INTERRELATIONS BETWEEN MICROORGANISMS AND PLANT ROOTS IN THE RHIZOSPHERE[1]

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I. INTRODUCTION

Plants affect microorganisms in soil in many ways. They remove water and mineral salts from the soil and, on death, leave their roots in the soil and the aerial parts on the soil surface. There is a decrease in the amounts of soluble mineral substances and a desiccating effect on the soil. During periods of growth, the roots permeate the soil, affecting its structure and the movement of gases and water. The roots attract various organisms, some of which feed on them directly. Roots absorb oxygen and release carbon dioxide, thus increasing the amounts of carbonates. The root area is a critical one for terrestrial plants and the site of intense chemical and biological activity in soil.

This region of contact between root and soil where the soil is affected by roots was designated the "rhizosphere" by Hiltner in 1904. He recognized that there were many microorganisms in this region, and it was his opinon that the rhizosphere microorganisms play important roles in plant development. Where reference is made to soil in comparison to rhizosphere in the following discussion, reference is made to that portion of the soil that is not the rhizosphere. The rhizosphere has indefinite dimensions, depending on the soil and plant. The greatest effects of the plant appear on the root surface and in the soil in contact with the root, but effects may extend for several millimeters beyond the root where fungus mycelium penetrates the soil from the rhizosphere which is the food base. Clark (26) proposed the term "rhizoplane" for the external root surface and closely adhering particles of soil and debris. The term rhizosphere is used herein because it has a wider acceptance than the term rhizoplane.

Most rhizosphere microorganisms are saprophytes, but not all of their relations to plants are incidental. Some microorganisms live on the root surface, whereas others penetrate the roots. Some are restricted to the cortical cells, but others go deeper, passing between the cells and invading them. Some are innocuous and others are destructive or have favorable effects on development of the host. In this discussion attention will be focused on the nonsymbiotic and nonpathogenic microorganisms.

The early literature has been discussed in comprehensive reviews by Katznelson *et al.* (59) and Clark (26). Consequently, in this report principal attention is directed to recent observations.

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II. THE EFFECTS OF PLANTS ON MICROORGANISMS IN THE SOIL

A. Abundance of Microogranisms in the Rhizosphere

In a generally overlooked report on interrelations between plants and microorganisms, Hoffmann (48) reported in 1914 that bacteria were generally more numerous adjacent to plant roots than in soil a foot or more distant from the plants. Numbers were greater adjacent to the roots in 27 of 32 tests, but the differences were smaller than those noted in more recent experiments. Many subsequent studies concerned with populations clearly established that microbial cells were much more abundant in the rhizosphere than in the soil (116–119). According to the plate method, the increase in numbers of aerobic bacteria was much higher than that of actinomycetes and filamentous fungi. Certain bacterial types were more affected by rhizosphere conditions than others. For example, *Agrobacterium radiobacter* was affected proportionally more than the general bacterial population. However, cells of this bacterium represented but a small portion of the total bacterial population.

The fact that the microbial population is dense on plant roots and that this occurs at all stages of plant growth and not only after the plants have died has been verified repeatedly. The importance of the phenomenon is twofold: the plant roots affect microbial development, and the plant in turn is affected by the increased activity of the microorganisms in the rhizosphere. Details of the causes and effects are still obscure. The information is suggestive but, save for exceptions, is inadequate to indicate the extent to which plant development is affected by the rhizosphere microorganisms, and whether plant growth is enhanced or impaired.

Evidence that there is a more abundant population of microorganisms in the rhizosphere than in soil rests not only on plate counts but on visual evidence. By means of the Cholodny buried slide technique it has been shown (120, 123) that not only large roots but small roots and root hairs have large numbers of bacteria on the surface and inside of the cells as well. In some cases the bacteria occur as a mantle of cells about the root hairs. Filaments of actinomycetes and filamentous fungi were occasionally detected also. With death of the root parts a mixture of cells of diverse microorganisms appears.

This effect was demonstrated by Linford (70) by other procedures. In one case roots were stained after their removal from soil. He also noted development of large colonies about newly formed roots of corn, lettuce, cowpea, and pineapple plants. In another case, seedlings were grown in small soil boxes with windows consisting of cover glasses. The roots that grew against the glass windows could be examined in place microscopically. Essentially the same results were obtained by both procedures; large colonies of bacteria developed about the young growing roots. Another staining method was used by Rovira (103) who determined the degree of bacterial development on roots of seedlings grown in a mineral solution on quartz sand. The results were like those of Linford in that the bacteria were abundant during the earliest period of root growth.

From determinations of numbers of bacteria on the seeds and roots of seedlings of tomato and oats, Rovira observed that bacteria were present on the dry seeds, and that they developed rapidly on the roots as the seeds germinated. The first oat roots had many bacterial cells, whereas on tomato roots the bacteria developed more slowly. He reported that root tips were free from bacteria, whereas other results (70, 120) suggest that this may not be the case invariably. Filamentous fungi were found occasionally on oat roots but were absent from tomato roots. Rovira clearly indicated that there was extensive bacterial development on seeds of oats and tomatoes almost from the start of germination.

Metz (82) noted that, when seeds germinated in soil under nonsterile conditions, bacteria produced mantles of growth about the roots and root hairs, more with roots of Cruciferae than Gramineae.

The nonsporulating, gram-negative rods are the most prominent group of bacteria in the rhizosphere, as determined both by direct staining and by tests of the bacteria recovered from agar plates. This has been verified repeatedly.

Gyllenberg (39) reported that the composition of the rhizosphere bacterial population of oats was similar from seedling stage to maturity, whereas the soil population differed from the rhizosphere population throughout most of the

period but became similar to it late in the season. This was ascribed to migration of the bacteria from the rhizosphere into the soil. It was observed also that the rhizosphere population of grasses was more abundant than that of trees (40).

Agnihothrudu (2) noted that fungi occurred predominantly as spores in soil (70 to 90 per cent) whereas they occurred mostly as vegetative material in the rhizosphere (>70 per cent). According to Garrett (34), root-inhabiting phytopathogenic fungi typically produce a spreading epiphytic growth over the surface of the root preceding and following root invasion. This occurs also with ectotrophic mycorrhizal fungi.

It has been reported that roots of varieties of some plants susceptible to wilt disease support larger bacterial populations than nonsusceptible varieties of the same plants. This was observed for black root rot of tobacco and for flax wilt by Lochhead *et al.* (77), Timonin (133), and West and Lochhead (141), and for Panama disease of bananas by Harper (45). Observations of Rombouts (101) failed to support the conclusions of Harper.

Buxton (21) found that exudates from roots of three varieties of peas had different effects on spore germination of three strains of *Fusarium oxysporum* f. *pisi*, a fungus causing pea wilt. The exudates had a greater depressing effect on the strains to which the plants were resistant than on those to which they were susceptible. Similar results were obtained with extracts of the rhizospheres of the three pea varieties (22).

Numerous rhizosphere studies indicate that the abundance of microbial cells is affected by the kind of plant and its stage of development and vigor. The greatest effect occurs during periods of active plant development and the effect disappears promptly on death of the plant. Therefore, it is an effect associated with normal growth.

In addition to bacteria, other groups of microorganisms are also more abundant in the rhizosphere than in soil. There is more development of both actinomycetes and fungi but generally the increase is proportionally less than that of the bacteria. Nevertheless, where mycorrhizae are formed, root and soil conditions are such that the fungi are the dominant root microorgansims and they have important favorable effects on development of various plants, coniferous trees in particular (42). According to Thornton (132), there were more fungi in the rhizosphere of wheat and clover than in soil, and the kinds of fungi recovered from the roots varied at different stages of plant growth. According to Tolle and Rippel-Baldes (134) the ratio of numbers of fungi in rhizospheres of cereals to numbers in soil varied from 1.4:1 to 3.0:1. Similar kinds of fungi were isolated from thoroughly washed roots of oats, wheat, rye, and barley.

Both protozoa and nematodes are frequently more numerous in the rhizosphere. The destructive effects of the latter are being increasingly recognized. Algae are little affected and may even be less numerous in the rhizosphere than in the soil.

B. Physiological and Nutritional Groups of Bacteria in the Rhizosphere

The results already mentioned indicate that the microbial population of the rhizosphere is quantitatively different from that of soil. Other results show that the population differs qualitatively. Katznelson (58) and Katznelson *et al.* (63) noted that the aerobic cellulose-decomposing bacteria, anaerobic gas-producing bacteria, and anaerobic bacteria in general, as well as ammonifying and denitrifying bacteria, were more abundant in the rhizospheres of wheat and mangels, whereas the nitrifying and anaerobic cellulose-decomposing bacteria were less numerous and *Azotobacter* showed no rhizosphere effect.

The abundance of the various groups of microorganisms does not necessarily reflect a proportional activity in the transformations implied by their designations, nor does it exclude this. For example, there is no evidence that denitrification is rapid in the rhizosphere, although it may be more rapid than in soil owing to the greater microbial activity which might provide local areas of anaerobiosis. Recent results of Skerman and MacRae (114, 115) show that there is no denitrification where there is a determinable quantity of oxygen in solution.

In order to characterize the bacterial population of soil, Lochhead and associates determined the ability of the isolated cultures to grow in media varying in complexity, that is, a simple medium and media containing accessory growth substances. Thus they estimated the relative abundance of soil bacteria of each nutritional group. These studies have provided a wealth of information on the soil population and the effects

of various soil factors on the proportional abundance of bacteria of the different groups. Many of the studies have been concerned with microorganisms of the rhizosphere. In brief, the following procedure was used. The soil material was plated on a soil-extract-agar medium and all of the bacterial colonies (frequently about 100) on a certain area of a plate were isolated. The ability of each to grow on the following media was determined: (a) basal medium containing glucose, nitrate, and mineral salts; (b) medium like (a) but supplemented with several amino acids; (c) medium like (b) but containing several vitamins also; (d) medium like (a) but supplemented with yeast extract; and (e) medium like (d) but containing soil extract also. On occasions, additional media were used. Ability to grow on the basal medium or requirement for growth factors served to characterize the bacterial population into a few groups that could indicate qualitative changes with soil treatment.

One of the most consistent results was the presence of a higher percentage of amino-acid-requiring bacteria in the group isolated from the rhizosphere than in the group obtained from the soil (75). It was reported also (139) that in the rhizosphere there was a higher percentage of bacteria that grew on the basal medium and a lower percentage of those with requirements for undetermined growth factors contained in soil extract. Young but not older plants had fewer bacteria requiring yeast extract. Among the preformed organic materials frequently required was the sulfur-containing amino acid methionine.

In referring to the predominance of amino-acid-requiring bacteria in the rhizosphere, Gibson (36) stated that this suggests leakage of amino acids but not of growth factors from the roots, whereas the work on mycorrhizal fungi indicates that growth factors, coming from the roots, are responsible to a considerable degree for the mantle of filaments of mycorrhizal fungi on the roots. Possibly this is an indication of differences in the materials exuded from roots of different plants.

It was found by Lochhead and Burton (74) that of 499 soil bacteria tested, a high proportion (27 per cent) required one or more vitamins preformed. The same authors (73) reported that the percentage of bacteria isolated from the rhizosphere that required vitamin B_{12} was somewhat smaller than that of bacteria obtained from soil. Nevertheless, the actual number of these bacteria was greater in the rhizosphere. It is presumed that both the vitamins and the amino acids required preformed were obtained either from the plant residues, from plant root excretions, or from excretions of associated microorganisms. Since vitamins and amino acids are susceptible to decomposition (110, 111, 121, 122) they are likely to be more available in those regions of the soil where there is extensive microbial development which would provide a continuing supply of the compounds.

Various bacteria recovered both from soil and rhizosphere produced extracellular amino acids and vitamins when cultivated on a simple medium with nitrogen supplied as nitrate (72, 76, 93) and it was presumed that, in associations of microorganisms in the rhizosphere, the fastidious bacteria would derive the required preformed organic compounds, at least in part, from the products of bacteria that excreted the materials. The percentage of the rhizosphere bacteria that excreted certain vitamins into the medium was not always greater than the percentage of the bacteria isolated from soil that did this, but the actual number was much greater. In one test of 30 cultures with simple nutritional requirements, 22 cultures liberated amino acids into the medium (93). Ten different amino acids were detected, but no more than 4 were produced by any one culture. It was concluded that excretion of amino acids is a fairly general property of soil bacteria that have simple nutritional requirements.

Rouatt and Katznelson (102) verified recently the fact that bacteria determined by the plate method were much more abundant in the rhizosphere than in the soil, that, of the bacteria isolated from plates, the percentage requiring amino acids was higher in the rhizosphere isolates than in the soil isolates, and that conditions were reversed for those bacteria that required yeast extract or both yeast extract and soil extract. Although the relative abundance of the bacteria of the various nutritional groups differed in the soil and rhizosphere, the cells of all groups were much more numerous in the rhizosphere. Possibly emphasis on the percentage increase in each group obscures the great increase in numbers of all of the nutritional groups. It is of interest that, of the isolates from both the rhizosphere and soil, the bacteria that grew on the simple basal me-

dium were much fewer than those having requirements for preformed organic substances.

Characterization of the bacterial flora of soil and rhizosphere by tests of cultures isolated from plates is affected by the procedure used, and different procedures yield different results. For example, by use of different methods from those of Lochhead, Taylor (125) found that the incidence of nutritional groups of bacteria isolated from several soils differed from that reported by Lochhead *et al.* King and Wallace (66, 138) used different physiological tests to characterize the bacterial population and found little consistent difference in the physiological groups in the cultures isolated from soil and rhizosphere. In general, there was little difference in the incidence of the groups of bacteria from rhizosphere and soil that reduced nitrate, fermented glucose, or hydrolyzed gelatin or starch. Some of the reasons for the differences in the results of Taylor and of Wallace and King from those of Lochhead *et al.* were discussed by Lochhead (71) and Lochhead and Rouatt (75).

There are various limitations inherent in any plating procedure for estimating numbers of microorganisms in soil or for isolating cultures for studies of physiological characteristics. By plating procedures one obtains colonies of only a small fraction of the soil bacterial population, probably less than 10 per cent. These organisms are the selected group that is able to grow on the medium used for their isolation. It is possible that important groups of bacteria are not recovered. Furthermore, whereas the ability of the bacteria to grow in various media characterizes the bacteria somewhat, little indication is obtained on what these bacteria do in the soil. As stated by Thornton (131): "When data have been derived from plate cultures, any generalizations based on these data, whether as regards the numbers or quality of the microflora, refer only to the species that will grow on the medium used."

C. Physiological Activity of the Rhizosphere Bacteria

From tests made with cultures isolated from the soil and rhizosphere, it was noted that the bacteria of the rhizosphere are physiologically more active than the soil bacteria. The mere fact that bacterial cells are more abundant in the rhizosphere than in the soil is evidence that they were more active physiologically in their natural environment, irrespective of their inherent physiological characteristics or what they did in the soil. Rovira (105) and Rouatt and Katznelson (102) observed that the rhizosphere isolates generally grew more profusely than the soil isolates in various media. Katznelson and Rouatt (60) also found that the rhizosphere bacteria were more active as reducers of methylene blue and resazurin in various media, in production of acid and gas from glucose, in liberation of ammonia from peptone, and in denitrification. The rate of oxygen uptake by the rhizosphere bacteria was more rapid with substrates of glucose, alanine, and acetate (64), and oxygen uptake by rhizosphere soil supplemented with casamino acids, mixtures of carbohydrates, or organic acids was more rapid than that of nonrhizosphere soil. Furthermore, oxygen uptake of unsupplemented rhizosphere soil was greater than that of control soil but this has doubtful significance because roots probably become incorporated with the rhizosphere soil in sampling.

Rovira (106) reported that root exudate of seedlings of oats and peas increased the activity of the soil population. He noted the following when air-dried soil was moistened with a solution of root exudate: there was an increase in the number of gram-negative bacterial rods but not of fungi; there was no effect on release of ammonia from soil or added peptone; there was no effect on release of phosphate from soil or added nucleic acid; there was an increase in the oxidation of added glucose. From this he concluded that the rhizosphere bacteria exert little effect on the rate of decomposition of the soil organic matter. If this is so, it would indicate that readily decomposable organic matter does not appreciably accelerate decomposition of the soil organic matter. This supports the conclusions of Pinck and Allison (95) but is contrary to those of Broadbent (16, 17), Broadbent and Norman (19), Broadbent and Bartholomew (18), and Hallam and Bartholomew (41).

The diverse tests of the rhizosphere populations provide additional evidence of a high level of microbial activity in the rhizosphere although most of the evidence has been obtained under conditions unlike those of the rhizosphere. Consequently, in interpreting the results one might consider the probability that what the microorganisms do in soil is different from what they do in the test media. There is no evidence that

the rate with which a culture decomposes some simple compound such as a sugar or amino acid is correlated with the rate with which it decomposed the compounds which supported it in the rhizosphere. Some of the organic nutrients in the soil may have been relatively resistant substances.

There is conflicting evidence regarding development of *Azotobacter* in the rhizosphere, but critical tests have indicated that there is little or no development of these nitrogen-fixing bacteria about roots (3, 4). Nevertheless, there are claims that inoculation of seeds with *Azotobacter* results in improved growth of various plants, including legumes. Clark (25) found that where roots of tomato were inoculated with cultures of *Azotobacter* before being planted, the numbers decreased during subsequent plant growth. According to Jensen (54), growth and nitrogen content of white clover and alfalfa were not increased by inoculation with *A. chroococcum* in agar or sand. Furthermore, root excretions failed to support appreciable growth of the bacterium, and the number of cells of *A. chroococcum* was little or no greater in planted sand or in rhizosphere material of the legumes than in unplanted sand. Recently, Parker (90) reported that significant amounts of nitrogen became fixed by nonsymbiotic nitrogen-fixing bacteria developing in the rhizosphere of nonleguminous plants on organic matter coming from the roots. It was stated that more nitrogen was fixed under grass crops than from addition of 3000 pounds of sugar per acre. Based on these results he concluded that, "The soil data presented and the microbiological evidence cited, together make a strong case for a reappraisal of the role of the free-living microorganisms in the nitrogen economy of the finer textured soils."

It was observed by Metz (82) that roots or root sap of various plants differed in their effects on *Azotobacter*. Some were strongly inhibitive, others weakly inhibitive, and still others were nontoxic. However, toxicity of the plant sap was not closely correlated with occurrence of *Azotobacter* about the roots under soil conditions.

More claims of practical significance of *Azotobacter* in soils may be expected because of the erroneous prevailing idea that bacteria of this genus are some of the most important microorganisms in the soil, and that, theoretically, conditions in the rhizosphere should be favorable for their development.

D. Organic Matter Excreted from Roots

The dense population of microorganisms in the rhizosphere is evidence that roots supply considerable quantities of readily decomposable organic matter. Information is still fragmentary on the excretion of organic matter by roots, the amounts and kinds of compounds, and the conditions affecting excretion. Among the organic materials reported as coming from roots are the following: amino acids, vitamins, sugars, tannins, alkaloids, phosphatides, and unidentified substances that are toxic or stimulatory to other plants and various microorganisms.

It has long been known that roots of legumes excrete materials that increase the number of cells of *Rhizobium* (142); this was recently verified by Purchase and Nutman (99). Virtanen developed a theory of nitrogen fixation based largely on detection of certain amino acids in excretions from nodules of legumes.

Preston *et al.* (98) recovered α-methoxyphenylacetate in the root exudate of plants after treating the tops with the substance. Similar results were obtained with 2,3,6-trichlorobenzoic acid and 2,3,5,6-tetrachlorobenzoic acid (69). The compounds placed on stems or leaves were absorbed, translocated to the roots, excreted, absorbed by roots of nearby plants, and translocated upward, inducing modification of the aboveground parts. Turner (135) reported that the bentonite about roots of legumes and nonlegumes became colored, apparently from organic materials coming from the roots. It was postulated that these were compounds of indole or salicylic acid. Fries and Forsman (33) found that cotyledons of pea seeds excreted various amino acids, purines, and pyrimidines during germination. Root sections exuded various constituents of nucleic acids, most of which were high molecular weight phosphorylated compounds.

Bhuvaneswari and Subba Rao (12) identified tartaric acid, oxalic acid, D-xylose, and D-fructose in exudates of roots of sorghum and mustard. Malic acid, citric acid, D-glucose, and maltose were also detected in exudates of mustard roots.

Scopoletin (6-methoxy-7-oxycumarin) is excreted by roots of germinating oats (30), and more was released under conditions somewhat unfavorable for seedling growth (80). The fact

that culture filtrates of certain microorganisms increased the amount of substance excreted is suggestive in that it indicates that development of microorganisms in the rhizosphere may affect the amount and kind of organic matter that is excreted.

When Metz (82) added root fragments or root sap to agar plates and tested their effects on development of microorganisms, he observed that generally there was no inhibitive effect, but there was toxicity in some cases. The results were variable, but occasionally the root or root sap of a plant was nontoxic to bacteria isolated from roots of this plant and it was more toxic to bacteria from roots of another plant or to bacteria isolated from soil. More frequently, root bacteria, irrespective of origin, were less sensitive than soil bacteria. In one plant the sap obtained from the roots during the vegetative period of plant growth was less inhibitive than that obtained from the plant in the fruiting stage. Inhibition of bacteria was greatest from root sap of *Chelidonium majus*, *Crepis virens*, *Hieracium pilosella*, *Hypericum perforatum*, and *Viola tricolor*.

Rovira (104) observed that considerable amounts of organic matter were excreted from roots of seedlings. Most of the organic matter obtained from the roots was excreted material and there were smaller amounts of cell debris. More organic matter was produced by the roots of the older seedlings but the amount was not proportional to the root size. In a test of the effects of the root exudate on development of various bacteria, Rovira found that cultures from both soil and rhizosphere were stimulated, irrespective of the complexity of the basal medium, but in general the cultures isolated from the rhizosphere grew more abundantly than those from soil. It was Rovira's opinion that most of the organic matter was excreted by the roots although some might have arisen by autolysis of sloughed-off cells.

Metz (82) reported that soil from the rhizospheres of the plants *Hypericum perforatum* and *Chelidonium majus* inhibited growth of a gram-positive rod. A thermolabile substance having antimicrobial action is produced by potato buds (56).

The fact that spores of various fungi fail to germinate in soil even though they germinated in pure water led Dobbs and Hinson (29) to conclude that soils contain inhibitive substances, the influence of which must be counteracted before the spores can germinate. The inhibitive effect may be overcome by glucose (29, 53) and by other required nutrients such as vitamins (20). As Garrett (34) indicated, many substances stimulate germination of spores of some fungi (87) whereas one or few compounds stimulate germination of other spores (51). Whatever is required by the spores of root-inhabiting parasites is provided by the roots of the host, and sometimes by roots of nonsusceptible plants (87). Spores of soil-inhabiting fungi are similarly affected. Jackson (53) observed that spores of *Gliocladium roseum* and *Paecilomyces marquandii* failed to germinate in plant-free soil but did so in soil in close proximity to roots of seedling peas. Similar effects were obtained with seedlings of pea, wheat, and lettuce on spores of three cultures of *Fusarium*. The results of Tolle and Rippel-Baldes (134) indicate that the effects of roots on spore germination are selective in that spores of fungi isolated from roots of cereal plants germinated in the rhizosphere of the cereals but not in rhizospheres of unrelated plants or in soil. Furthermore, roots of the cereals failed to promote germination of spores of all fungi.

According to Buxton (21, 22) the exudate of roots of certain varieties of peas and also the extracts of the rhizospheres of these plants lowered germination of races of *Fusarium oxysporum* to which the pea varieties were resistant but not those to which they were susceptible. The evidence suggests that root exudates of the pea varieties differed. Furthermore, with any one variety the exudate differed with age of the plant. "Results of the inoculation experiments described here show that soil can be made inhibitory to a particular race by previously cropping it with a pea cultivar which resists that race, but how long such inhibition persists, and whether or not similar methods might be of value for controlling certain races of the fungus, are problems which need further investigation" (22).

Recently, considerable attention has been directed to the excretion of amino acids from roots. Kandler (57) reported that the following amino acids were excreted from excised roots of corn grown in the absence of microorganisms: asparagine, alanine, serine, aspartic acid, valine, glutamic acid, leucine, and glutamine. There was a

proportionality between the concentration within the roots and the amount excreted. Corn embryos excreted only a small amount of glutamine in one case. Fries and Forsman (33) found that amino acids and various other nitrogenous compounds were excreted by roots of pea seedlings. Parkinson (91) grew plants free of microorganisms in sand that was periodically perfused and detected several amino acids in the perfusate. Katznelson *et al.* (61, 62) found amino acids in leachings from the nonsterile sand substrate of various plants, but considerably larger amounts were obtained when the plants wilted owing to desiccation of the sand just before it was leached. Nine amino acids were identified, namely, glutamic acid, aspartic acid, proline, leucine, alanine, cysteine, glycine, lysine, and phenylalanine. Also there was reducing material, possibly glucose, a pentose, and another substance. Small amounts of ninhydrin-positive substances were detected in solutions in which wheat and peas were grown free from microorganisms. The various solutions stimulated growth of amino-acid-requiring bacteria.

Rovira (104) grew seedlings of peas and oats in sterile sand and then extracted the sand with water to recover the soluble organic substances after the seedlings had grown for 10 and 21 days. Many amino acids were detected in the washings, a greater variety of amino acids and larger amounts from peas than from oats. Twenty-two amino compounds were detected from peas and 14 from oats. Both glucose and fructose were detected in the washings from both plants at 10 days but practically none at 21 days. Ultraviolet-absorbing and fluorescent materials were also present. Mucilaginous material was noted on roots of the oat seedlings (103). The sulfur compounds methionine, cystine, and taurine were either not detected in the root exudate or were present in very small amounts. In tests of the response of growth of bacteria in diverse media supplemented with the root exudates, there was evidence that in some cases the enhanced growth was due to amino acids, but the principal effect was ascribed to vitamins or other growth factors (105).

Andal *et al.* (5) reported that the four amino acids, aspartic acid, glutamic acid, tryptophan, and lysine were excreted from roots of germinating rice, from varieties both susceptible and resistant to root-rot by *Fusarium moniliforme*. Four other amino acids (cystine, methionine, asparagine, and tyrosine) were present in the exudate of the resistant varieties but they were absent from exudate of the susceptible ones.

It has been mentioned previously that amino acid-requiring bacteria have been found to comprise a larger portion of the bacterial population of the rhizosphere than of soil. Frequently, they were more abundant than the bacteria of the other nutritional groups of Lochhead. It is not surprising, therefore, that the high incidence of the amino acid-requiring bacteria in the rhizosphere has been ascribed, in part, to the availability of amino acids in the root exudates.

E. Factors Affecting Development of Microorganisms in the Rhizosphere

Norman (88) refers to the rhizosphere effect as an enrichment phenomenon owing to the nutritional circumstances in the zone. Concerning the rhizosphere, Bawden (7) wrote as follows: "The rich population here may in part reflect the action of the root exudates in stimulating dormant microbes to activity, but there are probably many other contributing factors. Some of the microbes may be living on sloughed-off root hairs or epidermal cells, and others on sugars and amino acids secreted by the thin-walled living cells; as the root grows and respires, it must alter the soil locally, both physically and chemically, and so present habitats in which microbes with special needs can multiply. It is important to remember that, even with a dense stand of plants, the soil is still far from filled by roots and, on microbial standards, much of the soil microflora remains remote from living tissues."

The growth of root-inhabiting parasitic fungi and mycorrhizal fungi on roots of the host is due to something provided by the host, otherwise the fungi would grow from the roots into the soil instead of being restricted to the root surface (34). The effect of the root need not be that of providing the major organic nutrients, because these are derived from invaded root tissue from which the spreading mycelium develops. In some cases this may be an effect of reaction because it was observed by Thom and Humfeld (129) that the rhizosphere is more nearly neutral in reaction than the soil. The concentration of carbon dioxide in the rhizosphere is also one of the numerous factors affecting develop-

ment of fungus parasites on roots and affecting root penetration by them (34).

In the rhizosphere the microbial population differs both quantitatively and qualitatively from that in the soil, and conditions are changing continuously because the rhizosphere population is determined to a large extent by the root metabolism, which is affected by both soil and atmospheric conditions and the stage of plant growth. Furthermore, the rhizosphere effects vary with different plants. Also, the soil conditions, as they are affected by moisture, temperature, aeration, reaction, and fertility, affect microorganisms through their influences on plant development. Plant vigor is also affected by illumination, air humidity and temperature, and disease. All of these effects will be reflected in the microbiological status of the rhizosphere. To some degree the rhizosphere population is also affected directly by soil conditions.

III. THE EFFECTS OF RHIZOSPHERE MICROORGANISMS ON PLANTS

The opinion has been expressed that each kind of plant has a typical rhizosphere population and that, therefore, inoculation with rhizosphere microorganisms will hasten plant growth and secure vigorous plant development. There is still very little information about the kinds of microorganisms in the rhizospheres of different plants and their significance in development of the plants. However, there is no convincing evidence that inoculation with rhizosphere microorganisms is of practical value, with the exception of legume bacteria and mycorrhizal fungi. Furthermore, the activities of some rhizosphere microorganisms, such as phytopathogens, are unfavorable to plant development. The majority of the rhizosphere microorganisms are saprophytes and convert both organic and inorganic material in the rhizosphere. The products of these transformations may be beneficial or injurious. Certain rhizosphere organisms have more direct effects on plants through symbiosis or parasitism.

Bawden (7) stated: "Resistance to infection is the normal condition of plants, and susceptibility the exception." In our present ignorance of the mechanisms of resistance and pathogenicity (65) this is more surprising than may be apparent, because there is a host of saprophytes that should be able to destroy the plant and, indeed, one another except for some unknown protective effect. Virtanen *et al.* (137) found antifungal agents in all of several cereal and food plants tested and were of the opinion that the substances were important in providing resistance of the plants to fungus attack. Two of the antifungal agents were identified as 2(3)-benzoxazolinone and 6-methoxy-2(3)-benzoxazolinone.

The influence of environmental effects on susceptibility of the plant to microbial attack was recently illustrated by Harley and Waid (43), who observed effects of light intensity on development of beech seedlings and the composition of the microbial population of the roots. High light intensity favored seedling vigor and development of mycorrhizal fungi, whereas low intensity resulted in poorer plant development and in appearance of pathogenic types of fungi on the roots. The degree of light intensity determined whether or not the root fungi had favorable or unfavorable effects on the plant, by affecting plant composition. Bjorkman (13) found that high light intensity and low levels of nitrogen and phosphorus promoted the formation of mycorrhiza of forest trees apparently by increasing the amount of carbohydrate in the roots. The fact that mycorrhiza of certain pine species form after the first leaves appear (52) and that with alfalfa, infection by the nodule bacteria occurs with appearance of the first leaf (130), is additional evidence that plant composition, as influenced by photosynthesis, affects the rhizosphere population.

The effects of microorganisms on plants in the rhizosphere may be diverse, in that microorganisms affect soil structure and availability of plant nutrients. Furthermore, soil microorganisms may favor or inhibit development of one another.

A. Occurrence of Microorganisms in Plants

Whereas saprophytic microorganisms are prominent on root surfaces there is some evidence that they are not confined to the tissue surfaces. There may be invasion by microorganisms other than the pathogens and the symbiotic bacteria and fungi, but the significance of this penetration is obscure. Bacteria have been isolated from roots more frequently than from other plant parts but they have been isolated also from stems, leaves, and flowers of healthy plants. However, it is not known whether the microbial cells merely survive or make limited development in the tissues.

It was reported by Jodidi and Peklo (55) that seeds of English rye grass and certain kinds of wheat and barley contain smut fungi located in the aleurone layer, that they affected seed composition, and that they were normal seed associates. Sathe and Subrahmanyan (109) failed to find microorganisms in the seeds of diverse plants and concluded that there was no evidence of any symbiotic or mutual relation between seeds and microorganisms. Hennig and Villforth (47) found bacteria in leaves, stems, twigs, and roots of 28 different apparently healthy plants in all stages of development. Whereas some fruits and seeds seemed to be sterile, others yielded bacteria, yeasts, and fungi in studies of Marcus (79). Sanford (108) recovered bacteria from the interior tissues of mature potato tubers and from the stele of apparently healthy stems of potato and garden bean and from tap roots of alfalfa and sweet clover. Fungi were seldom found in sound potato tissue. Tervet and Hollis (126) and Hollis (50) also isolated bacteria from potato stems. Bacteria of five different genera other than *Rhizobium* were isolated from roots of red clover and subterranean clover by Philipson and Blair (94).

In a doctor's thesis, "Bacteria in their relation to vegetable tissue," H. L. Russell (107) reported in 1892 studies on the fate of bacteria injected into plant stems. Some of the saprophytic bacteria persisted for more than a month but they had no noticeable effect on the plant. Animal pathogens survived for only short periods. There was some upward movement of the bacteria but practically no downward movement. His findings, indicating that the environment of a healthy plant was not favorable for development of the bacteria, still apply to the great majority of microorganisms.

B. Influence of Organic Materials on Plant Development

Organic materials in the rhizosphere may have various effects on plants. They may be absorbed and stimulate the plant, injure it, or affect the food value of the plant. The development of pathogens in the rhizosphere may also be influenced by the organic compounds in the rhizosphere.

Cholodny (24) made the interesting observation that gaseous substances that were produced by microorganisms in soil were capable of inducing root growth, geotropic reactions of roots, and production of root hairs. Various volatile organic compounds are produced by microorganisms, including ethylene and other hydrocarbons, methyl thiol, and dimethyl disulfide. Information is lacking, however, on the kinds of volatile organic materials that are produced in soil and their effects on plants. Furthermore, it is not known whether the condition observed by Cholodny was exceptional or common.

Becker *et al.* (8, 10, 11), Becker and Gyot (9), Winter and Schönbeck (145), and Schönbeck (112) found that extracts of cereal stubble fields or of soil to which cereal straw had been added were toxic to wheat seedlings. Furthermore, extracts of cereal straw and of green and mature leaves of various plants were toxic to seedlings and to germination of seeds of many plants (112, 143, 144, 146). Leaf extracts were also toxic to bacteria, those of dead leaves being more inhibitive than those of green leaves (147). The effects were interpreted as indicating that various plant residues have direct inhibitive effects on microorganisms in soil and on plant development.

It has been reported that on removal of old peach trees there is toxic material that interferes with development of newly planted young trees (92). The root bark contains the cyanoglucoside, amygdalin, from which the toxic factor was apparently liberated as a result of microbial action. Whether the toxicity was due to hydrocyanic acid, benzaldehyde, or some other products produced from amygdalin is unknown. Both hydrocyanic acid and benzaldehyde were toxic but they might not persist in the soil.

Residues of quack grass were reported to be toxic to alfalfa by Kommendahl *et al.* (68) and both filtrate and residue of rhizomes of the plant had inhibitive effects. The substance was thermolabile. Toxicity, possibly caused by organic substances, has been reported also for several other plants (67).

In an investigation of the unfavorable effects of stubble mulching on crops, McCalla and Duley (81) found evidence that the plant residues contained substances toxic to plants. In soil mulched with wheat straw that was kept wet, there was 44 per cent germination of corn, whereas in unmulched soil germination was 92 per cent. Seeds soaked in extracts of sweet clover or wheat germinated abnormally; the roots were short and

in some cases grew upward. Various nitrogenous organic materials were incorporated in agar which was inoculated with soil. Seeds that were placed on the agar surface frequently germinated abnormally. The factor responsible for the effects was not stated, but the possibility that it was free ammonia or high alkalinity was not excluded. Inhibitive materials such as coumarin may have been contained in the plant residues or produced by microorganisms.

The likelihood that effects such as those observed by McCalla and Duley were due to organic substances of microbial origin is supported by results of Stille (124) who found that culture solutions of various bacteria, actinomycetes, and fungi were toxic to roots and root hairs of young seedlings. Although toxins were produced by phytopathogenic fungi, they were produced also by many saprophytes. Culture filtrates of fungi isolated from roots of cereal plants were both stimulating and toxic to root growth (134); at certain concentrations they promoted root development and at higher concentrations they were inhibitive. In these studies the cultural conditions of the microorganisms were so different from those in the rhizosphere that the results suggest rather than prove that microorganisms affect roots through organic substances produced in the rhizosphere.

In certain yellow podzolic soils (granite origin) of New South Wales, difficulty was encountered in securing inoculation of subterranean clover. Hely *et al.* (46) attempted to ascertain the reason for this. Seeds were germinated on agar slants of a mineral salts medium containing no nitrogen and inoculated with the legume bacteria and soil or soil dilutions, one or two weeks after adding the seeds. Nodulation was inhibited in tubes inoculated with material from the problem soil but not in those inoculated with soil from a field where there was normal nodulation. Furthermore, the inhibitive factor was transferable. It was concluded that, "in the soil under investigation, a microorganism (or -organisms) colonizes the rhizosphere of subterranean clover plants and prevents their normal multiplication and spread of nodule bacteria within."

The probability is great, however, that where legume seeds are inoculated with a culture of effective bacteria, there will be nodulation even though soil conditions are unfavorable for plant development and even for the bacteria. Some evidence for this was obtained by Hoffmann (49) who planted soybean seeds in a very poor acid soil (pH 4.5). The plants were less than a foot high at maturity and had very few leaves, but they set pods. Even under the adverse soil conditions, the roots became inoculated and the inoculated plants made more growth than the uninoculated ones.

Roots of legumes excrete organic materials that increase the number of legume bacteria in the rhizosphere and affect nodulation. Nutman (89) found that the excreted materials increased the rate of nodule formation and, at high concentrations, reduced the number of nodules.

Garrett (34) cited examples of antagonism of phytopathogenic fungi by the rhizosphere population, that by *Trichoderma viride* in particular. The possibility that antibiotics produced in the rhizosphere are a factor in immunity to phytopathogens was discussed by Metz (82) and he presented evidence that plants contain and excrete organic materials inhibitive to microorganisms. The fact that in the rhizosphere there is an abundance of microorganisms that are able to produce antibiotics stimulates interest in the subject. Nevertheless, the potential antibiotic-producing microorganisms are present not only in the rhizosphere but also in the soil (100). Agnihothrudu (1) isolated actinomycetes from rhizospheres of varieties of pigeon pea resistant and susceptible to wilt caused by *Fusarium udum*, and found greater numbers of cultures strongly inhibitive to the fungus in the rhizospheres of the resistant varieties. It was suggested that root excretions of the resistant plants contained materials favoring development of antagonistic actinomycetes. Thornton (132) reported that there were more streptomycin-resistant bacteria in the rhizosphere than in soil, particularly with older plants and those growing in "clover sick soil."

Antibiotic-producing cultures have been used with variable success to control plant pathogens (78, 140). The significance of antibiotics in control of root parasites has been discussed recently in an interesting report by Brian (14), where he stated: "While we can now say with some confidence that some microorganisms can produce antibiotics in soil in quantities sufficient to account for some observed biological antagonisms, we have yet to show that the two phe-

nomena are connected. The evidence is mainly indirect, probably necessarily so."

It is probable that some of the organic materials produced by microorganisms in the rhizosphere are absorbed by the plant, but the kind and amounts are not known. From what has been reported on uptake of organic materials it is probable that plants can absorb many different kinds of compounds, including those of large molecular weight. There are numerous reports concerned with the uptake of sugars, amino acids, and other organic compounds by plants (44, 136). The systemic action of bactericides, fungicides, and insecticides is based on the uptake of the organic compounds by the plant tissues and their persistence in the tissues (31, 86). The antibiotics, streptomycin, chloramphenicol, and griseofulvin have been identified in shoots of plants whose roots developed in solutions containing the compounds (27). The molecular weight of streptomycin is 581. The fact that the chemical structure of a compound is as important for uptake as molecular size was shown by Pramer (96, 97) who found that the ionized basic antibiotic, streptomycin, was rapidly absorbed by active transport whereas a neutral substance, chloramphenicol, was absorbed more slowly and in smaller amounts by diffusion.

The uptake of pesticides and herbicides by plants causes serious problems because the plants that contain them may be unsatisfactory as human foods and animal feed (37). Absorption of auxin (indolyl acetic acid) becomes evident from its typical effects on the plant. The manifestations of kinetin and gibberellic acid on plant growth are evidence of their entry into plant tissues. Although gibberellic acid is a product of the fungus *Gibberella fujikuroi*, the possibility that it is produced commonly by microorganisms in soil seems unlikely because Curtis (28) found that of approximately 1000 cultures of fungi and 500 of actinomycetes isolated from soil, none showed evidence of producing it when cultured in a corn steep, cerelose medium. Brian *et al.* (15) found that gibberellic acid could be absorbed by plants not only from nutrient solutions but from soil, but it underwent slow decomposition in unsterilized soil. There was evidence of decrease in amount on the sixth day, and it had apparently all disappeared in 31 days.

There is some evidence that exceedingly large molecules or particles may be absorbed by plants. Some years ago Moritz and vom Berg (85) and Moritz (84) reported uptake and translocation of ovalbumin in plants. *Vicia* plants were watered with a solution containing ovalbumin, which was later detected in the aerial parts of the plants by an anaphylactic technique. The guinea pig used as the test animal was either sensitized with leaf material and then tested against pure ovalbumin, or the animal was sensitized against pure ovalbumin and then tested against the leaves (see Chester (23)).

The uptake and translocation of animal virus by plants was reported by Skarnes (113). The plant roots were kept in a mineral solution to which the mouse encephalomyelitis virus was added, and the solution was renewed periodically. The aerial portions of the plants were tested for the virus by hemagglutination, by mouse infection, and by virus-neutralization tests. In one series of 112 tests with various plants, including tomato, bean, lettuce, radish, and pea, 14 (12 per cent) were positive. Most of the positive results were obtained with tomatoes. In most cases the virus was recovered in 3 to 7 days after adding the virus to the mineral solution. The implications of these results are important. If particles the size of the virus (20 to 50 mμ) enter plants through the roots and are translocated, it is possible that many of the organic compounds produced by microorganisms in the rhizosphere also penetrate plant tissues.

C. Effects of Microorganisms on Availability of Nutrients in the Rhizosphere

Associated with all microbial development are the two processes of assimilation and formation of inorganic products. In addition, there are secondary changes of other materials in the environment where the reactions occur. It is to be expected, therefore, that the availability of plant nutrients would be affected by microbial activity in the rhizosphere. Some interesting evidence of this has been obtained. Some years ago (1918–1919) Fred and Haas (32) clearly demonstrated that the microorganisms on roots increased markedly the solvent effect of roots on calcium carbonate. This was apparently due to the carbon dioxide of microbial origin. Others, as Metzger (83), found that the amount of bicarbonate was greater in the rhizosphere than in the soil.

Some of the most striking evidence of the solvent effects of microorganisms in the rhizosphere

has been provided by Gerretsen (35). The plants were grown in sand moistened with mineral solution, and the phosphorus was supplied as the mono-, di-, and tri-calcium salts, bone meal, rock phosphate, and ferrophosphate. Where microorganisms were present, compared to sterile conditions, there was greater plant growth and more phosphate was absorbed from the various phosphorus compounds. The effects of the roots on solubility of phosphate was shown by use of sand cultures, where the plants were grown in such a way that they passed over the surface of a glass sheet on which there was an agar film in which the insoluble phosphate was dispersed. During plant growth numerous clear areas appeared at certain spots around the roots where the phosphate became dissolved, and this was ascribed to microbial activity. In some cases microbial development at the root surface had the opposite effect, that is, it increased the amount of insoluble material.

Plant growth affects the availability of nitrogen by its influence on growth of microorganisms in the rhizosphere. It was clearly shown by Goring and Clark (38) that less nitrogen is available to the plant than would have been transformed to nitrate in the soil if the soil had not been planted. A significant amount of nitrogen becomes assimilated by the rhizosphere microorganisms and is immobilized temporarily. Therefore, the crops reduced the amount of nitrogen available for plant growth. The inhibitive effect began to become apparent in 9 weeks, following which it was strong. Quoting from Bartholomew and Clark (6): "Nitrogen immobilization in the soil, believed to be accomplished by the rhizosphere microflora and by nonrecoverable root sloughings, accounted for an appreciable portion of the added nitrogen (fertilizer nitrogen)." During 16 weeks after cropping, the mineralization of nitrogen exceeded that of uncropped soil and the excess was approximately the same as the deficit during the preceding period of plant growth.

These results have been verified by others, including Harmsen and van Schreven (44). The immobilization of nitrogen occurs about all living roots but becomes particularly prominent in permanent grassland.

Conditions leading to immobilization of nitrogen are generally those where the percentage of nitrogen in the organic matter is low. The results suggest, therefore, that most of the organic matter coming from roots, whether as root exudate, sloughed-off cells, or whole roots, is low in nitrogen or is nonnitrogenous. If this is the case one might expect that microorganisms which can decompose these substances would be a significant portion of the rhizosphere population. Search for them is complicated by lack of knowledge of the actual substrates upon which they develop. It has been mentioned previously that amino acids are important excretory products of roots. From the results of Goring and Clark and others, however, it seems probable that the amount of carbohydrates and other nonnitrogenous organic material that is provided microorganisms by the roots greatly exceeds the quantity of amino acids.

Theron (127) and Theron and Haylett (128) suggested that the reduction in the amount of available nitrogen in the rhizosphere was due at least in part to toxicity to the nitrifying bacteria of organic matter coming from the plant roots, but the explanation mentioned previously seems to be more adequately supported by the facts. The significance of the phenomenon is thoroughly discussed by Harmsen and van Schreven (44).

IV. CONCLUDING REMARKS

The information derived from numerous sources in many different ways bears evidence that the rhizosphere is the seat of active microbial development and, for a soil microbiologist, one of the most interesting regions of the soil. It is also an important region because it is here that the principal effects of the soil are expressed on the plant. It is here that the diverse activities of microorganisms have their greatest influence on plant development. The relations between microorganisms and plants are most obvious in the intimate associations between plants and the legume bacteria, mycorrhizal fungi, and pathogens. The studies of microorganisms in the rhizosphere indicate that, in addition to these intimate associations of plants and microorganisms, there are other important relationships between microorganisms and plants even where the roots do not become invaded by the microorganisms. It is probable that for most plants these rhizosphere effects are more important than those where the plant tissue is invaded.

Whereas there is considerable information about microorganisms in the rhizosphere, what is known is more general than absolute. The rhizosphere effect is definitely established but its

significance is obscure. The extent of the absorption of organic materials by plants, particularly the organic compounds of microbial origin, and their effects on plant growth, are practically unknown. There is even limited information on the factors affecting uptake. There are suggestive results on the beneficial and injurious effects of the rhizosphere microorganisms on plants, but the effects have yet to be evaluated. The same applies to the antagonistic and stimulating effects of the rhizosphere microorganisms on one another. The possibilities of controlling the population have been almost completely unexplored.

V. REFERENCES

1. AGNIHOTHRUDU, V. 1955 Incidence of fungistatic organisms in the rhizosphere of pigeon-pea (*Cajanus cajan*) in relation to resistance and susceptibility to wilt caused by *Fusarium udum* Butler. Naturwissenschaften, **42**, 373.
2. AGNIHOTHRUDU, V. 1955 State in which fungi occur in the rhizosphere. Naturwissenschaften, **42**, 515–516.
3. ALLISON, F. E. 1947 Azotobacter inoculation of crops. I. Historical. Soil Sci., **64**, 413–429.
4. ALLISON, F. E., GADDY, V. L., PINCK, L. A., AND ARMIGER, W. H. 1947 Azotobacter inoculation of crops. II. Effect of crops under greenhouse conditions. Soil Sci., **64**, 489–497.
5. ANDAL, R., BHUVANESWARI, K., AND SUBBA RAO, N. S. 1956 Root exudates of paddy. Nature, **178**, 1063.
6. BARTHOLOMEW, W. V., AND CLARK, F. E. 1950 Nitrogen transformations in soil in relation to the rhizosphere microflora. Trans. Intern. Congr. Soil Sci., 4th Congr., Amsterdam, **2**, 112–113.
7. BAWDEN, F. C. 1957 The role of plant hosts in microbial ecology. In *Microbial ecology*. Cambridge University Press, New York.
8. BECKER, Y., GUILLEMAT, J., GUYOT, L., AND LELIÈVRE, D. 1951 Sur un aspect phytopathologique du problème des substances racinaires toxiques. Compt. rend., **233**, 198–199.
9. BECKER, Y., AND GUYOT, L. 1951 Sur les toxines racinaires des sols incultés. Compt. rend., **232**, 105–107.
10. BECKER, Y., GUYOT, L., MASSENOT, M., AND MONTEGUT, J. 1950 Sur la présence d'excrétats radiculaires toxiques dans le sol de la pelouse herbeuse à *Brachypodium pinnatum* du Nord de la France. Compt. rend., **231**, 165–167.
11. BECKER, Y., GUYOT, L., AND MONTEGUT, J. 1951 Sur quelques incidences phytosociologiques du problème des excrétions racinaires. Compt. rend., **232**, 2472–2474.
12. BHUVANESWARI, K., AND SUBBA RAO, N. S. 1957 Root exudates in relation to the rhizosphere effect. Proc. Indian Acad. Sci., **45**, 299–301. (Soils and Fertilizers, Commonwealth Bur. Soil Sci., **20**, 337 (1957)) .
13. BJORKMAN, E. 1949 The ecological significance of the ectotrophic mycorrhizal association in forest trees. Svensk Botan. Tidskr., **43**, 233–262. (Cited by Harley and Waid, ref. 40.)
14. BRIAN, P. W. 1957 The ecological significance of antibiotic production. In *Microbial ecology*. Cambridge University Press, New York.
15. BRIAN, P. W., ELSON, G. W., HEMMING, H. G., AND RADLEY, M. 1954 The plant-growth-promoting properties of gibberellic acid, a metabolic product of the fungus *Gibberella fujikuroi*. J. Sci. Food Agr., **5**, 602–612.
16. BROADBENT, F. E. 1947 Nitrogen release and carbon loss from soil organic matter during decomposition of added plant residues. Soil Sci. Soc. Am. Proc., **12**, 246–249.
17. BROADBENT, F. E. 1953 The soil organic fraction. Advances in Agron., **5**, 153–183.
18. BROADBENT, F. E., AND BARTHOLOMEW, W. V. 1948 The effect of quantity of plant material added to soil on its rate of decomposition. Soil Sci. Soc. Am. Proc., **13**, 271–274.
19. BROADBENT, F. E., AND NORMAN, A. G. 1946 Some factors affecting the availability of the organic nitrogen in soil—A preliminary report. Soil Sci. Soc. Am. Proc., **11**, 264–267.
20. BROWN, R. 1946 Biological stimulation in germination. Nature, **157**, 64–69.
21. BUXTON, E. W. 1957 Some effects of pea root exudates on physiologic races of *Fusarium oxysporum* Fr. f. *pisi* (Linf.) Snyder and Hansen. Brit. Mycol. Soc. Trans., **40**, 145–154.
22. BUXTON, E. W. 1957 Differential rhizosphere effects of three pea cultivars on physiologic races of *Fusarium oxysporum* F. *pisi*. Brit. Mycol. Soc. Trans., **40**, 305–316.
23. CHESTER, K. S. 1937 A critique of plant

serology. Part III. Quart. Rev. Biol., **12**, 294–321.
24. CHOLODNY, N. G. 1951 The soil atmosphere as a source of organic plant nutrients. Pochvovedenie, 16–29 (Chem. Abs., **45**, 4859, 1951).
25. CLARK, F. E. 1948 Azotobacter inoculation of crops. III. Recovery of Azotobacter from the rhizosphere. Soil Sci., **65**, 193–202.
26. CLARK, F. E. 1949 Soil microorganisms and plant roots. Advances in Agron., **1**, 241–288.
27. CROWDY, S. H. 1957 Antibiotics in the control of plant disease. Agr. Rev. (London), **2(12)**, 18–22.
28. CURTIS, R. W. 1957 Survey of fungi and actinomycetes for compounds possessing gibberellinlike activity. Science, **125**, 646.
29. DOBBS, C. G., AND HINSON, W. H. 1953 A widespread fungistasis in soils. Nature, **172**, 197–199.
30. EBERHARDT, F. 1955 Über fluoreszierende Verbindungen in der Wurzel des Hafers. Ein Beitrag zum Problem der Wurzelausscheidungen. Z. Botan., **43**, 405–422.
31. ENO, C. F. 1958 Insecticides and the soil. J. Agr. Food Chem., **6**, 348–351.
32. FRED, E. B., AND HAAS, A. R. C. 1919 The etching of marble by roots in the presence and absence of bacteria. J. Gen. Physiol., **1**, 631–638.
33. FRIES, N., AND FORSMAN, B. 1951 Quantitative determination of certain nucleic acid derivatives in pea root exudate. Physiol. Plantarum, **4**, 410–420.
34. GARRETT, S. D. 1956 Biology of root-infecting fungi. Cambridge University Press, New York.
35. GERRETSEN, F. C. 1948 The influence of microorganisms on the phosphate intake by the plant. Plant and Soil, **1**, 51–81.
36. GIBSON, J. 1957 Nutritional aspects of microbial ecology. In *Microbial ecology*. Cambridge University Press, New York.
37. GILPIN, G. L., PARKS, A. B., AND REYNOLDS, H. 1957 Flavor of selected vegetables grown in pesticide-contaminated soils. J. Agr. Food Chem., **5**, 44–48.
38. GORING, C. A. I., AND CLARK, F. E. 1948 Influence of crop growth in mineralization of nitrogen in the soil. Soil Sci. Soc. Am. Proc., **13**, 261–266.
39. GYLLENBERG, H. G. 1957 Seasonal variation in the composition of the bacterial soil flora in relation to plant development. Can. J. Microbiol., **3**, 131–134.
40. GYLLENBERG, E., AND HANIOJA, P. 1956 The "rhizosphere effect" of graminaceous plants in virgin soils. III. Comparison with the effect of other plants. Physiol. Plantarum, **9**, 441–445.
41. HALLAM, M. J., AND BARTHOLOMEW, W. V. 1953 Influence of rate of plant residue addition in accelerating the decomposition of soil organic matter. Soil Sci. Soc. Am. Proc., **17**, 365–368.
42. HARLEY, J. L. 1948 Mycorrhiza and soil ecology. Biol. Revs. Cambridge Phil. Soc., **23**, 127–158.
43. HARLEY, J. L., AND WAID, J. S. 1955 The effect of light upon the roots of beech and its surface population. Plant and Soil, **7**, 96–112.
44. HARMSEN, G. W., AND VAN SCHREVEN, D. A. 1955 Mineralization of organic nitrogen in soil. Advances in Agron., **7**, 299–398.
45. HARPER, J. L. 1950 Studies in the resistance of certain varieties of banana to Panama disease. Plant and Soil, **2**, 374–382, 383–394.
46. HELY, F. W., BERGERSEN, F. J., AND BROCKWELL, J. 1957 Microbial antagonism in the rhizosphere as a factor in the failure of inoculation of subterranean clover. Australian J. Agr. Research, **8**, 24–44.
47. HENNIG, K., AND VILLFORTH, F. 1940 Experimentelle Untersuchungen zur Frage der Bakteriensymbiose in höheren Pflanzen und ihrer Beeinflüssung durch Leitelemente. Biochem. Z., **305**, 299–309. (Cited by Philipson and Blair, ref. 86.)
48. HOFFMANN, C. 1914 A contribution to the subject of the factors concerned in soil productivity. Univ. Kansas Sci. Bull., **9**, 81–99.
49. HOFFMANN, O. L. 1942 Influence of inoculation upon growth of legumes under field conditions. Master's Thesis, Rutgers University, New Brunswick, New Jersey.
50. HOLLIS, J. P. 1951 Bacteria in healthy potato tissue. Phytopathology, **41**, 350–366.
51. HOOKER, W. J., WALKER, J. C., AND LINK, K. P. 1945 Effects of two mustard oils on *Plasmodiophora brassicae* and their relation to resistance to clubroot. J. Agr. Research, **70**, 63–78.
52. HUBERMAN, M. A. 1940 Normal growth and development of southern pine seedlings in the nursery. Ecology, **21**, 323–334.
53. JACKSON, R. M. 1957 Fungistasis as a factor in the rhizosphere phenomenon. Nature, **180**, 96–97.
54. JENSEN, H. L. 1942 Nitrogen fixation in

leguminous plants. II. Is symbiotic nitrogen fixation influenced by Azotobacter? Proc. Linnean Soc. N. S. Wales, **67**, 205–212.

55. JODIDI, S. L., AND PEKLO, J. 1929 Symbiotic fungi of cereal seeds and their relation to cereal proteins. J. Agr. Research, **38**, 69–91.
56. JORDANA, R. V. 1958 A bacteriostatic effect in potato tubers. J. Gen. Microbiol., **18**, XVI–XVII.
57. KANDLER, O. 1951 Papierchromatographischer Nachweis der Aminosäureausscheidung in vitro kultivierter Maiswurzeln. Z. Naturforsch., **6b**, 437–445.
58. KATZNELSON, H. 1946 The "rhizosphere effect" of mangels on certain groups of soil microorganisms. Soil Sci., **62**, 343–354.
59. KATZNELSON, H., LOCHHEAD, A. G., AND TIMONIN, M. I. 1948 Soil microorganisms and the rhizosphere. Botan. Rev., **14**, 543–587.
60. KATZNELSON, H., AND ROUATT, J. W. 1957 Studies on the incidence of certain physiological groups of bacteria in the rhizosphere. Can. J. Microbiol., **3**, 265–269.
61. KATZNELSON, H., ROUATT, J. W., AND PAYNE, T. M. B. 1954 Liberation of amino-acids by plant roots in relation to desiccation. Nature, **174**, 1110–1111.
62. KATZNELSON, H., ROUATT, J. W., AND PAYNE, T. M. B. 1955 The liberation of amino acids and reducing compounds by plant roots. Plant and Soil, **7**, 35–48.
63. KATZNELSON, H., ROUATT, J. W., AND PAYNE, T. M. B. 1956 Recent studies on the microflora of the rhizosphere. Trans. Intern. Congr. Soil Sci., 6th Congr., Paris, **C**, 151–156.
64. KATZNELSON, H., ROUATT, J. W., AND ZAGALLO, A. C. 1957 Manometric studies with rhizosphere and non-rhizosphere soils and with bacterial isolates from them. Bacteriol. Proc., p. 10.
65. KERN, H. 1956 Problems of incubation in plant diseases. Ann. Rev. Microbiol., **10**, 351–368.
66. KING, H. DE L., AND WALLACE, R. H. 1956 Morphological and physiological groups of soil bacteria from the roots of barley and oats. Can. J. Microbiol., **2**, 473–481.
67. KOCH, L. W. 1955 The peach replant problem in Ontario. I. Symptomatology and distribution. Can. J. Botan., **33**, 450–460.
68. KOMMENDAHL, T., LINCK, A. J., AND BERNARDINI, J. V. 1957 The toxic effect of quackgrass on growth of alfalfa (Abstract). Phytopathology, **47**, 526.
69. LINDER, P. J., CRAIG, J. C., JR., COOPER, F. E., AND MITCHELL, J. W. 1958 Movement of 2,3,6-trichlorobenzoic acid from one plant to another through their root systems. J. Agr. Food Chem., **6**, 356–357.
70. LINFORD, M. B. 1942 Methods of observing soil flora and fauna associated with roots. Soil Sci., **53**, 93–103.
71. LOCHHEAD, A. G. 1952 The nutritional classification of soil bacteria. Proc. Soc. Appl. Bacteriol., **15**, 15–20.
72. LOCHHEAD, A. G. 1957 Qualitative studies of soil microorganisms. XV. Capability of the predominant bacterial flora for synthesis of various growth factors. Soil Sci., **84**, 395–403.
73. LOCHHEAD, A. G., AND BURTON, M. O. 1956 Incidence in soil of bacteria requiring vitamin B_{12} and the terregens factor. Soil Sci., **82**, 237–245.
74. LOCHHEAD, A. G., AND BURTON, M. O. 1957 Qualitative studies of soil microorganisms. XIV. Specific vitamin requirements of the predominant bacterial flora. Can. J. Microbiol., **3**, 35–42.
75. LOCHHEAD, A. G., AND ROUATT, J. W. 1955 The "rhizosphere effect" on the nutritional groups of soil bacteria. Soil Sci. Soc. Am. Proc., **19**, 48–49.
76. LOCHHEAD, A. G., AND THEXTON, R. H. 1947 Qualitative studies of soil microorganisms. VII. The "rhizosphere effect" in relation to the amino acid nutrition of bacteria. Can. J. Research, **C25**, 20–26.
77. LOCHHEAD, A. G., TIMONIN, M. I., AND WEST, P. M. 1940 The microflora of the rhizosphere in relation to resistance of plants to soil-borne pathogens. Sci. Agr., **20**, 414–418.
78. MACH, F. 1956 Untersuchungen über die Möglichkeiten einer Bekämpfung phytopathogener Pilze mit saprophytischer Bodenphase (Vermehrungspilze) durch Superinfektion mit antagonistisch aktiven *Streptomyces*-Stämmen. Zentr. Bakteriol. Parasitenk., Abt. II, **110**, 1–25.
79. MARCUS, O. 1942 Über das Workommen von Mikroorganismen in pflanzlichen Geweben. Arch. Mikrobiol., **13**, 1–44. (Cited by Philipson and Blair, ref. 86).
80. MARTIN, P. 1958 Einfluss der Kulturfiltrate von Mikroorganismen auf die Abgabe von Scopoletin aus den Keimwurzeln des Hafers (*Avena sativa* L.). Arch. Mikrobiol., **29**, 154–168.
81. MCCALLA, T. M., AND DULEY, F. L. 1949 Stubble mulch studies. III. Influence of soil microorganisms and crop residues on the germination, growth and direction of

root growth of corn seedlings. Soil Sci. Soc. Am. Proc., **14**, 196–199.
82. METZ, H. 1955 Untersuchungen über die Rhizosphäre. Arch. Mikrobiol., **23**, 297–326.
83. METZGER, W. H. 1928 The effect of growing plants on solubility of soil nutrients. Soil Sci., **25**, 273–280.
84. MORITZ, O. 1934 Eie botanische Serologie. Beitr. Biol. Pflanz., **22**, 51–90. (Cited by Skarnes, ref. 105.)
85. MORITZ, O., AND VOM BERG, H. 1931 Serologische Studien über das Linswickenproblem. Biol. Zentr., **51**, 290–307.
86. NEWMAN, A. S., AND DOWNING, C. R. 1958 Herbicides and the soil. J. Agr. Food Chem., **6**, 352–353.
87. NOBLE, R. J. 1924 Studies on the parasitism of *Urocystis tritici* Koern., the organism causing flag smut of wheat. J. Agr. Research, **27**, 451–489.
88. NORMAN, A. G. 1955 The place of microbiology in soil science. Advances in Agron., **7**, 399–410.
89. NUTMAN, P. S. 1957 Studies on the physiology of nodule formation. V. Further experiments on the stimulating and inhibitory effects of root secretions. Ann. Botany, **21**, N.S., 321–337.
90. PARKER, C. A. 1957 Non-symbiotic nitrogen-fixing bacteria in soil. III. Total nitrogen changes in a field soil. J. Soil Sci., **8**, 48–59.
91. PARKINSON, D. 1955 Liberation of amino-acids by oat seedlings. Nature, **176**, 35–36.
92. PATRICK, Z. A. 1955 The peach replant problem in Ontario. II. Toxic substances from microbial decomposition products of peach root residues. Can. J. Botany, **33**, 461–486.
93. PAYNE, T. M. B., ROUATT, J. W., AND LOCHHEAD, A. G. 1957 The relationship between soil bacteria with simple nutritional requirements and those requiring amino acids. Can. J. Microbiol., **3**, 73–80.
94. PHILIPSON, M. N., AND BLAIR, I. D. 1957 Bacteria in clover root tissue. Can. J. Microbiol., **3**, 125–129.
95. PINCK, L. A., AND ALLISON, F. E. 1951 Maintenance of soil organic matter. III. Influence of green manures on the release of native soil carbon. Soil Sci., **71**, 67–75.
96. PRAMER, D. 1955 Absorption of antibiotics by plant cells. Science, **121**, 507–508.
97. PRAMER, D. 1956 Absorption of antibiotics by plant cells. II. Streptomycin. Arch. Biochem. Biophys., **62**, 265–273.
98. PRESTON, W. H., JR., MITCHELL, J. W., AND REEVE, W. 1954 Movement of alpha-methoxyphenylacetic acid from one plant to another through their root systems. Science, **119**, 437–438.
99. PURCHASE, H. F., AND NUTMAN, P. S. 1957 Studies on the physiology of nodule bacteria. VI. The influence of bacterial numbers in the rhizosphere on nodule initiation. Ann. Botany, **21**, N.S., 439–454.
100. RĚHÁČEK, Z. 1956 Spreading of actinomyces in the rhizosphere of some grains (English abstract). Československ. mikrobiol., **1**, 211–215.
101. ROMBOUTS, J. E. 1953 The microorganisms in the rhizosphere of banana plants in relation to susceptibility or resistance to Panama disease. Plant and Soil, **4**, 276–288.
102. ROUATT, J. W., AND KATZNELSON, H. 1957 The comparative growth of bacterial isolates from rhizosphere and non-rhizosphere soils. Can. J. Microbiol., **3**, 271–275.
103. ROVIRA, A. D. 1956 A study of the development of the root surface microflora during the initial stages of plant growth. J. Appl. Bacteriol., **19**, 72–79.
104. ROVIRA, A. D. 1956 Plant root excretions in relation to the rhizosphere effect. I. The nature of root exudate from oats and peas. Plant and Soil, **7**, 178–194.
105. ROVIRA, A. D. 1956 Plant root excretions in relation to the rhizosphere effect. II. A study of the properties of root exudate and its effect on the growth of microorganisms isolated from the rhizosphere and control soil. Plant and Soil, **7**, 195–208.
106. ROVIRA, A. D. 1956 Plant root excretions in relation to the rhizosphere effect. III. The effect of root exudate on the numbers and activity of microorganisms in soil. Plant and Soil, **7**, 209–217.
107. RUSSELL, H. L. 1892 Bacteria in their relation to vegetable tissue. Dissertation, Johns Hopkins University, Baltimore.
108. SANFORD, G. B. 1948 The occurrence of bacteria in normal potato plants and legumes. Sci. Agr., **28**, 21–25.
109. SATHE, T. R., AND SUBRAHMANYAN, V. 1931 The relation between seeds and microorganisms. J. Indian Inst. Sci., **14A**, 119–139.
110. SCHMIDT, E. L. 1951 Soil microorganisms and plant growth substances. I. Historical. Soil Sci., **71**, 129–140.
111. SCHMIDT, E. L., AND STARKEY, R. L. 1951

Soil microorganisms and plant growth substances. II. Transformations of certain B-vitamins in soil. Soil Sci., **71**, 221–231.

112. SCHÖNBECK, F. 1956 Untersuchungen über Vorkommen und Bedeutung von Hemmstoffen in Getreiderückständen innerhalb der Fruchtfolge. Z. Pflanzenkrankh. u. Pflanzenschutz, **63**, 513–545.
113. SKARNES, R. C. 1952 Studies upon the persistence of animal viruses in soil and their uptake by higher plants. Master's Thesis, University of Minnesota, Minneapolis.
114. SKERMAN, V. B. D., AND MACRAE, I. C. 1957 The influence of oxygen on the reduction of nitrate by adapted cells of *Pseudomonas denitrificans*. Can. J. Microbiol., **3**, 215–230.
115. SKERMAN, V. B. D., AND MACRAE, I. C. 1957 The influence of oxygen availability on the degree of nitrate reduction by *Pseudomonas denitrificans*. Can. J. Microbiol., **3**, 505–530.
116. STARKEY, R. L. 1929 Some influences of the development of higher plants upon the microorganisms in the soil. I. Historical and introductory. Soil Sci., **27**, 319–334.
117. STARKEY, R. L. 1929 Some influences of the development of higher plants upon the microorganisms in the soil. II. Imfluence of the stage of plant growth upon abundance of organisms. Soil Sci., **27**, 355–378.
118. STARKEY, R. L. 1931 Some influences of the development of higher plants upon the microorganisms in the soil. IV. Influence of proximity to roots on abundance and activity of microorganisms. Soil Sci., **32**, 367–393.
119. STARKEY, R. L. 1932 The application of available methods to studies of certain factors associated with changes in the soil population. Proc. Intern. Congr. Soil Sci., 2nd Congr., Leningrad-Moscow, **3**, 248–259.
120. STARKEY, R. L. 1938 Some influences of the development of higher plants upon the microorganisms in the soil. VI. Microscopic examination of the rhizosphere. Soil Sci., **45**, 207–249.
121. STARKEY, R. L. 1942 Transformation of riboflavin and pantothenic acid during decomposition of plant materials. Soil Sci. Soc. Am. Proc., **7**, 237–242.
122. STARKEY, R. L. 1944 Changes in the content of certain B-vitamins in organic materials decomposing under aerobic and anaerobic conditions. Soil Sci., **57**, 247–270.
123. STILLE, B. 1938 Untersuchungen über die Bedeutung der Rhizosphäre. Arch. Mikrobiol., **9**, 477–485.
124. STILLE, B. 1957 Schädigungen an Pflanzenwurzeln durch Kulturfiltrate von Mikroorganismen. Arch. Mikrobiol., **26**, 71–82.
125. TAYLOR, C. B. 1951 The nutritional requirements of the predominant bacterial flora of the soil. Proc. Soc. Appl. Bacteriol., **14**, 101–111.
126. TERVET, I. W., AND HOLLIS, J. P. 1948 Bacteria in the storage organs of healthy plants. Phytopathology, **38**, 960–967.
127. THERON, J. J. 1951 The influence of plants on the mineralization of nitrogen and the maintenance of organic matter in the soil. J. Agr. Sci., **41**, 289–296.
128. THERON, J. J., AND HAYLETT, D. G. 1953 The regeneration of soil humus under a grass ley. Empire J. Exptl. Agr., **21**, 86–98.
129. THOM, C., AND HUMFELD, M. 1932 Notes on the association of microorganisms and roots. Soil Sci., **34**, 20–36.
130. THORNTON, H. G. 1929 The role of the young lucerne plant in determining the infection of the root by the nodule-forming bacteria. Proc. Roy. Soc. (London), **B104**, 481–492.
131. THORNTON, H. G. 1956 The ecology of microorganisms in soil. Proc. Roy. Soc. (London), **B145**, 364–374.
132. THORNTON, H. G. 1957 Soil microbiology department. Rpt. for 1956 of Rothamsted Exp. Sta., p. 71–75.
133. TIMONIN, M. I. 1941 The interaction of higher plants and soil microorganisms. III. Effect of by-products of plant growth on activity of fungi and actinomycetes. Soil Sci., **52**, 395–413.
134. TOLLE, R., AND RIPPEL-BALDES, A. 1958 Untersuchungen über die Rhizosphäre von Gramineen. Zentr. Bakteriol. Parasitenk., Abt. II, **111**, 204–217.
135. TURNER, E. R. 1955 The reaction between bentonite and certain naturally-occurring compounds. J. Soil Sci., **6**, 319–326.
136. VAN RAALTE, M. H. 1954 The uptake of organic matter by plants. (In Dutch) Landbouwk. Tijdschr., **66**, 356–364. (Cited by Harmsen and van Schreven, ref. 41.)
137. VIRTANEN, A. I., HIETALA, P. K., AND WAHLROOS, O. 1957 Antimicrobial substances

in cereals and fodder plants. Arch. Biochem. Biophys., **69**, 486–500.

138. WALLACE, R. H., AND KING, H. DE L. 1954 Nutritional groups of soil bacteria on the roots of barley and oats. Soil Sci. Soc. Am. Proc., **18**, 282–285.

139. WALLACE, R. H., AND LOCHHEAD, A. G. 1949 Qualitative studies of soil microorganisms. VIII. Influence of various crop plants on the nutritional groups of soil bacteria. Soil Sci., **67**, 63–69.

140. WEINDLING, R., KATZNELSON, H., AND BEALE, H. P. 1950 Antibiosis in relation to plant diseases. Ann. Rev. Microbiol., **4**, 247–260.

141. WEST, P. M., AND LOCHHEAD, A. G. 1940 Qualitative studies of soil microorganisms. IV. The rhizosphere in relation to the nutritive requirements of soil bacteria. Can. J. Research, **C18**, 129–135.

142. WILSON, P. W. 1940 The biochemistry of nitrogen fixation, p. 73. University of Wisconsin Press, Madison.

143. WINTER, A. G., AND BUBLITZ, W. 1953 Über die keim- und entwicklungshemmende Wirkung der Buchenstreu. Naturwissenschaften, **40**, 416.

144. WINTER, A. G., AND SCHÖNBECK, F. 1953 Untersuchungen über die Beeinflussung der Keimung und Entwicklung von Getreidesamen durch Kaltwasserauszüge aus Getreidestroh. Naturwissenschaften, **40**, 168–169.

145. WINTER, A. G., AND SCHÖNBECK, F. 1954 Untersuchungen über wasserlösliche Hemmstoffe aus Getreideböden. Naturwissenschaften, **41**, 145–146.

146. WINTER, A. G., AND SIEVERS, E. 1952 Untersuchungen über die Beeinflussung der Samenkeimung durch Kaltwasserextrakte aus der Blattstreu verschiedener Gramineen. Naturwissenschaften, **39**, 191–192.

147. WINTER, A. G., AND WILLEKE, L. 1952 Untersuchungen über Antibiotica aus höheren Pflanzen. Naturwissenschaften, **39**, 45–46, 190–191.