SOIL PRODUCED ANTIBIOTICS—PLANT DISEASE AND INSECT CONTROL

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The soil is the natural habitant of coutless hordes of microorganisms, consisting of many kinds and types, living in a constantly changing environment. Most of them are active one way or another in reducing complex organic substances to simpler forms. Usually their existence produces a healthy soil and a more favorable environment for plant growth. The latter is brought about in part by increasing the food supply and improving the structure of the soil. Some of the organisms are injurious, however. They can cause a variety of diseases among cultivated plants. During recent years it has been discovered that the organisms which produce the "wonder" drugs (*e.g.*, penicillin) used in treating certain human ills also produce these antibiotics in the soil. Certain of these antibiotics have been found capable of destroying or inhibiting the development of a number of plant pathogens, viruses, and insect pests, both in the laboratory and under field conditions.

Presumably these antibiotics are produced in the soil for the protection of the microorganisms producing them. The apparent purpose is to enable the particular organisms to hold their own in competition with numerous others, perhaps by insuring the production of an uninterrupted supply of food and energy. This is accomplished through the elimination or control of plant pathogens, viruses, and insect pests that prey on their food producing plants.

These antibiotics may destroy the plant pathogens either directly by attacking them in the soil or by acting systemically. In the latter case, the antibiotics, after being absorbed by the roots, are translocated to other parts of the plant, rendering the penetrated tissues toxic (1, 2, 3). Certain plant pathogens, viruses, and insect pests which invade or attack the plants are thus destroyed.

Many of the organisms which cause plant diseases live in the soil, and it has long been known that the composition of the soil microflora can exert a controlling influence on them. This effect is connected closely with microbial antagonism. When a soil has been sterilized, competition between microorganisms is reduced or eliminated; when such a soil is invaded later by a parasite, the disease spreads much more quickly and is more virulent than in a natural nonsterilized soil. Thus, the microflora of the soil acts as a barrier to the invader.

Organic substances high in carbonaceous materials appear to be particularly favorable to the development of antibiotic producing microorganisms in the soil. The limited data available on this point suggest that it may have practical possibilities under field conditions in controlling plant diseases and insects by using suitable cropping systems and the proper utilization of the resultant plant residues.

It has been found that the most intensive microbiological activity occurs in the rhizosphere. The microbiological population and activity also vary widely within different parts of the rhizosphere. The antibiotic and associative interactions between groups of microorganisms are thought to be intensified in the rhizosphere by the effect of root growth and decay on the numbers and kinds present. This intensified activity is believed to account for the production of substances beneficial to higher plants when absorbed by their roots. This intensified microbial activity in the rhizosphere is thought to be related closely to other conditions existing in soils. There is a vast difference in the microflora of the rhizosphere of disease resistant and disease susceptible varieties and strains of crops grown in the same soil under identical conditions. Antibiotics produced in the soil have prevented some plant diseases by inhibiting or destroying pathogenic microorganisms, either in the soil or systemically when absorbed by roots of higher plants.

It appears that those genetic factors which impart disease or insect resistance to a plant may do so by enabling that plant to produce a more favorable microflora in the rhizosphere. The

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modified microflora of the rhizosphere of resistant varieties may be capable of producing antibiotics which destroy or inhibit the disease producing germs, the invading viruses, or the attacking insect pests.

ANTIBIOTIC PRODUCING SOIL MICROORGANISMS

The microflora of the soil is very complex. Almost all of the vast number of kinds and types of organisms are subject to numerous antagonistic as well as associative, or even symbiotic, interrelations. A radical change in the soil in one respect is followed by equally radical changes in many other respects until new conditions of equilibrium are obtained. This is especially true of the soil bacteria. If the type of food to support one type of organism is exhausted, that which is left will support some other kind. This shift in turn will cause numerous changes with far reaching results (4).

Tests by Lochhead and Landerkin (5) on the interaction of 90 strains of actinomycetes showed that a wide range of cross-antagonisms existed within a group of 11 strains found to be antagonistic to *Streptomyces scabies*. These strains were isolated by nonselective procedure from the rhizosphere of potatoes grown in scab infected soil which had been modified favorably by organic amendment. The possible importance of this in the study of specific antagonist-pathogen relationships is indicated, as well as that of the "rhizosphere effect" in modifying the microbial equilibrium in the soil. Some forms, such as the *Actinomycetes* spp., can produce such antibiotics as actinomycin, streptothrycin, and streptomycin (6, 7, 8, 9, 10, 11, 12). These substances may be beneficial, neutral, antagonistic, or inhibitive to other forms (13). They can destroy forms pathogenic to cultivated plants, and they also may produce antibiotics with insecticidal qualities (14). On the other hand, the same species of microorganism can manufacture more than one antibiotic. The different antibiotics produced by the same species of microorganism differ greatly from each other and affect a different range of organisms.

It has been shown that antibiotic producing strains of *Streptomyces rimosus* and *S. aureofaciens* inhibited sensitive organisms in soil (15). A streptomycin producing strain and a nonproducing mutant of *S. griseus* depressed the development of *Bacillus subtilis* (16). Penicillin had similar effects on *Pythium debaryanum*. The addition of *Trichoderma lignorum* to sterilized soil simultaneously with the pathogen *Rhizoctonia solani* prevented the damping-off of citrus seedlings (17). Numerous other instances of this type of biological control of plant diseases have been reported (18, 19, 20).

The effect of antibiotics operating in two or more combinations may be one of four types, namely, synergistic, additive, interfering, or indifferent, depending on the particular antibiotics involved (21, 22).

Meredith and Semeniuk (23) reported that 21 per cent of the strains of *Actinomycetes* isolated from two soils exhibited antagonism to *Pythium graminicola*, which causes seedling root necrosis. Under certain conditions the growth of *P. graminicola* was inhibited by the antibiotic produced by streptomyces. Claviformin, gliotoxin, penicillin, tyrothricin, eheiroline, and aspergillic acid were effective in inhibiting the growth of certain plant pathogenic bacteria and fungi (24).

Xylaria multiplex, an associated fungus, completely prevented the growth of *Chalara quercina*, the oak wilt fungus, when grown on laboratory media (25). Chloromycetin is synthesized by *Streptomyces venezuelae* and is active against many bacteria (26). This organism produced an antibiotic in the soil which had been sterilized previous to its infestation with actinomycetes. Alfalfa hay turned under was effective in causing *Streptomyces venezuelae* to produce chloromycetin in the soil. Under selected conditions, Meredith's actinomycete produced culture fluids toxic to many fungi (7). Musarin, the active principle, is a potent antifungal antibiotic. It is active against several important parasites and may be useful in combating a number of fungus diseases.

Certain antibiotic agents were found to exert selective fungistatic and even fungicidal properties (27). Actinomycin had a strong effect upon *Ceratostomella ulmi*, the causative agent of the Dutch elm disease. Penicillin cured crown gall on *Bryophyllum* (28). Actidione was highly active against certain yeasts, including the fungal pathogen *Cryptococcus neoformans* (29). Soils were found to contain substances of the actinomycin type which inhibit the growth of certain bacteria in culture media (30). These substances when isolated from the soil have both bacteriostatic and bacteriocidal effect upon certain bacteria.

Ahmad *et al.* (6) isolated a new antibiotic, antimycin A, from cultures of a *Streptomyces* species that is toxic to many yeasts and other fungi but is relatively inactive towards bacteria. Its effect on several of the more sensitive organisms is easily observable at concentrations of a few parts per billion. Waksman and Horning (31) isolated a number of fungi from soil which were antagonistic to bacteria. These fungi belonged to nine distinct taxonomic groups. Some produced antibiotics rapidly on laboratory media.

In studying the *in vitro* effect of aureomycin, chloromycetin, streptomycin, dihydrostreptomycin, penicillin, and bacitracin on bacterial strains of several species, Spencer (22) found that each antibiotic produced destructive action on the majority of the organisms in a sensitive strain. Dubos and Hotchkiss (9) reported that several species of aerobic sporulating bacilli isolated from soil, sewage, manure and cheese—as well as authentic strains obtained from type culture collections—exhibited antagonistic activity against unrelated microorganisms. Cultures of these organisms yielded tyrothricin which was bactericidal for most gram positive and gram negative microbial species.

ANTIBIOTIC PRODUCTION IN SOIL

Trichothecin was synthesized by the fungus *Trichothecium roseum* when grown in a variety of unsterilized soils (32); clay soils had a higher trichothecin producing capacity than sandy soils. It was adsorbed strongly by soil, but very little biological breakdown occurred. It has been demonstrated that chloromycetin can be produced in sterilized soil by *Streptomyces venezuelae* (26), and that the production and activity of this antibiotic were greatly increased by the addition of tryptone.

Johnson (20), after studying four types of bacteria antibiotic to certain smuts and fungi, suggested that antibiotic processes occur in nature. Martin and Gottlieb (15) found that antibiotic strains of *Streptomyces rimosus* and *S. aureofaciens* inhibited sensitive organisms in soil; the amount of antibiotic necessary for the inhibition of a sensitive organism in soil was dependent upon the pH of the soil solution. By the use of ether as an extracting agent, Waksman and Woodruff (12) demonstrated that soils contained substances of the actinomycin type, which inhibited the growth of certain bacteria in culture media. When isolated from the soil, these substances had not only a bacteriostatic but also a bactericidal action upon certain bacteria.

Siminoff and Gottlieb (16) found that both a streptomycin producing strain and a nonproducing mutant of *Streptomyces griseus* depressed the development of *Bacillus subtilis* in unamended and tryptone amended soil cultures. In studying the stability of ten antibiotics in Bangshot sand soils and in a neutral garden loam, Jeffreys (33) found that the rate of inactivation varied from soil to soil, but all exhibited a fair degree of stability in some of the soils. Gottlieb and Siminoff (26) found that chloromycetin could be produced by *Streptomyces venezuelae* when grown in unamended soil. Its production was greatly increased by the addition of tryptone and only slightly by the addition of green alfalfa hay. Oat straw and starch were ineffective. Gottlieb *et al.* (34) reported that both actidione and clavacin inhibited the growth of sensitive microorganisms in the soil. Actidione was produced in sterile soil by *Streptomyces griseus* when soybean meal was added. The amount of actidione synthesized in such soil depended on the concentration of soybean meal.

Aspergillus clavatus was antagonistic to *Bacillus subtilis* when sterile soil was infested with both of these organisms (34). Clavacin could not be found in soils infested with *A. clavatus* when different carbonaceous materials were added to the substrate. The presence of clavacin in soil caused the rise of a resistant microflora in the soil.

ANTIBIOTICS AND PLANT DISEASE CONTROL

In preliminary experiments with several plant species, Brian *et al.* (2) immersed the cut ends of shoots in an aqueous solution of griseofulvin (10 or 100 μg/ml). They reported that griseofulvin could be detected in the upper leaves in 7 to 14 days. These investigators also found that griseofulvin acted systemically, practically to control the fungus parasite, *Botrytis cinerea*, when grown in water culture nutrient containing griseofulvin. It also controlled *Alternaria solani* when used in nutrient solution on tomatoes grown in sand culture. Griseofulvin showed undoubted activity as a systemic fungicide. These investigations also produced results which indicated griseofulvin was produced in both sterilized and unsterilized soil.

Mitchell *et al.* (3) found that the application of a small amount of streptomycin sulfate to stems of primary leaves of bean plants, prior to inoculating the leaves with the halo blight organisms *Pseudomonas midicaginis* var. *phaseolicola*, prevented them from developing symptoms of the disease. Similar plants inoculated in a like manner developed very mild symptoms of the disease when dihydrostreptomycin sulfate was applied to their stems prior to inoculation. These antibiotics apparently were absorbed by the stems and translocated upward into the primary leaves in sufficient amounts to prevent growth and development of the organism. Similar treatment with these antibiotics prevented the development of the common bean blight caused by *Xanthomonas phaseoli* (E.F.Sm) Dowson. Streptomycin sulfate was absorbed by the stems of bean seedlings and translocated to trifoliate leaves 6 to 8 inches from the point of treatment in sufficient amounts to reduce the incidence of the infection and severity of symptoms produced by the blight organism.

Rhide *et al.* (35) found that of the 20 species of *Xanthomonas* tested, *X. cassiae* and *X. begoniae* were not inhibited by any of the actinomycetes. The other species were inhibited by one or more cultures of the actinomycetes. Of the 64 cultures of actinomycetes under study, seven proved antibiotic to three or more species of the bacteria tested.

Zoril (36) protected carnations against attacks from *Fusarium culmorum* and *F. oxysporum f. dianthi* by applying *Trichoderma lignorum* to the soil in which carnation plants were growing one week prior to the addition of the pathogens. The organism was parasitic on the fusaria.

In small scale greenhouse and field experiments with *Helminthosporium victoriae* blight of Vicland oats, Leben *et al.* (37) showed that a partially purified preparation of the antibiotic helixin B resulted in disease control in most tests. In similar experiments with Wisconsin Barbless barley, seed treatments with the antibiotic controlled the seedling blight incited by *H. sativum*. Seed treatment with the antibiotic was effective in limited field tests for controlling wheat bunt, oat smut, and covered smut of barley. Helixin B preparations acted across an air space to inhibit the germination of spores of a test fungus.

Zaumeyer (38) successfully stopped and controlled the halo blight of beans by spraying the foliage with water containing small amounts of streptomycin. Allen and Haenseler (18) reduced seed decay and damping-off by inoculating the soil heavily with a species of *Trichoderma*. Garden peas showed a similar but less marked response. These workers thought the *Trichoderma* may have had an antagonistic effect on the growth of *Rhizoctonia* and *Pythium*. The presence of an active principle, toxic or lethal to *Rhizoctonia*, was demonstrated in a liquid medium in which *Trichoderma* had grown for five days. The filtrate from a five day old *Trichoderma* culture was lethal to *Rhizoctonia* when used both in full strength or when diluted to 40 per cent or less with nontoxic diluents. *Rhizoctonia* failed to grow in a freshly prepared filter sterilized filtrate from a five day old *Trichoderma* culture but grew luxuriantly in a similar filtrate after the toxic principle had been inactivated or destroyed.

Patulin, previously obtained from *Penicillium patulum* Bainier, was shown by Anslow *et al.* (39) to be a metabolic product of the apple-rot organism, *P. expansum* (Link) Thom. It completely inhibited the growth of various species of *Pythium* at dilutions of approximately 1:400,000.

Anwar (19) found that 48 out of a total of 86 isolates of bacteria, actinomycetes, and fungi from soil were antibiotic to *Helminthosporium sativum* and 12 to *Fusarium lini* when grown on potato-glucose agar. The metabolic products of some of the bacteria, actinomycetes, and fungi inhibited the growth of *H. sativum* when added to agar medium.

A soil isolate of *Bacillus subtilis*, when added to steamed soil with *H. sativum*, completely protected barley seedlings against root rot. *B. subtilis* protected field plots of barley from heavy infection by *Helminthosporium*. *Trichoderma lignorum* and *Penicillium* sp. also gave partial protection from disease. Whiffen (40) reported that actidione, produced by *Streptomyces griseus*, was highly active against certain yeasts, including the fungal pathogen *Cryptococcus neoformans*. Young and Brandt (25) found that *Xylaria multiplex* completely prevented the growth of *Chalara quercina* (oak wilt disease) in agar plates.

Sugar beet pulp incorporated into a soil containing *Phytophthora parasitica* and inoculated with *Penicillium patulum* prevented damping-off of tomato seedlings (41). In this study, antibiotics were produced in a partially sterilized soil, and *Aspergillus terreus* produced them in sterilized soil. Timothy hay was a satisfactory source of organic matter for antibiotic production in autoclaved soil.

Brown and Boyle (28, 42) cured crown gall on *Bryophyllum* with crude penicillin. The galls were punctured in a number of places, and penicillin soaked antiseptic cotton was wrapped around them. The cotton was wetted frequently with crude penicillin. These workers found in laboratory studies that penicillin depressed *Erwinia carnegieana* Stranding, which has caused extensive destruction of the giant cactus in Arizona and New Mexico. Evidence was obtained indicating that *Corynebacterium spedeonicum* was also susceptible to the action of penicillin. Good control of damping-off of citrus seedlings due to *Rhizoctonia solani* was secured by adding *Trichoderma* sp. to a sufficiently acid, sterilized soil (17). *Trichoderma koningi* and *T. album*, as well as all isolates of *T. lignorum* tested, were found to attack the *Rhizoctonia*.

A number of fungi and bacteria exerted a marked antagonistic action against the pathogen *Ophiobolus graminis* on infected wheat seedlings (27). In addition to the living cultures, the culture filtrates were also effective. Growth of *Helminthosporium sativum* and *Fusarium graminearum* on sterilized soil was suppressed completely by adding small amounts of unsterilized soil or by simultaneous inoculation with harmless fungi and bacteria. Wheat seedlings produced from seeds inoculated with this soil were free of infection. Although *H. sativum* sporulated rapidly in sterilized soil, it did not do so in unsterilized soil; sporulating was inhibited in the unsterilized soil by microorganisms.

Leben and Keitt (43) demonstrated antibiotic properties of antimycin against certain phytopathogens and found that it was an extremely potent fungicide, producing inhibitory effects against *Nigrospora sphaerica*. A species of *Streptomyces* was antagonistic on agar to the 33 fungi tested but was not antagonistic to a number of bacteria. The antibiotic, which was tentatively named antimycin, was produced in shake flasks and by tank fermentation. Hessayon (44) found that small concentrations (0.00018–0.0018 units/ml) of trichothecin stimulated the growth of *Fusarium oxysporum* var. *cubense* when grown on laboratory media. In higher concentrations (0.0018–10 units/ml) the effect was fungistatic. Above these amounts there was a killing effect, and with 44 units/ml the effect was wholly fungicidal. He was able to produce trichothecin in a sterile soil by *Trichothecium roseum*. Risbeth (45) found that *Fomes annosus*, the fungus causing root rot of *Pinus sylvestris* and *P. nigra* var. *calabrica*, was suppressed on laboratory medium by *Trichoderma viride*.

Cooper and Chilton (46) reported that the four major sugarcane soils of Louisiana showed a consistent difference in the number of *Actinomyces* spp. antibiotic to *Pythium arrhenomanes*, one of the important fungi causing root rot of sugarcane. The number of these organisms present was in direct relation to the amount of organic matter in the soil; the Yahola silt loams had the highest number, the Yazoo silt loams the next, the Iberia silt loams and first terrace soils were third, and Sharkey clays the lowest. Comparative yield tests during five years with sugarcane on the various soils gave yields correlating with the number of species of antibiotic *Actinomyces*. In greenhouse tests in sterile soil, certain of the *Actinomyces* increased in the soil and reduced root rot in corn.

Stessel *et al.* (47) isolated 170 antagonistic organisms from approximately 70,000 colonies obtained from soil during a screening test designed to discover antagonists suitable for plant disease control. Approximately 80 per cent of these were actinomycetes. Of the one hundred and two kept for further studies, a few organisms were antagonistic to four or more of the 10 phytopathogens tested.

Gregory *et al.* (48) obtained from 26 samples of soil, peat, and chaff, 31 bacteria, 29 actinomycetes, and 14 fungi which were strongly antagonistic to *Pythium debaryanum*. Of the most potent cutlures, three actinomycetes and one bacterium were innocuous to *Rhizobium meliloti* and *R. trifolii*. The antibiotics, actidione and fradicin, were also active against *Pythium* sp. but inactive against *Rhizobium* sp. Clavacin (*Penicillium patulum*) and gliotoxin (*Trichoderma lignorum*) were toxic to species of both *Pythium* and *Rhizobium*.

Penicillium patulum, *Trichoderma lignorum*, *Streptomyces* sp., strain A67, and *Bacillus* sp., strain B6, were the antibiotic producers. Each organism produced potent antibiotic activity in sterile soil containing suitable organic material. *Bacillus* sp. B6 produced more activity in suitably treated soil than in liquid culture. *P. patulum* and *Bacillus* sp. B6 produced appreciable amounts of antibiotic in nonsterile soil containing 0.5 per cent soybean meal, 0.5 per cent glucose, and 0.2 per cent corn steep liquor. Whiffen *et al.* (49) found that the growth of the fungal pathogen, *Cryptococcus neoformans*, was inhibited in a 1:100 dilution of a beer of *Streptomyces griseus* containing 130 μg per ml of streptomycin, that the organism was not inhibited by 285 μg per ml of highly purified streptomycin.

In testing a group of 197 cultures of actinomyces for their antibiotic activity against *Ceratostomella ulmi*, Lechevalier *et al.* (50) found a strain of *Streptomyces griseus* which produced an antibiotic candicidin that was active against yeasts, yeast fungi, and *C. ulmi*. Crude candicidin was fungicidal against growing and resting cells of *Candida albicans* in the concentration of 1 to 5 mcg/ml and had no injurious effects upon the germination of pea seeds in concentration of 125 mcg/ml. Spraying young bean plants once a week with an aqueous suspension of crude candicidin (660 mcg/ml) resulted in a decrease of mildew infection.

Sanford and Bradfoot (51) working with 26 cultures of fungi and 40 of bacteria, nearly all of which were isolated from the soil, found six cultures of fungi and 15 of bacteria that suppressed the pathogenicity of *Ophiobolus graminis* to a degree varying from almost zero to a 10 per cent infection rate. It was concluded that the toxicity of the living cultures or of their filtrates was the chief factor in suppressing the virulence of the pathogen, and that many soil inhabiting fungi and bacteria are effective in suppressing the pathogenicity of *Ophiobolus graminis*. By the addition of other fungi to the soil, Luijk (52) found that it was possible to inhibit the parasitism of *Pythium volutum* on *Agrostis stolonifera*. *Pullularia pullulans* formed substances growth inhibiting to *Pythium* species when grown on laboratory media. The rotting of grass seedlings by *Pythium* was inhibited by products formed by *Pullularia*, and neither seeds nor the seedlings were damaged by these substances. *Pythium* species were antagonistic mutually in nonsterile conditions.

Applications of fresh organic matter destroyed certain plant pathogens originally in the soil (53). Corn, normally immune to *Phymatotrichum* root rot, was attacked and killed readily when grown in sterile culture without its normal complement of root surface bacteria (54). Browning root rot of cereals, formerly a serious disease of summer-fallow crops on the Canadian prairies, is seldom encountered. A change to farming practices of returning practically all of the crop residues to the land and liberal use of phosphate fertilizer are credited with this change (55). It is contended that the fertilizer stimulates faster growth of the seedlings. Incorporation of crop residues and trashy fallow act mainly through the complex microbiological activities that prevent a flare-up of the parasitic soil fungi (*Pythium* sp.) at the time the host plants are in the seedling stage.

The antibiotic griseofulvin is produced by the fungus *Penicillium nigricans*, which is abundant and widely distributed in natural and agricultural soils (56, 57), as well as by the less common *Penicillium griseofulvum* (2). It can be taken into plants through their root systems and then can be translocated to other parts. It has marked fungistatic properties and is active as a systemic fungicide. Similar activity is possessed by certain aryloxyaliphatic acids (58) and 4-nitrosopyrazoles (59). The use of systemic compounds was effective in treating Dutch elm disease (60, 61).

CARBOHYDRATE LEVEL AND DISEASE CONTROL

Soybeans grown as a cover crop and incorporated into strawberry root rot soil caused a striking reduction in the incidence of root rot and a drastic shift in the bacterial equilibrium of the soil according to West and Hildebrand (29, 62). A red clover cover crop turned under had little effect on either the severity of the disease or the general microflora of the soil. Their study of "rhizosphere effects" revealed that the characteristic differences between the resultant bacterial equilibrium of the soils in which the two leguminous plants were grown could not be attributable to influences exerted by the latter in the living state. However, the bacterial types favored during decomposition in experimental cultures of tissues of red clover and soybean, each inoculated with root rot soil, were identical with those isolated from root rot soil with which red clover and soybean, respectively, had been incorporated. In contrast with the putrefactive decomposition of red clover, soybeans apparently underwent a carbohydrate breakdown that could be reproduced essentially in culture by the substitution of glucose for soybean tissues. Beneficial changes in the bacteriology of actual root rot soils could be induced by the decomposition of pure carbohydrate in the place of soybean. The favorable alteration in the bacterial equilibrium was accompanied by a corresponding modification of the fungus flora such that potentially pathogenic forms were replaced by presumably innocuous ones. These carbohydrate treated soils were capable of producing strawberry plants with well developed healthy root systems. The ability of soybeans to control strawberry root rot, therefore, seemed to depend primarily on a carbohydrate type of breakdown in diseased soil causing a highly favorable shift in the microbial equilibrium. The decomposition of red clover, on the other hand, did not induce these salutary effects under the same conditions.

After altering the amount and source of carbon in the laboratory media, Luijk (52) concluded that carbon was the most important substance causing inhibitory effects. He thought that the inhibiting substance was a metabolic product of the carbon compounds.

Grossbard (63) showed that sugar beet pulp was a very satisfactory material for the production of an antibiotic by *Penicillium patulum*. *P. patulum* produced an antibiotic when cultured on sterilized soil to which either wheat straw or glucose or sugar beet pulp was added. *Aspergillus terreus* and *Streptomyces antibioticus* also produced antibiotics when grown on wheat straw. The susceptibility of cotton plants of fruiting age to *Phymatotrichum* root rot, both in potted soil and in the field, was related to the carbohydrate content of the bark (54). With increasing carbohydrate concentration, resistance was increased, and plants sufficiently high in carbohydrates withstood attack.

The correlation between carbohydrate concentration and the resistance of cotton to root rot was believed to reflect antibiotic protection at the higher carbohydrate level. The microbiological equilibria on the surface of cotton roots were altered markedly as the carbohydrate concentration within the roots was increased. The number of certain organisms tended to increase through successive carbohydrate levels whereas others decreased. The existence of an important interaction was demonstrated between root-surface saprophytes and the parasitic activity of the root fungus, *Phymatotrichum omnivorum*, by means of experiments with maize. After inoculation with *P. omnivorum*, maize plants growing on sterile sand-bentonite substances were rapidly attacked and killed. The roots of maize plants on otherwise similar but nonsterile substrates remained healthy even though paralleled for long distances by strands of the fungus.

Clark (64) noted that the *Pseudomonas* types of bacteria from soil differed from those associated with roots. Most of the soil isolates did not utilize sucrose or hydrolyze starch, whereas the majority of root isolates fermented the more complex sugars.

ANTIBIOTICS AND VIRUS CONTROL

Antibiotics produced by a number of soil inhabiting organisms have been found to inhibit certain plant viruses. Two general theories, accounting for the mechanism by which this is accomplished, have been advanced. According to the first, the inhibitor enters into a loose but reversible combination with the virus (65). According to the second, the inhibitor alters the physiology of host cells so they no longer support virus multiplication (8, 11).

Bawden and Pirie (65) found that the addition of trypsin immediately reduced the infectivity of potato virus "X" without affecting the flocculating power with antiserum. Incubation with crystalline preparations of trypsin and pepsin destroyed both the infectivity and the power of reacting with antiserum.

Filtrates of 5 different strains of *Neurospora sitophila* and one strain of *N. crassa* were tested for ability to inhibit infection by Southern bean mosaic, tobacco mosaic, and tobacco necrosis viruses by Slagle *et al.* (11). All filtrates were highly effective in reducing the number of infections, depending upon the host plant used. It was effective immediately after mixing with virus, no time reaction being involved. It inhibited infection whether applied directly to the virus or to host-plant leaves either before or up to 30 minutes after inoculation, and when applied to the leaf surface opposite to that on which the virus was inoculated as well as to the same surface.

Trichoderma sp. grown in liquid medium produced a substance which had caused up to 90 per cent reduction in the infection capacity of the tobacco mosaic virus, measured in number of local lesions on *Nicotiana glutinosa* half leaf inoculation (10). Under the experiments, the fungus was grown in liquid medium and the filtrates used in inactivation tests on the virus. It was concluded from the fact that the liquid filtrate showed inactivation power after only two days of culture, that the inactivation is of the nature of a secretion of the fungus into the liquid medium. The inactivation proceeded within two minutes after mixture of the culture extract with the virus; no better results were obtained if the mixtures were allowed to stand for a longer period of time.

Stanley (66) prepared a trypsin-virus solution which when sprayed or rubbed on the leaves failed to produce lesions on plants of *Phaseolus vulgaris* but produced many lesions when tested on plants of *Nicotiana glutinosa* having about an equal susceptibility to untreated virus. He concluded that this showed that trypsin in some way affects the cells of plants of *P. vulgaris*. Trypsin spread or rubbed on the leaves of plants of *Nicotiana glutinosa*, *N. tabacum* var. Turkish, and *Phaseolus vulgaris* markedly lowered their susceptibility to virus. A number of other viruses were affected similarly by trypsin. They were a masked and a yellow strain of tobacco mosaic, aucuba mosaic, severe etch, tobacco ring spot, ordinary cucumber mosaic, and a yellow strain of cucumber mosaic virus. Trypsin was thought to inhibit infection by killing injured cells that otherwise could serve as entry points for virus (67) or by forming a loose complex with the tobacco mosaic virus (65). It reduced the infection of tobacco mosaic virus, and its effect was immediate.

ANTIBIOTICS AND INSECT CONTROL

Recent discoveries, relating to the production in the soil of antibiotics by microorganisms and of the systemic action of certain insecticides when absorbed by plants (1, 68, 69, 70, 71, 72), may lead to other discoveries showing nature has built into the soil, by means of organic matter and microorganisms, a system which may be utilized to protect plants against diseases and insects. It was shown earlier in this paper how some of the antibiotics that are produced in the soil function in keeping certain soil plant pathogens from running rampant. The possible role of these antibiotics in controlling insects is no less intriguing.

Insecticidal qualities of certain plants have been known for centuries. To date, more than 2,000 species of higher plants are said to have value as insect killers (73). The active principle includes alkaloids, pyrethrins, wilfordine, nicotine, anabasine, sesamin, oils, asarinin, nornicotine, glycoside, conine, scarbin, and others. The insecticidal principle may be present in the leaves and leaflets, roots, bark and wood. The insects affected by these insecticides include a wide variety. Only recently, however, was it discovered that certain microorganisms in the soil produced antibiotics of insecticidal qualities and that substances are assimilated by higher plants through their roots and are systemic in nature.

Kido and Spyhalski (14) isolated antimycin A from cultures of an unidentified species of *Streptomyces*. The initial tests showed that the antibiotic caused mortality to some insects which ingested the material. The toxicity of antimycin A was not confined to members of the Insecta, as it showed efficacy for the control of the red spider mite, *Tetranychys* sp.

Davich and Apple (74) obtained satisfactory control of aphides on peas by use of systemic insecticides. The systemic insecticides used were octamethyl pyrophosphoride, trialkyl selenophosphate and trialkyl thio-phosphate. They were effective in controlling pea aphides when used in proper amounts and applied to the soil as a spray prior to planting the peas or to the leaves of the growing plants. Beck (75) reported that antimycin A had insecticidal possibilities for some species of insects and mites. The antibiotic inhibited either the succinoxidase system or some other essential step in the oxidative metabolic cycle of cockroaches. Inhibition of the oxidative cycle readily explains the depression of oxygen consumption by the poisoned insects.

SYNERGISM

Bigger (21) reported that the antibacterial action of six substances—sulfathiazole, penicillin, streptomycin, chloromycetin, boric acid, and *p*-aminosalicylic acid—acting in pairs on *Escherichia coli* in sympathetic medium was synergistic in most cases. Synergism was demonstrated with every combination except one; antagonism was demonstrated with five combinations. In only three cases was it marked and permanent, and in only one of these (boric acid and sulfathiazole) was each substance antagonistic to the other.

A study *in vitro* effect of penicillin and bacitracin mixtures against several strains of α- and γ-hemolytic streptococci indicated synergistic action between the two antibiotics instead of merely an additive effect (76). The synergistic effect varied from strain to strain. It was very pronounced with some cultures while less dramatic with others.

RHIZOSPHERE AND MICROBIAL ACTIVITY

The rhizosphere is shedding light on the importance of microorganisms in the production of crops as its nature and function become more clearly understood. It has been shown that plant roots support on their surfaces a population of bacteria which differs from that of the surrounding soil. It is of much greater density and differs widely in the type of its dominant species. Roots have been shown to provide the food supply of the rhizosphere organisms, partially in the form of soluble compounds which are excreted and partially as cellular structure such as root caps, root hairs, rootlets and epidermal and cortical cells, all of which are being sloughed off. The rhizosphere microflora has been shown to vary with the stage of growth of the plant and with the species and even with the variety of the plant; indications are that it is probably of importance in several respects (53).

The rhizosphere is a unique zone, exerting on many soil organisms a powerful stimulation which varies with the type, variety, age and vigor of the plant and the type, treatment and moisture content of the soil in which it grows (77). Any factor contributing to a modification of growth and physiological behavior of the plant then may be expected to initiate changes in the root microflora. Inasmuch as the nutrient material coming from the roots is primarily responsible for this immediate microbial concentration, this mantle of microorganisms attached to the root surface is perhaps the most important part of the rhizosphere.

Bakerspigel and Miller (78) found bacteria to be more abundant in the rhizosphere of iris, beans, and potatoes than in soil more distant from the rhizosphere. The rhizosphere microorganisms seemed about as sensitive to antibiotics as from control samples. Tests by Lochhead and Landerkin (5) on the interaction of 90 strains of actinomycetes, isolated by nonselective procedure from the rhizosphere of potatoes grown in scab infested soil which had been modified favorably by organic amendment, showed that a wide range of cross-antagonisms existed within a group of 11 strains, found to be antagonistic to *Streptomyces scabies*. The possible importance of this in the study of specific antagonist-pathogen relationships is indicated, as well as that of the rhizosphere effect in modifying the microbial equilibrium in soil.

The rhizosphere microflora varies both quantitatively and qualitatively from that of soil farther away from the plant as a rhizosphere is created following germination of a seed. A given antagonist, present in or added to the soil, finds itself in a changed environment. This might affect its capacity to thrive and exert its antibiotic action. To be effective, an antagonistic microorganism must be able to maintain its existence when exposed to the competition and antagonism of the indigenous soil microflora, as well as to exert its antibiotic action when the microbial balance is shifted and different competitions and antagonisms come into play through action of the growing plant.

Lochhead and Thexton (79) found that one of the most characteristic rhizosphere effects on bacteria of different nutritional requirements was the preferential stimulation of bacteria requiring amino acids for maximum growth. Organisms for which amino acids were either essential or stimulative were increased proportionately in the rhizosphere. No similar effect was noted with reference to bacteria responding to growth factors.

Three main processes are believed to take place in the complex and intricate relationships between the microorganisms of the rhizosphere and plant root (2). Excretion of soluble nutrients and sloughing off of dead tissues by the roots encourages the development of a microflora greater in numbers and qualitatively different from that of soil farther away from the roots. This higher level of microbial activity is thought to intensify the antagonistic and associative interactions between groups of microorganisms in the rhizosphere. Also, it is thought to influence the development of the plant through its effects on the roots.

In a study carried on with diseased and healthy soils, Timonin (80) found that a susceptible variety of oats harbored in its rhizosphere a denser population of manganese oxidizing, casein hydrolyzing, and denitrifying bacteria than the rhizosphere of a resistant variety when grown in the same soil under identical conditions. Furthermore, it was found that, on application of soil fumigants, such as chloropicrin, cyanogas, calcium cyanide, and formaldehyde, the bacteria capable of oxidizing manganese were greatly reduced or completely eradicated. The plants grown in such soils were free from symptoms of manganese deficiency disease and showed a marked increase in yield of grain. Application of straw mulch resulted in a denser population of manganese oxidizing and cellulose decomposing organisms, and more severe symptoms of the disease than the untreated soil.

In comparative studies of the rhizosphere of resistant and susceptible varieties of flax, Timonin (81) showed that the incidence of species of *Alternaria*, *Cephalosporium*, *Fusarium*, *Helminthosporium*, and *Verticillium* was relatively lowered and that of species of *Mucor*, *Cladosporium*, *Penicillium*, and *Trichoderma* increased by the "rhizosphere effect" of the resistant variety as compared with the susceptible variety or control soil. He also found that when the solutions in which plants had been grown were allowed to diffuse through collodion membranes into the soil, the microbial activities in the vicinity of the membrane were stimulated in a manner analogous to that in the natural rhizosphere of corresponding plants. The solution after growth of the susceptible variety, when added to liquid or solid media, induced a greater stimulation of growth of *Fusarium* and *Helminthosporium* than the solution after growth of the resistant variety. Chemical analysis indicated that the solutions after growth of resistant variety contained from 25 to 37 mg hydrocyanic acid per plant grown, whereas the solution after growth of the susceptible variety contained only a trace. Potassium cyanide when added to Crone's solution exerted an effect on the growth of the same fungi analogous to that produced by the solution after growth of the resistant variety.

Differences in rhizosphere effects may be obtained also with different varieties of one plant species. Obraztzova (82) found that the variety of tea-bush did not influence numbers of microbes but did affect their quantitative composition. Timonin (81) noted higher numbers of bacteria and, to a lesser extent, of fungi in the rhizosphere of varieties of flax and tobacco plants susceptible to soil-borne plant pathogens, though free from the disease, than in those of corresponding resistant varieties. Data by Lochhead and co-workers (83, 84, 85) support this observation. It was suggested that these results reflected inherent differences in physiological function, making, in the case of susceptible plants, conditions somewhat more favorable for general bacterial development. In this connection the interesting work of Eaton and Rigler (54) showed that as the carbohydrate content of cotton roots increased the total bacterial count on the root surface decreased, suggesting that it is possible to produce within a single variety of cotton differences in bacterial numbers and root rot susceptibility somewhat analogous to those that exist between different varieties of flax and tobacco.

Thom and Humfeld (86) reported considerable variations in absolute counts and the rhizosphere: soil (R:S) ratios of rye in three different soils. A Keyport clay loam gave ratios of 6 and 5 for fungi and bacteria as compared with 15 and 16 on roots of plants in a Collington fine sandy loam. In a study of corn growing in different soils varying from pH 4.5 to 8.1, bacteria were found to be more numerous under slightly acid or neutral conditions, and fungi were more abundant on roots in the most acid and most alkaline soil, whereas actinomycetes were not affected by soil differences. Adati (87) showed that, on the whole, R:S ratios were highest on loam with sand, clay, and humus soils next in order of decreasing effectiveness. This order held for the three major groups of organisms studied. The influence of soil type on the rhizosphere microflora was observed also by other investigators (78).

Chromogenic forms occurred in greatest relative abundance in tobacco rhizospheres; other plants showed similar though not such striking differences (73). A somewhat more active microflora was observed in the rhizosphere of susceptible varieties of flax and tobacco. Bacteria requiring or stimulated by amino acids were relatively more abundant in the rhizosphere of both flax and tobacco plants than in the control soil, especially in the rhizospheres of susceptible varieties (84). Similar observations were made with bacteria requiring growth factors in addition.

Working with flax, Timonin (88) reported that of 19 genera isolated, nine were obtained from both rhizosphere and control soil and ten from the rhizosphere only. Certain genera—*Alternaria*, *Aspergillus*, *Cephalosporium*, *Fusarium*, *Helminthosporium*, and *Verticillium*—were numerically more abundant in the rhizosphere of a variety of flax susceptible to root rot, whereas others, such as *Mucor*, *Cladosporium*, *Hymenula*, *Penicillium*, *Scolecobasidium*, and *Trichoderma*, were more abundant in the rhizosphere of a resistant variety.

In the rhizosphere, the phenomena of association, antagonism, and competition for oxygen and food are perhaps even more intense than in the soil proper, owing to the denser population at the root surface and its greater physiological activity. Here the influence of root physiology and excretions may play an important role, directly by stimulating or repelling the pathogens or indirectly by affecting the rhizosphere microflora (89). Lochhead and co-workers (79, 85) showed that plants susceptible to certain root rots induce a greater rhizosphere effect than corresponding resistant varieties, suggesting that resistance to a certain disease may be linked with a selective action of root excretions upon the saprophytic soil microflora. This favors types which may be more and in other cases less antagonistic towards pathogenic organisms (85). In this connection, it was demonstrated (88) that a wilt resistant variety of flax excreted hydrocyanic acid into the surrounding medium, thus exercising a selective power upon the fungus flora of the rhizosphere. The cyanide depressed pathogenic fungi, such as *Fusarium* and *Helminthosporium*, but appeared to favor *Trichoderma viride*, an organism mentioned frequently for its ability to suppress development of other fungi (27, 90, 91). On the other hand, by-products of growth of a susceptible variety stimulated growth of two pathogens. Eaton and Rigler (54) showed that cotton plants with low carbohydrate concentration were attacked most severely by *Phymatotrichum omnivorum*, had largest number of bacteria and lowest number of blue-green fluorescent bacteria in their rhizospheres, and suggested that the resistance of the cotton to root rot reflected antibiotic protection at the high carbohydrate level. In further work, they demonstrated that the immunity of maize plants to *Phymatotrichum* root rot is attributable to protection afforded by its root-surface microflora.

Since soil treatment exerts marked effects on the soil microflora, the question naturally arises as to whether similar effects may be expected in the rhizospheres. Katznelson (84) obtained definite evidence of stimulation of certain groups of organisms in the rhizosphere of mangels growing in manured soil. By means of the qualitative-nutritional approach, Hildebrand and West (62) working with strawberries showed that the incorporation of soybean and red clover into the sterilized soil altered the bacterial equilibrium in both soil and rhizosphere so that untreated root rot soil gave bacterial balance index[2] values of −46 and +10 for rhizosphere soil apart from the root, respectively, as compared with +44 and +35 for steam treated soil. General agreement was noted between this index and severity of the disease. No such correlation, however, was observed in relation to manganese deficiency disease of oats (80). Hildebrand and West suggested that the organisms responsible for lowering the bacterial balance index may actually be involved in production of the disease symptoms. However, Katznelson (84) pointed out that it was possible that these organisms were secondary rather than primary invaders, increasing in abundance on the root following infection by other organisms, such as nematodes or fungi, or as a result of treatment and thus giving a low bacterial balance index.

West and Hildebrand (29) and West and Lochhead (92) showed, in addition, that the decomposition of soybean or glucose in root rot soil or the addition of acetic acid resulted again in a favorable alteration of the bacterial equilibrium and permitted growth of strawberry plants with well developed healthy root systems. These treatments also modified the fungus flora on the roots of strawberry seedlings grown in the treated soil so that potentially pathogenic forms (*Fusarium*, *Rhizoctonia*, *Cylindrocarpon*) were replaced by presumably harmless ones (*Penicillium*, *Mucor*, actinomycetes and yeasts). Steaming reduced bacterial and actinomycete numbers in soil very markedly for about three months, yet the number on the healthy roots of tomatoes was as high as on those of untreated soil, implying a protective and ameliorating influence of the plant on the soil microflora in the rhizosphere. Katznelson (84) in general obtained results very similar to those of West and Hildebrand (29), particularly with fungi. That dried blood added to root rot soil induced healthy root formation was of particular interest.

Zukovskaya (92a), working with potatoes, flax and clover, found that the microbial population of the rhizosphere was 100 times as great as in the soil away from the root. Changes of this microflora coincided with the development of the plant. She also claimed that each plant, grown in the same soil, enhanced the activity of a specific microflora of its own.

[2] The equilibrium between presumably "harmful" bacteria and the innocuous, normally occurring rhizosphere types is designated as the *bacterial balance index*.

Using flax, Berezova (93) concluded that different types of organisms were associated with plants at different stages of growth. Some were found only during certain stages of development, some during all stages, and others were present only at maturity.

DISEASE RESISTANCE

It has been known for many years that some varieties of plants are resistant to certain diseases while others grown under identical conditions are not. This resistance factor is traceable to specific genetic characteristics (46, 94–102). Plant breeders use this knowledge in breeding resistance into new varieties. More recently the same situation has been found to be true with certain insects (103).

Thus far no satisfactory explanation has been advanced as to just how a specific gene, a gene pair, or combination of genes actually imparts resistance to either disease or insect. Apparently it has been assumed that this influence is exerted within the plant. Recent discoveries in other, but related fields, indicate that this influence may be exerted by influencing the microflora of the rhizosphere. These findings indicate this genetic influence may determine the quality of substances excreted or sloughed off by the growing roots. These products in turn determine the composition of the microflora of the rhizosphere.

Walker and collaborators (104) showed that certain brown-skinned onion varieties are resistant to smudge (*Colletotrichum citcinans*) because of the presence of protocatechuic acid and catechol in the tissue. Greathouse and Rigler (105, 106) also demonstrated this principle in their characteristic series of *Phymatotrichum omnivorum* to different phenolic compounds and alkaloids. According to Johnson and Schaal (107) resistance to common scab (*Streptomyces scabies*) of potato varieties is related closely to the presence in the superficial layer, especially in and near the lenticels, of chlorogenic acid, a phenolic compound, and possibly also of tyrosinase. Chlorogenic acid or its quinone also accumulates in the periderm and around injuries whether mechanical or parasitic in origin.

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