitrogen balance of certain organic soil amendments. Soil Sci. Soc. Am. Proc. 27:164-167.

33. Dekker, J. 1963. Antibiotics in the control of plant diseases. Ann. Rev. Microbiol. 17:243-262.

34. Devay, J. E. 1956. Mutual relationships in fungi. Ann. Rev. Microbiol. 10:115-140.

35. Durbin, R. D. 1959. Factors affecting the vertical distribution of Rhizoctonia solani with special reference to CO_2 concentration. Am. J. Bot. 46: 22-25.

36. Easton, G. D. 1964. The results of fumigating Verticillium and Rhizoctonia infested potato soils in Washington. Amer. Pot. J. 41:296. (abstr.)

37. Elsaid, H. M., and J. B. Sinclair. 1964. Adapted tolerance to organic fungicides by isolates of Rhizoctonia solani from seedling C. Hon. Phytopathology 54:518-522.

38. Fulton, N. D., and Katharina Bollenbacher. 1959. Pathogenicity of fungi isolated from diseased cotton seedlings. Phytopathology 49:684-689.

39. Fulton, N. D., B. A. Waddle, and K. Bollenbacher. 1962. Varietal resistance to seedling diseases in cotton. Phytopathology 52:10. (abstr.)

40. Garrett, S. D. 1940. Soil conditions and the take-all disease of wheat. V. Further experiments on the survival of Ophiobolus graminis in infected wheat stubble buried soil. Ann. Appl. Biol. 27: 199-204.

41. Garrett, S. D. 1956. Biology of root infecting fungi. Cambridge University Press. 293 p.

42. Garrett, S. D. 1962. Decomposition of cellulose in soil by Rhizoctonia solani Kuehn. Trans. Brit. Mycol. Soc. 45:115-120.

43. Glover, T. 1856. Accidents and diseases of the cotton plant. In Report on Agriculture by the Commissioner of Patents for the year 1855. pp. 230-234. U. S. Patent Office Report. Wash. 488 p.

44. Glynne, M. D. 1951. Effects of cultural treatments on wheat and on the incidence of eyespot, lodging, take-all and weeds. Ann. Appl. Biol. 38:665-668.

45. Gottlieb, D., and P. Siminoff. 1950. The role of antibiotics in soil. Phytopathology 40:11. (abstr.)

46. Gottlieb, D., and P. Siminoff. 1952. The production and role of antibiotics in the soil. II. Chloromycetin. Phytopathology 42:91-97.

47. Gottlieb, D., and P. Siminoff. 1953. The activity of antibiotics in the soil, pp. 449-450. In Proceedings of the Seventh International Botanical Congress. Stockholm.

48. Gottlieb, D. 1961. An evaluation of criteria and procedures used in the description and characterization of the Streptomycetes. A cooperative study. Appl. Microbiol. 9:55-65.

49. Gregory, K. F., O. N. Allen, A. J. Riker, and W. H. Peterson. 1952. Antibiotics as agents for the control of certain damping off fungi. Amer. J. Bot. 39:405-415.

50. Griffin, G. J. 1964. Long term influence of soil amendments on germination of condia. Can. J. Microbiol. 10:605-612.

51. Grossbard, E. 1948. Production of an antibiotic substance on wheat straw and other organic materials in soil. Nature 161:614.

52. Grossbard, E. 1951. Antibiotics and microbial antagonism in plant pathology. Endeavour 10: 145-150.

53. Hessayon, D. G. 1951. "Double-action" of Trichothecin and its production in the soil. Nature, Lond. 168:998-999.

54. Houston, B. R. 1945. Cultural types and pathogenicity of Corticum solani. Phytopathology 35: 371-393.

55. Huber, D. M. 1963. Investigations on root rot of beans. Ph.D. Diss. Mich. State Univ. E. Lansing, 97 p.

56. Huber, D. M., R. D. Watson, and G. W. Steiner. 1965. Crop residues, nitrogen and plant disease. Soil Sci. 100:302-307.

57. Jefferys, E. G. 1952. The stability of antibiotics in soils. J. Gen. Microbiol. 7:295-312.

58. Johnson, L. F., E. A. Curl, J. N. Bond, and H. A. Fribourg. 1959. Methods for studying soil micro-flora-plant disease relationships. Burgess Publ. Co., Minneapolis 15, Minn. 178 p.

59. Katznelson, H., J. V. Rouatt, and T. M. B. Payne. 1954. Liberation of amino acids by plant roots in relation to desiccation. Nature 174:1110-1111.

60. Katznelson, H., J. V. Rouatt, and T. M. B. Payne. 1955. The liberation of amino acids and reducing compounds by plant roots. Plant and Soil 7:35-48.

61. Kalyansundarm, R. 1958. Production of fusaric acid by Fusarium lycopersici Sacc. in the rhizosphere of tomato plants. Phytopathol. Z. 32:25-34.

62. Kaufman, D. D., and L. E. Williams. 1962. Polyethylene bags for the study of soil microorganisms. Phytopathology 52:16.

63. Kaufman, D. D., and L. E. Williams. 1964. Effect of mineral fertilization and soil reaction on soil fungi. Phytopathology 54:134-139.

64. Kernkamp, M. F., D. J. deZeeuw, S. M. Chen, B. C. Ortega, C. T. Tsiang, and A. M. Khan. 1952. Investigations of physiologic specialization and parasitism of Rhizoctonia solani. Minnesota Agr. Exp. Tech. Bull. 200. 36 p.

65. Kerr, A. 1959. Some interactions between plant roots and pathogenic fungi. Australian J. Biol. Sci. 9:45-52.

66. Knudson, L. 1920. The secretion of invertase by plant roots. Am. J. Bot. 7:371-379.

67. Kommedahl, T. 1952. The incidence of Fusarium and Rhizoctonia-infected plants in wheat, corn, and oats. Phytopathology 42:468-469.

68. Kommedahl, T., and T. D. Brock. 1954. Studies on the relationship of soil mycoflora to disease incidence. Phytopathology 44:57-61.

69. Kommedahl, T. 1966. Relation of exudates of pea roots to germination of spores in races of Fusarium oxysporium f. pisi. Phytopathology 56: 721-722.

70. Kunkel, R., and Weller, M. 1964. Effect of soil fumigation and quality of russet burbank potatoes in the Columbia Basin of Washington. Am. Pot. J. 41:299. (abstr.)

71. LeClerg, E. L. 1934. Parasitism of Rhizoctonia solani on sugar beets. J. of Agr. Res. 49:407-431.

72. LeClerg, E. L. 1939. Methods of determination of physiologic races of Rhizoctonia solani on the basis of parasitism of several crop plants. Phytopathology 29:609-616.

73. Lehman, S. G. 1938. Seed infestation with Glomerella and Fusarium in the 1936 cotton crop in North Carolina. Pl. Dis. Reptr. 22:4-6.

74. Liu, Shu-Yen, and E. K. Vaughan 1965. Control of Pythium infection in table beet seedlings by antagonists. Phytopathology 55:986-989.

75. Lochhead, A. G., and M. O. Buxton. 1956. The incidence in soil of bacteria requiring Vit. B_{12} and the terregen factor. Soil Science 82:237-245.

76. Maier, C. R., and E. E. Staffeldt. 1963. Cotton seedling disease complex in New Mexico. New Mex. Agr. Exp. Sta. Bull. 474. 41 p.

77. Maloy, O. C., and W. H. Burkeholder. 1959. Some effects of crop rotation on Fusarium root rot of bean. Phytopathology 49:583-587.

78. Maloy, O. C. 1960. Physiology of Fusarium solani f. phaseoli to saprophytic survival in soil. Phytopathology 50:56-61.

79. Martin, N., and D. Gottlieb. 1952. The production and role of antibiotics in the soil. III. Terramycin and Aureomycin. Phytopathology 42: 294-296.

80. Martin, M., and D. Bottlieb. 1955. The production and role of antibiotics in the soil. V. Antibacterial activity of five antibiotics in the presence of soil. Phytopathology 45:407.

81. Martin, P. 1958. Einfluss der Kulturfiltrate von Mikroorgismen auf die abgabe von skopoletin aus den Keimwurzeln des Hafers (Avena sativa L.) (In German, English summary). Arch. Mikrobiol. 29: 154-186.

82. Matsumoto, T. 1921. Studies in the physiology of fungi. Physiologic specialization of Rhizoctonia solani Kuehn. Ann. Missouri Botan. Gard. 8:1-62.

83. Maurer, C. L., and R. Baker. 1965. Ecology of plant pathogens in soil. II. Influence of glucose, cellulose, and inorganic nitrogen amendments on development of bean root rot. Phytopathology 55: 69-72.

84. Menon, S. K., and L. E. Williams. 1957. Effect of crop, crop residues, temperature, and moisture on soil fungi. Phytopathology 47:559-564.

85. Menzies, J. D. 1957. A dipper technique for serial dilutions of soil for microbial analysis. Soil Sci. Am. Proc. 21:660.

86. Milne, A. 1961. Definition of competition among animals. In F. L. Milthorpe (ed.) Mechanisms in biological competition, Symp. Soc. Exptl. Biol. 15:40-61. Cambridge University Press, London.

87. Neal, D. C. 1953. Bacteria and fungi on cotton seedlings, pp. 311-314. In Plant diseases. U. S. D. A. Yearbook.

88. Norman, A. G. 1955. The effect of polymyxin on plant roots. Arch. Biochem. Biophys. 58:461-467.

89. Oswald, J. W., and O. A. Lorenz. 1956. Soybeans as a green manure crop for the prevention of potato scab. Phytopathology 46:22. (abstr.)

90. Paharia, K. D., and T. Kommendahl. 1954. A modified plating technique for the study of soil fungi. Phytopathology 44:502.

91. Papavizas, G. C., and C. B. Davey. 1959. Isolation of Rhizoctonia solani from naturally infested and artificially inoculated soil. Pl. Dis. Reptr. re:404-410.

92. Papavizas, G. C., and C. B. Davey. 1960. Rhizoctonia disease of bean as affected by decomposing green plant materials and associated microflora. Phytopathology 50:516-522.

93. Papavizas, G. C., and C. B. Davey. 1961. Saprophytic behavior of Rhizoctonia in soil. Phytopathology 51:693-699.

94. Papavizas, G. C., and C. B. Davey. 1962. Activity of Rhizoctonia in soil as affected by carbon dioxide. Phytopathology 52:759-766.

95. Papavizas, G. C. 1963. Microbial antagonism in bean rhizosphere as affected by oat straw and supplemental nitrogen. Phytopathology 53:1430.

96. Park, D. 1963. The ecology of soil-borne fungus disease. Ann. Rev. Phytopathology 1:241-258.

97. Parkinson, D., and J. S. Waid. 1960. The ecology of soil fungi. Liverpool University Press. 324 p.

98. Powelson, R. L. 1964. Studies on Verticillium wilt of potatoes in Oregon. Am. Pot. J. 41:303. (abstr.)

99. Presley, J. T., and C. J. King. 1942. A root rot caused by Thielaviopsis basicola. Phytopathology 32:752-761.

100. Presley, J. T. 1954. Cotton diseases and methods of control. U.S.D.A. Farmer's Bull. 1745. 34 p.

101. Quastel, J. H., and H. Lees. 1944. A new technique for studying soil sterilization. Chem. and Ind. 26:238.

102. Rangaswami, G., and S. Ethiraj. 1962. Antibiotic production by Streptomyces sp. in unamended soil. Phytopathology 52:989-991.

103. Rangaswami, G., and P. Vidyasekaran. 1963. Antibiotic production by Streptomyces sp. in corn rhizosphere. Phytopathology 53:995-997.

104. Ranney, C. D. 1962. Fungi involved in the seedling disease complex of cotton in the Yazoo-Mississippi delta. Pl. Dis. Reptr. 46:122-123.

105. Rauschkolb, Roy. 1966. University of Arizona Agr. Exp. Sta., Safford, Arizona (Pers. communication).

106. Ray, W. W. 1943. Effect of cotton seed dusting on emergence of seedlings in the soil infested with Rhizoctonia. Phytopathology 33:51-55.

107. Richards, B. L. 1923. Soil temperature as a factor affecting the pathogenicity of Corticum vagum on the pea and bean. J. Agr. Res. 25:431-450.

108. Riker, A. J., and Regina S. Riker. 1936. Introduction to research on plant diseases. Planographed by John S. Swift Co., St. Louis, Chicago, New York, Cincinnati, Cleveland. 117 p.

109. Rosen, H. R. 1925. Fusarium vasinfectum and the damping off of cotton seedlings. Phytopathology 15:486-488.

110. Rouatt, J. W., and R. G. Atkinson. 1950. The effect of the incorporation of certain cover crops on the micro-biological balance of potato scab infested soil. Can. J. Res. 28:140-152.

111. Rovira, A. D. 1956. Plant root excretions in relation to the rhizosphere effect. I. The nature of root exudates from oats and peas. Plant and Soil 7:178-194.

112. Rovira, A. D. 1956. A study of the development of the root surface microflora during the initial stages of growth. J. Appl. Bacteriol. 19:72-79.

113. Rovira, A. D. 1959. Root excretions in relation to the rhizosphere effect. IV. Influence of plant species, age of plant, light, temperature and calcium nutrition on exudation. Plant and Soil 11:53-64.

114. Rovira, A. D., and J. H. Harris. 1961. Plant root excretions in relation to the rhizosphere effect. V. The exudation of B-group vitamins. Plant and Soil 14:199-214.

115. Rovira, A. D. 1965. Interactions between plant roots and soil microorganisms. Ann. Rev. Microbiol. 19:241-266.

116. Rovira, A. D. 1965. Plant root exudates and their influence upon soil microorganisms, pp. 170-182. In K. F. Baker and W. C. Snyder (ed.) Ecology of soil-borne pathogens. Univ. of Calif. Press, Berkeley.

117. Rushdi, M. K., and W. F. Jeffers. 1952. Variation in Rhizoctonia solani. Phytopathology 42:473. (abstr.)

118. Sanford, G. B. 1938. Studies on Rhizoctonia solani Kuehn. III. Racial difference in pathogenicity. Can. J. Res. C-16:53-64.

119. Sanford, G. B. 1938. Studies on Rhizoctonia solani Kuehn. IV. Effect of soil temperature and moisture on virulence. Can. J. Res. C-16:203-215.

120. Sanford, G. B. 1946. Soil-borne diseases in relation to the microflora associated with various crops and soil amendments. Soil Science 61:23-30.

121. Sanford, G. B. 1947. Effect of various soil supplements on the virulence and persistence of Rhizoctonia solani. Sci. Agr. 27:533-544.

122. Sanford, G. B. 1956. Factors affecting the formation of sclerotia by Rhizoctonia solani. Phytopathology 46:281-285.

123. Sanford, G. B. 1959. Root-disease fungi as affected by other soil organisms, pp. 367-376. In C. S. Holton et al. (ed.) Plant pathology, problems and progress 1908-1958, University of Wisconsin Press, Madison, Wisc.

124. Schmidt, E. L. 1951. Soil microorganisms and plant growth substances. I. Soil Science 71:129-140.

125. Schroth, M. N., and W. C. Snyder. 1961. Effect of host exudates on chlamydospore germination of the bean root rot fungus Fusarium solani f. phaseoli. Phytopathology 51:389-393.

126. Schroth, M. N., and F. E. Hendrix, Jr. 1962. Influence of nonsusceptible plants on the survival of Fusarium solani f. phaseoli in soil. Phytopathology 52:906-909.

127. Schwegman, J. C. 1953. Temperature pathogenicity relationships in Rhizoctonia solani of cotton. Pl. Dis. Reptr. 37:178.

128. Scott, M. R. 1956. Studies on the biology of Sclerotium cepivorum Berk. I. Ann. Appl. Biol. 44:576-583.

129. Shatla, M. N. 1963. Identification of three physiologic races of Rhizoctonia solani isolated from seedling cotton. Phytopathology 53:889.

130. Shatla, M. N., Mohamed-Nagi, and J. B. Sinclair. 1963. Tolerance of pentachloronitrobenzene among cotton isolated Rhizoctonia solani. Phytopathology 53:1407-1411.

131. Shatla, M. N., and J. B. Sinclair. 1964. Effect of PCNB on isolates of Rhizoctonia solani under field conditions. Phytopathology 54:626. (abstr.)

132. Shatla, M. N., and J. B. Sinclair. 1965. Effect of PCNB on Rhizoctonia solani under field conditions. Pl. Dis. Reptr. 49:21-23.

133. Shatla, M. N., and J. B. Sinclair. 1965. Rhizoctonia solani: Physiologic specialization among isolates from cotton. Pl. Dis. Reptr. 49:711.

134. Siegle, H. 1961. Uber Mischinfektionen mit Ophiobolus graminis und Didymella exitialis. In German, English summary). Phytopathol. Z. 42: 305-348.

135. Siminoff, P., and D. Gottlieb. 1951. The production and role of antibiotics in the soil. I. The fate of streptomycin. Phytopathology 41:420-430.

136. Sinclair, J. B. 1965. Cotton seedling diseases and their control. Louisiana Agr. Exp. Sta. Bull. 590. 35 p.

137. Snyder, W. C., M. N. Schroth, and T. Christou. 1959. Effect of plant residues on root rot of bean. Phytopathology 49:755-756.

138. Staten, G. 1942. Cotton seed treatment in New Mexico. New Mexico Agr. Exp. Sta. Bull. 290. 36 p.

139. Storey, J. F. 1941. A comparative study of strains of Rhizoctonia solani Kuehn with special reference to their parasitism. Ann. Biol. 28:445-470.

140. Sulochana, C. B. 1962a. Amino acids in root exudates of cotton. Plant and Soil 16:312-326.

141. Sulochana, C. B. 1962b. B-vitamins in root exudates of cotton. Plant and Soil 16:327-334.

142. Tolmsoff, W. J., and R. A. Young. 1959. The influence of crop residues and fertilization on the development and severity of Verticillium wilt of potatoes. Phytopathology 49:114. (abstr.)

143. Toussoun, T. A., Shirley M. Nash, and W. C. Snyder. 1960. The effect of nitrogen sources and glucose on the pathogenesis of Fusarium solani f. phaseoli. Phytopathology 50:137-140.

144. Tsao, P. H. 1963. Effect of certain fungal isolation agar media on Thielaviopsis basicola and on its recovery in soil dilution plates. Phytopathology 54:548-555.

145. Ullstrup, A. J. 1939. The occurrence of the perfect stage of Rhizoctonia solani in plantings of diseased cotton seedlings. Phytopathology 29: 373-374.

146. Waksman, S. A. 1959. The Actinomycetes. Vol. I. Nature, occurrence and activities. Williams and Wilkens Co., Baltimore. 327 p.

147. Waksman, S. A. 1961. The Actinomycetes. Vol. II. Classification, identification and description of genera and species. Williams and Wilkins Co., Baltimore. 363 p.

148. Waksman, S. A., and H. A. Lechevalier. 1962. The Actinomycetes. Vol. III. The antibiotics of actinomycetes. Williams and Wilkins Co., Baltimore. 430 p.

149. Walker, M. N. 1928. Soil temperature studies with cotton. III. Relation of soil temperature and soil moisture to the soreshin disease of cotton. Florida Agr. Exp. Sta. Bull. 197. 21 p.

150. Weindling, R., P. R. Miller, and A. J. Ullstrup. 1941. Fungi associated with diseases of cotton seedlings and bolls, with special consideration of Glomerella gossypii. Phytopathology 31:158-167.

151. Weindling, R. 1959. Role of parasitism in microbial antagonisms, pp. 623-626. In Recent Adv. Bot. (Canada) 9th Intern. Botan. Congr., Montreal. Univ. of Toronto Press, Toronto.

152. Winke, K. E. 1962. The influence of nitrogen on the root disease of bean caused by F. solani f. phaseoli. Phytopathology 52:757. (abstr.)

153. West, P. M., and A. A. Hildebrand. 1941. The microbiological balance of strawberry root rot as related to the rhizosphere and decomposition effects of certain cover crops. Can. J. Res. 19:199-210.

154. Whaley, J. W. 1964. Physiological studies of antagonistic actinomycetes from the rhizosphere of desert plants. Ph.D. Diss. Univ. of Ariz., Tucson. 193 p.

155. Whaley, J. W., and Alice M. Boyle. 1967. Antibiotic production by Streptomyces species from the rhizosphere of desert plants. Phytopathology 57:347-351.

156. Wilhelm, S. 1951. Effect of various soil amendments on the inoculum potential of Verticillium wilt fungus. Phytopathology 41:684-690.

157. Winfield, R. W. 1942. The effect of cotton seed dusting on emergence of seedlings in soil infested with Rhizoctonia. Phytopathology 32:15. (abstr.)

158. Wood, R. K. S., and M. Tveit. 1955. Control of plant diseases by use of antagonistic organisms. Bot. Rev. 21:441-492.

159. Woodruff, N. C. 1927. A disease of cotton roots produced by Fusarium moniliforme Sheld. Phytopathology 17:227-238.

160. Wright, J. M. 1956. Production of gliotoxin in soils. Nature 177:896.

161. Wright, J. M. 1956. Biological control of a soilborne Pythium inspection by seed inoculation. Plant and Soil 8:132-140.

162. Wright, Neal. 1961. A programed-controlled environment plant-growth chamber. Univ. of Ariz. Agr. Exp. Tech. Bull. 148. 31 p.

163. Zentmeyer, G. A. 1963. Biological control of Phytophthora root rot of avacado with alfalfa meal. Phytopathology 53:1383-1387.