THE BOTANICAL REVIEW

VOL. XIV NOVEMBER, 1948 No. 9

SOIL MICROORGANISMS AND THE RHIZOSPHERE[1]

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INTRODUCTION

In 1904 Hiltner (28) introduced the term "rhizosphere" to designate that portion of the soil which is subject to the influence of the plant root system, and noted that this soil supported greater microbial activity than soil more distant from the roots. This observation was confirmed subsequently by many investigators whose work up to 1929 has been thoroughly reviewed (93). The present article concerns studies carried out since that time.

Much of the work in this field has dealt with the physiology of the plants and their roots (carbon dioxide evolution, absorption of minerals, root excretions) in relation to their influence on the rhizosphere. It is intended here to emphasize the microbiological aspects of this phenomenon, considering root physiology only insofar as it is necessary to interpret the recorded activities of soil microorganisms in the rhizosphere. Reviews of root activity and physiology will be left for the qualified plant physiologist (68). However, as a background for the elucidation of the microbiological phenomena involved, the inclusive statement of Starkey (93) concerning the influence of higher plants on the rhizosphere may perhaps be appropriately quoted here: "Among the various modifications of the soil which the plants may provoke are the following: lowering the concentration of certain mineral nutrients in the soil due to their absorption, partial desiccation of the soil by absorption of water, increase in soil carbonates following root excretion of carbon dioxide, contribution of microbial foods by sloughed off root portions or excretions. Some of these effects may

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directly affect the soil population and others indirectly by modifications of the physical composition of the soil".

The organisms in the rhizosphere in turn may exert profound influences upon the plant, which include the multiplicity of reactions carried out by soil microorganisms in general: liberation of plant foods by decomposition of soil organic matter and by the solvent action of carbon dioxide and other acids which they produce; transformation of nitrogen and sulfur compounds (ammonia, hydrogen sulfide) into readily available forms which may be beneficial or toxic to plants, affecting the oxygen tension in the immediate vicinity of the roots; competition with higher plants for available nitrogen and minerals; synthesis of vitamins and auxins; and entering with higher plants into various associations which may be beneficial or harmful (120, 133). In view of the increased density of organisms in the root zone it is to be expected that associative and antagonistic relationships will be particulary pronounced with resulting favorable or unfavorable effects on the plant (21, 122).

One of the most important considerations in the study of the microorganisms of the rhizosphere is obviously that concerned with the extent of the zone of root influence. It is to be expected that the effects of root growth and physiology will diminish with the distance from this organ, but whether the most characteristic picture of the microflora of the rhizosphere may be obtained from the soil adjacent to the root, from that on the intact root itself, or from root scrapings, is still controversial. Whereas in most cases soil at the immediate root surface is used, it has been suggested (105) that a more characteristic rhizosphere microflora is obtained by using macerated root tissue after the roots have been washed in several changes of sterile water. Various workers (24, 80) have made distinctions between the "outer" rhizosphere (soil adhering to the root) and the "inner" rhizosphere (root surface). The localization of microorganisms on roots and around root hairs, as demonstrated by the contact slide method (30, 33, 62, 99), supports the practise of utilizing the roots themselves in rhizosphere studies to obtain the most characteristic picture of the microflora.

In order to demonstrate a "rhizosphere effect" on numbers of microorganisms it is necessary to make comparative counts on soil more distant from the root. The extent to which the roots influence the soil microflora may then be expressed by the rhizosphere: soil (R: S) ratio, that is, the number of organisms in the rhizosphere soil divided by the number in the soil at a distance from the root (calculated on a dry weight basis). Counts of numbers on the root surface alone give ordinarily no indication of the influence of the plant and are of use mainly in comparing effects of different plants grown under identical conditions. Environmental factors may be such as to reduce numbers of organisms in both soil and rhizosphere (low temperature, excess moisture), but whether the "rhizosphere effect" is reduced or not can be determined only by means of rhizosphere and soil counts (41). For evaluating the influence of soil type, treatments and other factors on the root surface microflora, this ratio is of fundamental importance (41).

Comparative tests with control soils are particularly important in demonstrating a selective action of roots on definite types or groups of microorganisms. The mere isolation of species from the rhizosphere, as reported by various workers (80,83,115), is without significance in the absence of data from soil apart from the plant.

METHODS OF STUDY

The microflora of the rhizosphere may be studied by means of cultural methods or by direct microscopic examination. Since the former yield by far the most significant information, a general procedure utilizing them is described below.

Plants are carefully removed from the field or greenhouse pot and adherent soil gently broken to release the root system which is then shaken to remove superfluous soil and immersed in a known volume of sterile water in a weighed flask (10, 110*a*). After thorough shaking, suitable dilutions are made and the roots removed and washed, the wash water being collected in the original dilution flask. The moisture in the original flask is then driven off, the contents weighed and the number of organisms computed per gram oven-dry "rhizosphere soil", allowance being made for the amount of material removed in perparing dilutions.

There is some question as to whether or not the roots themselves should be used as the basis for calculation of numbers of organisms in the rhizosphere, although this would be impossible in comparisons of fibrous and fleshy roots. Clark (12) contends that an appreciable error may be involved in the use of rhizosphere soil for this purpose, especially where plants are obtained from soils of varying moisture content, since this would affect the quantity of soil adhering to the roots. As there are few comparative data on this point, it may be best to include dry weights of both roots and rhizosphere soil in any presentation of results.

It is preferable to use the complete root system where possible. However, when roots are bulky or particularly voluminous (mangels, legumes, *etc.*) representative portions may be used (110*b*). Samples of soil apart from the root should be studied concurrently, as it is only by this means that a "rhizosphere effect" can be demonstrated. The media and methods employed for estimating numbers and types of organisms in the rhizosphere will depend of course on the kinds of organisms under investigation.

The microscopic procedure involves either use of the contact slide technique of Rossi-Cholodny or direct examination of roots (30, 33, 59, 62, 99, 102, 112). The former involves burying glass slides or cover glasses in soil so that roots of plants come in contact with them, removing at suitable intervals and examining, usually after staining. As Starkey (99) states, "the method provides a means of obtaining a more accurate conception of the development of microorganisms in response to root growth, the types of organisms affected, the localization of the individuals and groups and the sequence of morphological types". However, as ordinarily conducted this procedure does not permit culturing of the organisms, nothing can be ascertained regarding their physiology, specific bacteria cannot be recognized on the basis of morphology alone, and, in addition, the sequence of development is problematical. Furthermore, as Linford (62) points out, contact slides do not retain nematodes, nor can protozoa be recognized; again, certain fungi present in abundance on roots do not adhere to slides. Many of the larger compact colonies are also removed with the root from the slide. By means of flooding these slides with selective media Isakova[4] (33) extended this procedure to permit the culturing of the adherent organisms.

Roots may also be removed and examined under the microscope (107) or observed *in situ* by means of specially constructed observation boxes (61, 62). The glass coverslips used in this procedure may later be stained. This method yields interesting

[4] The Russian literature was assembled by M. I. Timonin.

information concerning localization of various microbes of the soil on roots and root hairs but is subject in general to the same criticisms as the contact slide method.

QUANTITATIVE EFFECTS OF HIGHER PLANTS ON SOIL MICROORGANISMS

That bacteria, actinomycetes and fungi of the soil find the root zone a more congenial environment for development than soil apart from the root is now generally accepted. Very little has been done with the soil microfauna in this connection or with soil algae. Linford (60, 62) reported that protozoa and microphagous nematodes congregate and multiply in the root zone. By means of cultural methods, Katznelson (41) noted R: S ratios as high as 23 for protozoa on plants in manured soil. Algae have not been mentioned in studies on the rhizosphere until recently when it was reported (41) that a distinct effect occurred in the rhizospheres of mature but not young mangels in both fertilized and unfertilized soil.

As may be expected, numbers of organisms in the root zone are influenced by many factors: nature and age (or stage of growth) of plant; type, moisture content, reaction, and treatment of the soil. Frequently, it is impossible, however, to determine which of these phenomena is responsible for the observed effects. Soil treatment, for example, may stimulate plant growth which, in turn, may cause an increase in numbers of organisms on the root surface. An increase in number of organisms may be expected following certain soil treatments, but a "rhizosphere effect" need not necessarily result unless the plant itself is stimulated in some manner.

Nature and Age of Plant. Comparisons of rhizosphere counts of various plants grown under identical conditions are fairly numerous. These counts, however, are not always comparable, since they are usually obtained from plants of the same age but certainly not at the same stage of development because of different growth rates and maturity periods. Insofar as the major groups of soil microorganisms are concerned, different plants exert considerably different effects which are also influenced by different stages of growth (98). Starkey (93) noted that alfalfa roots stimulated filamentous fungi only slightly but that eggplant exerted an appreciable effect. The latter also caused greatest increases in actinomycetes and bacteria, whereas sugar beet and corn were the least effective. On comparing various other plants he (94) observed that numbers of actinomycetes and filamentous fungi were slightly higher on the roots. However, considerably greater increases of bacteria occurred, rape being particularly effective, potatoes least. In no case was the R: S ratio more than 3. This can be explained by the fact that the samples were not taken from the root but from the soil surrounding it, a procedure which undoubtedly diluted the numbers of organisms in the rhizosphere. In general, most of the plants did not exert a great effect at the early stage of growth, and the maximum effect generally appeared after the plant had attained appreciable size, had reached the limit of vegetative growth or had bloomed or started to degenerate. After death of the plant a rapid decline in numbers of bacteria occurred. Potatoes and oats particularly showed rapid increases in numbers after the early stage of growth and extensive decline after maturity and death. Biennials showed a marked effect, even in the later stages of growth. In later studies Starkey (96) reported much greater numbers of organisms in the rhizosphere, especially on the immediate plant root, and a marked decline with distance from it. Again the bacterial population was affected to a much greater extent than actinomycetes and filamentous fungi. Sweet clover exerted a profound effect, yielding an R: S ratio of 50 at 356 days and 217 at 437 days. Actinomycetes were stimulated to a maximum ratio of 8.6 by sweet clover at 437 days. Younger plants such as bean exerted a slight effect, mangel beets and corn none. Filamentous fungi were affected slightly more than actinomycetes; again sweet clover was most effective, yielding a ratio of 10 at 437 days. Of the first-year plants the greatest effect was obtained with corn (R: S ratio of 13). It appears then that greater numbers of microorganisms, especially bacteria, were found on roots of legumes, though non-legumes also exerted a distinct effect.

In a study of the microflora of the roots of a variety of crops Gräf (24) distinguished between the "inner" rhizosphere (root surface), the "outer" rhizosphere (soil adhering to the root), and soil distant from root (control). Monthly determinations indicated that total bacterial counts were as expected, highest on the root surface. A pronounced increase in numbers occurred at the root surface during the period of growth followed by a decline during the ripening period and a further decline after harvest. She noted too that the increase in total numbers was least in the case of grains and strongest with legumes and rape. Thom and Humfeld (107) found bacteria to be stimulated on roots of alfalfa, rye and vetch more than fungi, and fungi on the whole more than actinomycetes. In the same soil they found that alfalfa stimulated bacteria more than rye; in another soil rye was more effective than vetch. Fungi and actinomycetes were stimulated to an equal degree by alfalfa and rye in the first soil, but no rhizosphere effect on actinomycetes occurred with the second soil.

Krassilnikov *et al.* (58) noted that the rhizosphere of cultivated peanuts harbored from 10 to 100 times as many organisms as soil apart from the root; this condition persisted until the flowering stage. Towards maturity numbers of bacteria diminished, and those of fungi and actinomycetes began to increase. In studies with wheat, maize, sunflower and soya he and his co-workers (49, 56, 57) observed marked increases in total numbers of microorganisms in the rhizosphere. That of soya had greatest numbers, maize and sunflower less and wheat least. Counts were highest with sunflower and soybean during the early part of the growing period and in the later part of the season. Evidence of a distinct "rhizosphere effect" by roots of the tea-bush was obtained by Obraztzova (78). With age of plant there was an increase in the numbers of microbes on the roots; a two-year old plant yielded twice as many organisms as an old flowering bush. There was apparently no essential difference between the kind of microorganisms in the rhizosphere of the tea-bush or grass. Manzon (74), in a study of a variety of leguminous and non-leguminous plants, concluded that the numbers and activity of the bacterial population of the rhizosphere varied greatly, depending both on the species of the plant and on the phase of its development. Highest bacterial numbers were obtained with legumes.

In studies of sandy desert soils Sabinin and Minina (84) observed an extraordinary example of the influence of root systems on the soil microflora. Soil at a short distance from the root system appeared to be sterile. The microbial life of these soils develops only in conjunction with the root systems of the plants.

By means of the contact slide technique, Starkey (99) did not observe any striking differences between mangel beet, barley, maize, rape, vetch and soybean. The most obvious change, an increase in abundance of organisms, was noted as the plants matured. Clark and Thom (13) found that the total bacterial count on roots of cotton in treated or untreated soil increased from July to August, then decreased. However, since no R : S ratios were computed, it is not evident to what extent the plant itself influenced these counts. Clark (10) found that the rhizosphere populations in manured soil were generally greater than in untreated soil, though the effect of manuring was much more pronounced in soil apart from the root. In a further study (11) he found wheat roots to support somewhat larger numbers of bacteria than barley and oats. Total counts in the rhizospheres of beans, radishes, lettuce and corn were highest in this order, though for these crops the "rhizosphere effect" can not be estimated in the absence of data from control soils.

Adati (1) made an extensive study of numbers of bacteria, actinomycetes and fungi in the rhizospheres of different crops growing in sand, loam, clay and humus soils. Counts were given for control soil, for rhizosphere soil directly surrounding the roots and for soil at an unspecified distance from them. Much higher numbers of all organisms were found in the rhizosphere directly adjacent to the root, decreasing with distance from it. In general, numbers were highest on legume roots, a ratio of 197 for bacteria being obtained with peas. Bacteria were stimulated to the greatest extent, fungi usually next and actinomycetes least, although R : S ratios for this last group of organisms were in certain instances as high as 62. Ratios varying from 2 for oats to 45 for flax were reported in a study of a variety of crops (65). Timonin (111), on comparing the microbial populations of the rhizosphere of seedlings of wheat, oats, alfalfa and peas, found that bacteria and actinomycetes (together) were 7 to 71 and fungi 0.75 to 3.1 times as great in number in the rhizosphere as in the soil. The latter group of organisms was somewhat more abundant in the rhizosphere of oats and barley and the former in that of alfalfa. In general, an increased rhizosphere effect was observed with advancing age of the seedlings. Zukovskaya (136), working with potatoes, flax and clover, found that the microbial population of the rhizosphere was 100 times as great in the soil as from the root. Changes of this microflora coincided with 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. Using flax, Berezova (3) 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. This author is of the opinion that there are two distinct zones in the rhizosphere which support different microbial populations; one is the rhizosphere soil, containing aerobic bacteria, fungi and actinomycetes, and the other is the root surface with denitrifying organisms of the *Radiobacter* and *B. macerans* group. Borodulina (8) observed that the microflora of the seed coat develops and establishes itself in the plant rhizosphere; beneficial as well as harmful organisms can multiply in this zone, a fact which finds application in bacterial treatment of seed. Working with mangels in field plots, Katznelson (41) reported distinct effects of age of plant on bacteria, fungi, actinomycetes, algae and protozoa and on specific groups of organisms to be referred to later. For example, R: S ratios of bacteria on plants in untreated soil were 35, 45 and 113 at 80, 117 and 142 days; ratios for actinomycetes increased from 3 to 30 during this period, algae from 0.4 to 29.

Different rhizosphere effects may also be obtained with different varieties of one plant species. Obraztzova (78) found that the variety of tea-bush did not influence numbers of microbes but did affect their quantitative composition. Timonin (112) noted higher numbers of bacteria and, to a less 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 (65, 67*a*, 131) 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 it is interesting to note the recent work of Eaton and Rigler (18) who showed that as the carbohydrate content of cotton roots increased, the total bacterial count on the root surfaces 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".

Environmental Factors. The influence of factors such as the type of soil, moisture content, reaction, treatment and temperature on the soil microflora has been thoroughly presented (120). Much less work has been done on the effects of these factors on the rhizosphere microflora. Thom and Humfeld (107) report considerable variations in absolute counts and 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, fungi more abundant on roots in the most acid and most alkaline soil, whereas actinomycetes were not affected by soil differences. Adati's (1) work, referred to previously, showed that on the whole, R: S ratios were highest on loam (not sand, as claimed) with sand, clay and humus soils 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 has also been observed by other investigators (11), although it is not yet clear whether the effect is direct or indirect (*i.e.*, through influence on plant growth).

In general, soil treatment does not appear to affect numbers of organisms in the rhizosphere (10, 13, 30, 42, 78, 112). Liming was reported, however, to increase numbers by 25% (78). Manuring, though invariably inducing large increases in the microbial population of the soil, did not appear to exert a similar effect on the root population (10, 13). The effect of any treatment must also be evaluated in terms of its influence on the rate of growth of the plant (11, 41). For example, Katznelson (41) observed that numbers of bacteria, fungi, actinomycetes and protozoa reached a maximum in the rhizosphere of rapidly growing, vigorous mangel plants in manured soil at the height of the growing season. However, plants in unfertilized soil grew much more slowly, reaching near the end of the growing period a size comparable to that of the mangels in the manured soil at the peak of the growing season, and, as may be expected, exerted their full effect on the root microflora only when full grown. The importance of the R: S ratio in interpreting results is clearly demonstrated in this work. Counts of actinomycetes on mangel roots reached a peak at 117 days and decreased at 142 days; the R: S ratio was 23 and 30 for these two sampling periods, indicating an actual increase in the "rhizosphere effect".

It was also observed that at 142 days, counts of algae were considerably higher in the rhizosphere of plants in manured soil than in that of plants in unfertilized soil, yet the R : S ratios 15 and 29, respectively, indicated that the roots in the latter soil exerted a greater effect than those in the former.

Soil moisture content has been observed to influence numbers of organisms in the rhizosphere. On comparing soil and rhizosphere counts at different soil moisture contents, Clark (10) found that the R: S ratio did not vary appreciably over a range of 12 to 20% moisture (about 9), but at 24.5% the ratio dropped to 4.5. Under field conditions (11) it was somewhat more difficult to assess the influence of moisture, since ratios varied considerably. In studies on the influence of soil moisture content on numbers of microbes in the rhizosphere of flax, Timonin (112) observed that a lower moisture content of the soil (30% of the total moisture-holding capacity as compared with 60%) increased the density of the microbial population in the rhizosphere. This effect was especially striking with bacteria, next with fungi and least if at all with actinomycetes. Similar results were obtained (113) with "artificial rhizospheres" (collodion membranes), especially as related to the fungous population; there was little effect with actinomycetes. It has been suggested (10, 12) that these effects may be due more to increased or decreased adherence of soil particles to roots (in wet and dry soil, respectively) than to actual changes in numbers of organisms in response to altered environmental and nutritional conditions. However, more information is required on this point before it can be generally accepted.

Soil sterilization with various agents such as steam, formaldehyde and chloropicrin, is analogous to soil treatment in certain respects (120, 124). Since these agents are used to eliminate a disease factor from soil they should perhaps be left for consideration along with root rots in general. However, certain peculiar effects have been obtained which may be mentioned at this point. Katznelson and Richardson (42) found, for example, that steaming reduced bacterial and actinomycete numbers in soil very markedly for about three months, yet the numbers on the healthy roots of tomatoes were as high as on those in untreated soil, implying a protective and ameliorating influence of the plant on the soil microflora in the rhizosphere. Similar results were obtained with certain treatments, *e.g.*, dried blood in connection with control of strawberry root rot (43).

A number of investigations have been published concerning the influence of soil amendments on the quantitative and qualitative rhizosphere microflora in relation to root rot diseases of plants. Since the root rot condition introduces another factor contributory to the rhizosphere population, these findings will be considered in a separate section.

Carbon Dioxide Evolution. The abundance of organisms about plant roots presupposes intense activity in that region. This activity has been measured in terms of carbon dioxide evolution. It appears generally accepted that larger quantities of this gas are evolved from soils under plants than from unplanted soil. Considering the contribution of the plant in this connection, it is obvious that carbon dioxide arising from its growth may come from at least two sources, *viz.*, from the plant roots, as a product of root cell respiration, and from microbial activity about the roots (124) in decomposing organic excretions and "sloughed off" portions of the roots. Which of these sources supplies the greater amount of this gas depends on prevailing environmental conditions, stage of growth and nature of the plant. As much as 45% of this gas may be the product of microbial activity (124).

Slight effects were noted (95) in the early stages of growth and abundant formation of carbon dioxide when plants reached the stage of extensive vegetative development; following maturity, less gas was produced. Biennials were found to exert a prolonged effect, owing to their longer growth period. In general, then, the influence of plants on the formation of carbon dioxide was similar to the changes he reported of the bacterial populations of the rhizospheres of these plants (94). In further studies Starkey (96) reported greater amounts of carbon dioxide arising from soils obtained from regions of principal root development than from soils apart from the root; this was true with old (biennials) as well as with young (first year) plants. Again the correlation between intense carbon dioxide evolution and abundance of soil microbes was evident.

Stille (102) compared carbon dioxide evolution by mustard growing in sterile sand and sand inoculated with a soil suspension, and concluded that carbon dioxide production was due largely to microorganisms. Analysis of the data of Barker and Broyer (3) indicates that under aerobic conditions of growth the average carbon dioxide evolution by squash roots receiving manganese in sterile culture was 0.279 and in nonsterile culture 0.549 mgs., or slightly over 50% due to the roots. However, under anaerobic conditions the sterile system yielded 0.108 and the nonsterile system 0.289 mg., or 37% from the roots. It is clear from this limited information that no definite conclusions can be reached regarding the proportion of carbon dioxide produced by plant roots or the rhizosphere microflora. That the amount evolved is appreciable is quite apparent, and its influence on root feeding and microbial activity in the rhizosphere is of unquestioned importance (120, 124).

QUALITATIVE EFFECTS OF HIGHER PLANTS ON SOIL MICROORGANISMS

Perhaps even more significant than the quantitative effects of higher plants is their qualitative influence on the soil microflora. Studies on this phase of the rhizosphere problem are increasing in number, with emphasis almost exclusively on bacteria, but are still not abundant. In general, the procedures involved in these investigations have been along two broad lines: those in which numbers of specific groups or species are determined on selective media by plating or dilution methods, and those in which a non-selective medium is used and all colonies on a plate or a representative sector of a plate are picked and studied from the point of view of cultural, morphological or physiological behavior; by this means quantitative, as well as qualitative, data may be obtained, as well as information on the relative incidence of specific types of organisms. In addition, studies of prevailing types of bacteria adhering to buried slides have also yielded useful information. Little data exist, however, on the selective action of roots on the fungus flora of the soil and practically none concerning actinomycetes, protozoa, nematodes and algae with the exception of the parasitic forms within these groups which are involved in root diseases (6, 14, 21, 27, 91).

Since plants differ in chemical composition, physiology and nutrition, it is conceivable that they would attract specific groups or species of organisms which may be beneficial, harmful or without known effects. Decomposition of "sloughed off" root fragments may be carried out by specific organisms whose nature is governed by the chemical composition of these root parts, and excretion of specific and perhaps characteristic substances may favor the development of a rhizosphere microflora typical of a particular plant (20, 31, 32, 34, 51, 79*a*). Hiltner (28) held the view that this selective action is favorable for the plant, which is far from being the case, as the multitude of root rot diseases attests. Any factor contributing to a modification of growth and physiological behavior of the plant may, then, be expected to initiate changes in the root microflora. Such factors as age and nature of the plant, moisture content, reaction and type of the soil have been shown above to alter the "rhizosphere effect" on numbers of the broad groups of soil organisms. The following section deals with this "rhizosphere effect" on the incidence of specific groups or species of soil microorganisms. Bacteria have been studied most extensively in this connection and will be considered first.

Bacteria: Morphologic and Taxonomic Types. In this section it is intended to group all organisms without known or specific physiological function, such as "dye-tolerant bacteria", Gram-negative rods, spore-formers or specific types, as *Alcaligenes radiobacter.*[5] Organisms of the Radiobacter group and related forms usually appearing on the same selective medium employed (*Aerobacter* and *Rhizobium* types) have been widely mentioned as being stimulated in the root zone and especially in that of legumes (15, 24, 90, 93). For example, Starkey (94) found that organisms of the *B. radiobacter* group, producing mucoid colonies in nitrogen-free mannite agar, increased in the rhizosphere more than other groups studied; oats and rape showed the most pronounced effects, but potatoes exerted only slight influence. As plants reached maturity the rhizosphere effect diminished, though beans exerted their selective power up to the final stages of growth. Sweet clover also exerted a prolonged effect, as might be expected from a biennial. Even more striking results were obtained with this group by use of glycerol-nitrate agar; the influence of rape was particularly great; an R: S ratio of 86 was obtained at 44 days, decreasing to 9 at the final sampling period. Again with most plants the increase in rhizosphere effect was greatest at the height of the growing period followed by a decrease at the final stages of growth. In general,

[5] Specific names of bacteria are based on Bergey's Manual (5th Ed.) except where derived from original articles in which case the name used is that employed by the author quoted.

young plants exerted only a slight effect. Despite these large ratios, the absolute increases in most cases are much lower than with the other organisms studied, as this group of bacteria constitutes a small proportion of the total soil or rhizosphere population. Later Starkey (96), in a comparison of the effect of biennials and annuals at different distances from the roots on numbers of the *B. radiobacter* group, observed a marked selective action, especially with sweet clover; an R: S ratio at 356 days of about 254 was obtained which increased to 2,218 at 437 days. These large ratios were considered due to the inclusion of *Bacillus radicicola* in this group. Again the Radiobacter group increased proportionally more than any of the other groups studied.

The weight of evidence suggests that Gram-negative, non-spore-forming bacteria (Radiobacter types, fluorescent and "dye-tolerant" forms) multiply extensively in the rhizosphere of a wide variety of plants, whereas spore-forming bacteria do not (10, 11, 48, 50, 56, 57, 65, 78, 136). The latter can develop on roots (13, 102) and occur in greater abundance on those of certain plants (oats, radishes) than on others (barley, corn, beans, lettuce) (11). Certain spore-formers (*B. brevis, B. circulans, B. polymyxa*) were reported to comprise a more important fraction of the *Bacillus* population in the rhizosphere than in the soil; *B. polymyxa* appeared to be relatively more abundant on cereal roots and *B. megatherium* on cotton roots (11). Some plants actually repress these organisms in the rhizosphere (11, 65). Distribution of these bacteria on plant roots is also affected by the nature of the soil (11).

As may be expected, the nature and age of the plant affect numbers of Gram-negative, non-spore-forming rods also. "Dye-tolerant" bacteria were found to be more abundant on cotton than on wheat roots, and fluorescent bacteria highest on barley and radish roots (11). Lochhead (65) also observed that Gram-negative short rods were relatively more abundant, and coccoid forms less abundant in the rhizosphere of varieties of flax and tobacco susceptible to certain root diseases. Starc (92) reported that *B. fluorescens* developed rapidly on the root hairs of maize. Eaton and Rigler (18) noted the preponderance of blue green fluorescent bacteria on cotton roots with a high carbohydrate content. The early stages of growth appear to favor extensive development of these organisms (11, 56, 57, 136), although they are very abundant even in the later stages (50). Numbers of Gram-negative short rods in the rhizosphere are also affected by soil and climatic conditions (50).

Examination by the buried slide technique (30, 99, 100, 112) lends further support to the observed preponderance of Gram-negative non-spore-forming rods and the scarcity of spore formers in the rhizosphere.

Mycobacteria have been reported to occur in great abundance in the rhizosphere of certain plants (56, 57, 78). Thom *et al* (108) noted that the majority of organisms isolated from wheat roots in chicken-manured soil were Gram-positive corynebacteria, inhibited by crystal violet, reducing nitrate and generally requiring organic nitrogen for growth. Similar forms, though without the capacity to reduce nitrates, predominated on the roots of wheat on alfalfa-treated soil. Lochhead (65) reported that the *Corynebacterium* group and proactinomycetes were relatively less abundant in the rhizosphere of various plants than in soil apart from the root, but that *Mycoplana* was preferentially stimulated.

Valleau *et al.* (117, 118) found that certain bacterial pathogens of tobacco plants, such as *Bacterium tabacum,* causing wildfire, and *Bacterium angulatum,* causing angular leaf spot, occur in nature on the roots of pasture and crop plants. They remain indefinitely and apparently form colonies on the small rootlets of the plants. *Xanthomonas vesicatoria* also appears to grow and overwinter on wheat roots (16).

Bacteria: Physiological Groups. Perhaps even more important for an understanding of the interrelationships between plant roots and the soil microflora is an appreciation of the physiological activity and nutritional requirements of the organisms congregating at the root-soil interface. Carbon dioxide evolution from soil has frequently been used as an index of microbial activity and has been shown to be greater in soil adjacent to roots than in soil beyond the zone of root influence (74, 93, 100). This effect is related to the abundance of organisms in these soils and to availability of organic substances for decomposition, the rhizosphere soil containing more of both than soil apart from the root. Thom (106) pointed out that most of the organisms associated with the rhizosphere belong to species active in the decomposition of fresh organic matter and not to forms involved in the breakdown of humus residues in soil. Clark (11) 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.

By means of plating on a non-selective medium and isolations Lochhead (65) found a more active bacterial flora in the rhizosphere than in the soil apart from the root. A higher percentage of forms growing well on nutrient agar, of liquefying types, and of those causing acid or alkaline reactions in dextrose were found in the rhizosphere. A noticeably greater percentage of motile bacteria and of chromogenic types was also noted on the root surfaces. Chromogenic forms occurred in greatest relative abundance in tobacco rhizospheres; other plants showed similar though not such striking differences. He observed a somewhat more active microflora in the rhizosphere of susceptible varieties of flax and tobacco.

Bacteria: Nutritional Groups. The method employed in studies of the qualitative nature of soil bacteria by Taylor and Lochhead (104) and Lochhead (65) was applied by West and Lochhead (131) to a study of the nutritional requirements of the organisms isolated from the non-selective (soil extract agar) medium as described earlier. Different cultural media, ranging from a simple inorganic basal medium to more complex substrates, containing amino acids, amino acids + growth factors, or yeast extract, were used. The roots of even young seedlings of flax and tobacco favored the development of bacteria dependent upon a supply of thiamine, biotin and amino acids for growth. Organisms growing in the basal medium were relatively more numerous in the flax rhizosphere than in the control soil; tobacco rhizospheres did not show this effect. Bacteria requiring or stimulated by amino acids were relatively more abundant in the rhizospheres of both plants than in the control soil and especially so in the rhizospheres of susceptible varieties (131). Similar observations were made with bacteria requiring, in addition, growth factors. Organisms requiring yeast extract were relatively more numerous in the control soil than in the plant rhizospheres in most cases. Greater differences were found to exist between the rhizosphere and the control soil where the latter was poorer than where it was richly supplied with organic matter whose decomposition would obviously yield amino nitrogen and growth factors for the soil bacteria. These results were confirmed (42) with greenhouse tomatoes and later (43) with strawberry plants. However, organisms requiring known growth factors did not appear to be preferentially stimulated in the rhizosphere (43, 67). Further studies (132) involved a slight modification of the media described above and the development of a means of assessing the equilibrium existing between these nutritional groups in soil—the Bacterial Balance Index (B.B.I.). This index was calculated by assigning a negative value to the percentage occurrence of Gram-negative bacteria growing abundantly in a simple inorganic medium, a positive value to those organisms requiring amino acids and growth factors, and adding the resulting figures. By this method it was shown that the rhizosphere of Premier strawberry seedlings had an index of +63 as compared with +35 in the control soil. This principle was later applied (29, 130) in a study of the rhizosphere of strawberry plants in relation to root rot and will be considered.

A more extensive differentiation of the nutritional groups of bacteria was proposed by Lochhead and Chase (66), which involved a determination of growth response in seven cultural media of increasing complexity. In an application of this procedure to a study of mangels, it was found (67) that bacteria with simple requirements and those responding to amino acids were preferentially stimulated in the rhizosphere. It was also reported that culture filtrates of bacteria growing in the simple inorganic medium provided greater stimulation to organisms requiring amino acids and were more antagonistic to bacteria requiring growth factors. Thus at least a partial explanation for the increased relative incidence in the rhizosphere of bacteria requiring amino acids may be found in associative action of the rhizosphere microflora, although other phenomena, such as root excretions and decomposition of root fragments, undoubtedly also play an important rôle in this connection. In addition to the relative increase in the rhizosphere of organisms with simple requirements and of those requiring amino acids for maximum growth, another characteristic effect is the relative suppression of bacteria dependent upon the more complex nutrients provided in soil extract (42, 43, 67, 126), findings confirmed by recent work (126) with greenhouse oats, wheat, flax, timothy, red clover and alfalfa.

Nitrogen-Fixing Bacteria—Rhizobia. Due to technical difficulties there are comparatively few actual counts of rhizobia in soil and in the rhizosphere of plants. However, it has been shown by a number of investigators (20) that growth of plants, especially legumes, results in greater numbers of rhizobia in the soil. It has been noted previously that organisms of the *A. radiobacter* group are abundant in the rhizospheres and particularly so in the rhizospheres of leguminous plants. Such counts undoubtedly include organisms such as *Rhizobium* (93), and it may not be unjust to infer that this group is also selectively stimulated. In subsequent work Starkey (95) suggests that the abundance of *B. radiobacter* and related forms about legume roots is due partially at least to the fact that *Bacillus radicicola* is included in the group and undoubtedly makes up a considerable portion of the bacteria developing from samples taken from legume roots. Rhizobia have been isolated from the root zones of a large number of plants in Russia (83), although there was no indication of their relative incidence in soil or in the rhizosphere. Plants of the family Pinaceae did not yield these organisms whereas roots of members of Polygonaceae supported largest numbers. Lochhead (64) noted distinctly larger numbers of *R. trifolii* in the rhizosphere of clover than in control soil; R: S ratios over 100 were obtained.

Koreniako (46) reported that root excretion of some plants (clover, peas) stimulate, while those of others (corn, flax) retard development of nodule bacteria in the rhizosphere. Nodule bacteria developed well in the rhizosphere of wheat, cotton and other non-leguminous plants. Later work (54, 55) reported that some *Pseudomonas* and *Achromobacter* types inhabiting the root zone influence strongly the development of nodule bacteria (*R. trifolii*) in the rhizosphere of clover. Certain species stimulated growth of the nodule bacteria as did *Azotobacter,* others inhibited and still others were without effect. The authors claimed, too, that less virulent strains of nodule bacteria are activated by mixing with the stimulating organisms. A further suggestion was made that nitrogen fixation occurs in clover cultures in the absence of nodules but that the presence and development of bacteria activating this process around and on the root is necessary. The claim that these activating bacteria produce biological catalyzers penetrating the plant and stimulating the process of nitrogen fixation by plant tissue requires further substantiation in the light of the widely accepted concept that both symbionts, the legume and the nodule bacteria, participate in this process (20, 133, 134).

Azotobacter. Despite the interest in these organisms from a theoretical and practical point of view, there is surprisingly little information of a quantitative nature concerning their natural occurrence in the rhizosphere of plants. Beijerinck as early as 1908 found larger numbers of *Azotobacter* in soil close to the roots of legumes than in soil from roots of non-legumes (93). Kostychev *et al.* (47) noted the accumulation of *Azotobacter* in the vicinity of tobacco roots. Starkey (94), however, could obtain no evidence of a selective action of roots of legumes or non-legumes on these nitrogen-fixing bacteria. Comparing samples from the rhizosphere and from soil apart from the root, Gräf (24) concluded that the most intensive nitrogen-fixation by *Azotobacter* and *B. amylobacter* occurred with the rhizosphere soil. It was strongest with samples from the root surface ("inner" rhizosphere) and also from the control soil in June, and greatest with the rhizosphere soil ("outer" rhizosphere) in July. A decline of intensity of fixation occurred with samples from all three zones in August. Soil from the rhizosphere of legumes exerted the most favorable influence; that from rape was also somewhat favorable, but samples from grains were without effect. Although these results imply greater numbers of nitrogen-fixers in the rhizosphere of some plants, they can also be interpreted on the basis of more active strains of *Azotobacter* rather than larger numbers in the rhizosphere. Others (115) isolated *A. chroococcum* from the rhizosphere of wheat, but in the absence of data on its incidence in control soil, no selective action by wheat roots can be deduced. The same objection applies to the much quoted work of Poschenrieder (80). Evidence was obtained merely on the presence of *Azotobacter* on roots of various cultivated and wild plants. The organism was almost always present on roots in the summer, though frequently absent in the fall, especially from plants which had been harvested. Comparing the "inner" and "outer" rhizospheres (washed roots and roots with adhering soil respectively) he found that the latter showed generally better development of *Azotobacter* than the former (various plants showed no *Azotobacter* in the "inner" rhizosphere). Extensive growth of fluorescent bacteria in cultures from the "inner" rhizosphere suggested that these organisms may have repressed *Azotobacter* in this zone. There is little to show that this organism is more abundant in the rhizosphere than in the control soil, or to support the statement that it is a "root bacterium". Rokitzkaya (83), in a study of the microflora of the root systems of plants belonging to 31 families, noted the presence of *Azotobacter* in all but three (Pinaceae, Betulaceae and Fagaceae). "Relative intensity" of development of *Azotobacter* was greatest with members of Polygonaceae, Rosaceae, Pomaceae and others. Krassilnikov (48) concluded that this organism was absent from the rhizosphere of wheat and developed only weakly in that of maize. By means of buried slides it was demonstrated (30, 99) that *Azotobacter*-like cells occur on roots of various plants. Lipman and Starkey (63) pointed out that there is considerable evidence that *Azotobacter* does not appear more commonly in the rhizosphere than elsewhere in the soil. However, Manzon (74) claims that fixation of free nitrogen is greater in the rhizosphere than in control soil, and others (88) report the accumulation of *Azotobacter* throughout the early stage of development of corn and buckwheat; at the flowering stage numbers were depressed and the organism gradually disappeared as the plant matured. In a study of growth of roots *in vitro* it was observed (53) that the presence of bacteria depressed growth, wheat roots being more sensitive to *Azotobacter* than pea roots; but that filtrates of *A. vinelandii* stimulated root growth. Sidorenko (89) also reported that oats, barley, sudan grass, sunflower and soybean stimulated growth of *Azotobacter* inoculated on seed, but that wheat depressed it; corn, soybean and alfalfa caused fluctuations according to the developmental stage of the plants, and sugar beet was inert in its effect on *Azotobacter* development. Clark (11) did not observe appreciable numbers of *Azotobacter* on the root surface of plants growing in soils themselves generally free of this organism, and Jensen and Swaby (36) could not demonstrate a marked prominence of *Azotobacter* in the rhizosphere of legumes. Even by means of heavy inoculation of roots of tomato seedlings, Clark (12) was unable to establish *Azotobacter* in the rhizosphere. However, Starc (92) reported increases in number of cells in sand and around roots of maize when tested in suitable media; yet by means of buried slides he could not detect them on the root hairs. More recently, Katznelson (41), using a dilution-plate method of counting

Azotobacter cells, demonstrated the presence of this organism in the rhizosphere of mangels and in soil apart from the root, but there was no evidence of a selective action by the roots. Further work (44) provided evidence of the presence of this organism in the rhizospheres of wheat, oats, timothy, flax, red clover and alfalfa, but only with flax was there an indication of a slight "rhizosphere effect". Timothy actually exerted a slightly depressing effect on the organism.

It would appear, therefore, that *Azotobacter* does not find the root zone of many, if not of most, plants a more congenial habitat than the soil proper, a fact which renders the procedure of seed treatment with this organism and the claims of resulting increases in crop yields somewhat suspicious. This question will be referred to later.

Anaerobic Nitrogen-Fixing Bacteria. Surprisingly little attention has been paid to the anaerobic nitrogen-fixing bacteria in the rhizosphere, especially in view of the fact that they are more abundant in soil and are more adapted to a wide variety of factors (moisture, temperature, reaction) than *Azotobacter* (120). Gräf (24) mentions *B. amylobacter* as occurring in the rhizosphere and in soil, as demonstrated by nitrogen fixation in mannite solutions, but there seems to be no evidence to show that this organism was actually isolated and identified. Bezssonoff and Truffaut considered *Clostridium pasteurianum* to be involved in supplying nitrogen to corn (38). Demidenko apparently did not observe a selective action on this organism by plant roots (63).

In a study of gas-producing anaerobes, very closely related to, if not identical with, *Clostridium pasteurianum* in the rhizosphere of mangels, Katznelson (41) observed a distinct rhizosphere effect throughout the growing season for roots in manured soil. In untreated soil this effect was observed considerably later in the growing season, at which time the R: S ratio was 200 as compared with 16 for plants in the treated soil. As many as 1.4 million bacteria per gram rhizosphere soil were found. In a later survey of various plants grown in the greenhouse (44), no marked stimulation of this group of organisms was observed except with flax which yielded an R: S ratio of 5.0 and 3.2 at two and four months, respectively. As with *Azotobacter,* counts of these gas-producing anaerobes appeared to be depressed by timothy. More work is required to elucidate the incidence and rôle of these organisms in the rhizosphere.

Ammonifying and Proteolytic Bacteria. Various spore-forming and non-spore-forming bacteria, such as *B. mycoides* and *Bact. fluorescens,* respectively, have been shown to play an active part in the breakdown of proteins and simpler nitrogenous compounds with liberation of ammonia (120). Certain of these bacteria are more effective together than when alone (123). As noted above, spore-formers, though occurring in the rhizosphere, are nevertheless comparatively scarce, but non-spore-forming organisms, of which fluorescent types are prominent, are the predominant forms on root surfaces. It is likely, then, that these are chiefly responsible for the evidence of large numbers of ammonifying bacteria on plant roots. For example, Gräf (24) noted that the decomposition of peptone and production of ammonia was strongest with samples from the root surface, increasing in intensity from May to July and falling sharply in August. Rape appeared to stimulate the process, grains showing only a slight effect and legumes least. Rokitzkaya (83) also demonstrated the presence of an active ammonifying flora in the root zone of 178 different plants. As might be expected, different plants exhibited different "intensities" of ammonification. Krassilnikov *et al.* (57) stated that the predominant forms in the rhizosphere consist of non-sporing rods with pronounced ammonifying capacity. Lochhead (65) observed that gelatin-liquefying types, and Timonin (114) that casein-hydrolyzing bacteria were more numerous in the rhizosphere of various crops than in control soils. Katznelson (41) demonstrated a striking stimulation of this group of bacteria on mangel roots from manured soil, an effect which increased with age of the plant and was more pronounced in treated than untreated soil. However, at the end of the growing season the R: S ratios for the roots in both soils were about the same (about 2,000). It is to be expected that in this zone of root influence, microbial activity would be intense, and it is logical to infer that such common soil processes as CO_2 evolution and ammonification would be particularly pronounced. Thom's (106) observation that most of the organisms associated with plant roots belong to species active in the decomposition of fresh organic matter is in line with the above findings.

Nitrifying Bacteria. Studies of the effect of plant growth on nitrification in soil are quite numerous, but investigation of numbers of nitrifying bacteria on the rhizosphere of plants practically non-existent. Most investigators have compared samples taken from planted soil with those from unplanted soil, and the results (15, 71, 93) indicate that nitrates accumulate more rapidly under legumes than under non-legumes and that, in addition, plants exert an accelerating effect on nitrification in the early stage and depress it at more advanced stages of growth. Starkey (95) compared the accumulation of nitrate-nitrogen in fallow soil with soil obtained from the roots of various plants. In general, nitrates increased more rapidly in the latter soils and were more abundant in soil under biennials than annuals, the effect extending throughout the growing season. Nitrate formation was greater in soil supporting biennials at the last sampling than at any previous period with any of the other plants. Rape exerted the most pronounced effects throughout and potatoes least; however, no marked difference between the effects of legumes and non-legumes was observed. The influence of plants on nitrification was thought to be due to the addition of organic matter with a narrow carbon-nitrogen ratio to the soil resulting in liberation of ammonia on decomposition (120, 121, 124). It is, of course, possible that the development of plants causes an increase in numbers and activity of the nitrifying bacteria in the soil, but this could not be detected by the methods used.

Gräf (24) noted that nitrification was lowest at the root surface of plants but slightly higher in the rhizosphere soil than in the soil at a distance from the root. Rape showed no effect, however, and legumes were less favorable than grains. The rate of nitrification rose from May to June, dropped sharply in July and rose again in August. In a later study of this problem Starkey (96) again demonstrated that nitrification of soil nitrogen is greater in soil from regions of extensive root development than from soil supporting less root development, the results being especially pronounced with plants in more advanced stages of growth and more striking on the whole in clover than in table or mangel beet soil. From these data he considers it reasonable to suppose that the "soil constituents required by nitrifying bacteria are present in proportionately great abundance in soil close to the root". In addition, he states that "it may be concluded that either the nitrifying organisms in these soils are more efficient transformers of ammoniacal nitrogen or that these soils support a greater abundance of the bacteria". However, even though nitrate accumulation in soils from roots is greater than in fallow soil, the actual content of nitrate nitrogen of the soil decreases as the root is approached, owing to utilization of this nitrogen by the plant (97).

Truffaut and Vladykov (115) reported the isolation of *Nitrosomonas* from wheat rhizosphere, and Rokitzkaya (83) observed nitrification in the root zone of all plants tested. In an attempt to enumerate these organisms in soil and rhizosphere of mangels, Katznelson (41) demonstrated a slight rhizosphere effect of plants in manured soil especially at the early stage of growth.

Denitrifying and Nitrate-Reducing Bacteria. These organisms have been studied somewhat more extensively than the nitrifying bacteria. Gräf (24) noted that "nitrate assimilation" was strongest at the root surface, especially with legumes; rye was the best of the grains in this connection, whereas wheat and rape exerted the least effect. The presence of large numbers of denitrifying bacteria in the root zone of many plants has also been noted by other investigators (48, 56, 57, 58, 78, 83). Manuring appeared to stimulate these forms in the rhizosphere of mangels (41), although plants in unfertilized soil supported large numbers. Denitrifying bacteria were also reported to be more abundant in the root zone of an oat variety susceptible to manganese deficiency (114). Nitrate-reducing organisms were found in great abundance on wheat roots in manured but not in alfalfa-treated soils (108) and have been observed to be relatively more abundant in the rhizosphere of susceptible than of resistant strains of flax and tobacco (65).

Cellulose-Decomposing Bacteria. Cellulose-decomposing organisms, especially anaerobic types, have also received little attention with regard to their incidence in the rhizosphere. That organisms capable of decomposing cellulose are present on the root surface has been demonstrated by a number of workers (41, 78, 83, 115). Rokitzkaya (83) did not succeed in isolating *S. Hutchinsoni* (*S. cytophaga*) from the root zone of ten out of 31 families of plants. Anaerobic cellulose decomposers were isolated from all plant types studied, though, as with most of the data presented in this paper, there is no indication of a selective effect of the roots. Krassilnikov *et al.* (56) reported marked increases in cellulose-decomposing bacteria in the rhizosphere of wheat, maize, sunflower and soya. The same authors (57) noted a simultaneous increase of total numbers of organisms and of aerobic cellulose-decomposing bacteria in the rhizosphere of sunflower and soybean as the plants developed. An increase in numbers in the early part of the growing period was followed by a drop and then by another peak in the later part of the season. They concluded that these organisms take part in the decomposition of degenerated root fragments and that the products of this process are simultaneously used by other organisms. Aerobic cellulose-decomposing bacteria were found by Katznelson (41) to be abundant in the rhizosphere of mangels growing in manured soil but much more so in that of mangels growing in unfertilized soil. A rhizosphere effect with oats has also been reported (114); a slightly higher R: S ratio in the rhizosphere of an oat variety susceptible to manganese deficiency disease was noted.

Manganese-Oxidizing Bacteria. This group of organisms has been studied chiefly in connection with manganese deficiency disease of plants, particularly "grey speck" of oats. Gerretsen (22) suggested that this disease might be related to the presence of manganese-precipitating bacteria in the soil, pointing out that these organisms might be especially active in the neighborhood of the root where easily decomposable organic matter is available.

Timonin (114) observed that a susceptible variety of oats harbored in its rhizosphere a denser population of manganese-oxidizing bacteria than did a resistant variety grown under identical conditions. Partial sterilization of the soil with various substances (chloropicrin, formaldehyde, *etc.*) reduced markedly the number of these bacteria in the rhizosphere (and soil) and resulted in great improvement of the plants; the disease symptoms were also greatly reduced.

Anaerobes. It is surprising that this group of organisms has received so little attention from investigators of the rhizosphere. Yet the amounts of carbon dioxide evolved by roots and by their rhizosphere microflora might be expected to create conditions favorable for development of facultative and possibly even obligate anaerobes. Velich (119) found an organism called *Clostridium gelatinosum* Laxa in greater abundance in soils close to the roots and on the root epidermis of beets, mangels, barley, rye, oats and wheat. Similar observations were made by Maassen and Grüber (69). Rokitzkaya (83) demonstrated the presence of *Clostridium* in the root zone of 31 families of plants. The "intensity" of its growth was greatest with plants belonging to Chenopodiaceae, Solanaceae and Valerianaceae, and least with those of Pinaceae, Betulaceae and Fagaceae, the same families which yielded no *Azotobacter*. Katznelson (41) observed a striking increase in numbers of anaerobic forms on the root surface of mangels from both manured and untreated soils and especially from roots in the latter. This work was later extended (44) to include wheat, oats, flax, timothy, red clover and alfalfa growing in the greenhouse. Again, the roots supported much greater numbers of these organisms than the soil at a distance from them. R : S ratios were observed to increase with age of plants except for timothy which showed a lower ratio as it matured. Little else can be stated at present concerning this group of organisms except that it warrants further investigation.

Fungi and Other Micro-Organisms. Although many quantitative studies of fungi in plant rhizospheres have been reported, few data of a qualitative nature are available. Thom and Humfeld (107) reported that corn roots in acid soils yielded predominantely *Trichoderma* colonies, whereas roots from alkaline soil seemed to be associated chiefly with penicillia of the biverticillate series (*P. luteum, etc.*). Timonin (111) concluded that there is no significant difference in the fungal population of the rhizosphere of seedling plants (wheat, oats, alfalfa and clover) or between the rhizosphere and the soil distant from the root. However, in later work with flax he (113) 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.

Katznelson and Richardson (42), in a study of the qualitative differences in fungi between the rhizosphere of tomatoes and the soil, obtained some evidence of a selective action by roots, especially among the "unidentified" fraction of the fungus population. Results varied with age of plant and soil treatment. In later work with strawberries these workers (43) also obtained evidence of qualitative differences among the fungi on the root surface as compared with those in the control soil. The untreated soil series yielded dark green *Penicillium, Aspergillus* and *Fusarium* types which were absent from the roots growing in it, which, however, yielded *Cladosporium, Chaetomium* and an unidentified non-sporulating fungus. Soil treatment affected this distribution, as was also found by West and Hildebrand (130) whose work will be discussed later. The rhizospheres of plants in soil treated with acetic acid supported a red-green form of *Penicillium* (26%), *Verticillium* (28%) and *Cladosporium* (4%), whereas the soils yielded none of these but contained *Aspergillus* (6%), *Trichoderma* (12%), *Oothecium* (16%), an unidentified fungus (24%) and other forms, such as *Mucor* and *Fusarium*. These results indicate a selective action of roots on soil fungi, but again more work is needed along this line.

The remaining groups of soil microorganisms, such as protozoa and algae, have scarcely been studied from a qualitative point of view. Attempts to determine whether roots of field mangels, or of wheat, timothy, oats, flax, red clover and alfalfa exerted a selective action on protozoa (41, 44) were not successful. Ciliates, flagellates and ameboid forms were all observed in both soils and rhizospheres. Blue green algae were noted (41) to be somewhat more abundant in the rhizosphere of mangels than in soil apart from the plant.

OTHER MICROBIOLOGICAL PHENOMENA ASSOCIATED WITH PLANT ROOTS

The great majority of the organisms discussed in the preceding pages develop readily in the soil or at the soil-root interface under suitable conditions. Whatever influence they may have on the root is exerted from without. Some of the organisms in the rhizosphere lead a passive existence, exerting little if any detectable effects. Others penetrate the root and establish a more intimate relationship with it which may be beneficial, harmless or injurious to the plant as a whole. Among the organisms concerned in favorable associations with plant roots, the nodule bacteria and mycorrhizal fungi are the most important. The invasion of plants, especially nonlegumes, by bacteria resulting in the so-called "bacteriorrhiza" (20, 28, 79*a*, 133) may involve true symbiosis or may result in an entirely passive association (93). Of the forms concerned in harmful effects, fungi causing root diseases are the most numerous and economically important. In general, "the extent of penetration and degree of the effects vary with the soil conditions which determine the vigor and degree of resistance of the plants" (93). The nature of the organisms concerned (virulence, physiological state) also exerts a profound influence on the extent of invasion and severity of the effect (21, 27). Since all these associations involve highly specialized organisms with specific characteristics it may perhaps be argued that they should not be considered representative of the general microbial population of the soil or rhizosphere (21, 96). Nevertheless, they are involved in the microbiology of root surfaces and consequently deserve mention.

The symbiotic relationship between the nodule bacteria or rhizobia with leguminous plants, resulting in the fixation of atmospheric nitrogen, is the most thoroughly understood and most exhaustively studied of the beneficial associations between plants and soil microorganisms. Attracted by some specific substance or substances excreted by the young legume root, the nodule bacteria, after inducing a deformation or curling of a root hair, penetrate the root, multiply in the cells of the host and stimulate production of the nodule. The various factors influencing these steps are thoroughly summarized elsewhere (20, 133). One of the most important of these is the presence of combined nitrogen, such as nitrate, in the soil. This nitrogen apparently does not so much affect the organism as it does the host plant; it does not prevent entry of the bacteria but does inhibit nodule formation and depresses nitrogen fixation. These effects are believed to involve the carbohydrate-nitrogen balance in the plant, a subject which has been reviewed exhaustively (77, 133). Not all strains of rhizobia are capable of penetrating the root, nor are all effective in stimulating nodule production, or in nitrogen fixation, once the nodule is formed; some strains are even parasitic (110). On the other hand, some legumes do not form nodules (20).

Nodules in roots of plants other than legumes, such as alder, silver-berry and red root, have also been reported (20, 120, 124). In the majority of cases the organisms concerned have not been clearly defined nor their rôle in this association (20, 120).

Invasion of many plants by various bacteria is not uncommon (20, 28, 31, 79*a*, 93, 96, 133). However, here again it has not been generally established to what extent these "bacteriorrhizal" formations are passive or beneficial. There is some question as to whether or not these are the true rhizosphere organisms as compared with other forms existing on the surface of the root or in the film of soil surrounding it. These may be the forms deriving some or slight benefit from the host, possibly contributing materially to it and detectable only by microscopic examination of roots or by culturing root fragments after washing the roots. Some investigators (31–35) feel that in these "bacteriorrhiza" we are dealing with an association similar to that of mycorrhizae.

The penetration of woody and herbaceous plants by certain fungi, resulting in "mycorrhizal" formations, is generally believed to be beneficial and in some instances essential to the host (9, 26, 120). Under unfavorable conditions, however, these fungi may even become parasitic (21, 72, 120). In some cases the fungus does not actually penetrate the root cells but forms a mass of mycelium around it, entering the root tissue chiefly between the cells; this type of mycorrhiza, very common among forest trees, especially evergreens, is called "ectotrophic". Other fungi penetrate root cells and may develop extensively therein; these are called "endotrophic" and are common in orchids and heather. According to various authors, mycorrhizal fungi supply their host with nitrogen, carbohydrates, water, phosphorus and other nutrients, auxins and enzymes and B vitamins (73, 75). However, "the nature and extent of the benefit to the host depends on various environmental conditions and the physiological processes involved in specific associations" (120). The benefit derived by evergreens from mycorrhizal fungi is of considerable practical importance in reforestation projects (21, 25, 81, 82).

A great variety of organisms occurring in the soil, such as nematodes, bacteria and fungi, can invade root tissue and cause serious damage to or even kill the plant (27). Attempts at control include use of inorganic and organic soil amendments (17, 21, 63, 120); partial sterilization with steam and other agents such as chloropicrin, formaldehyde (39, 76), and biological methods involving use of specific antagonists for particular pathogens (122, 127, 128); use of antibiotics (23, 122); or use of soil amendments designed to stimulate development of saprophytic organisms, thereby suppressing or eliminating the pathogen (85, 122).

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 rôle directly by stimulating or repelling the pathogen or indirectly by affecting the rhizosphere microflora (85). It has been pointed out earlier (65, 112) 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 up with a selective action of root excretions upon the saprophytic soil microflora thus favoring types which may be more and in other cases less antagonistic (directly or indirectly) towards pathogenic organisms" (65). In this connection it has been demonstrated (113) 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 (122, 127, 128). On the other hand, by-products of growth of a susceptible variety stimulated growth of the two pathogens. Eaton and Rigler (18) showed that cotton plants with low carbohydrate concentration were most severely attacked 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 but that resistance of seedling cotton plants to *P. omnivorum* may be chemical.

Since soil treatment exerts marked effects on the soil microflora (saprophytic and parasitic), the question naturally arises as to whether similar effects may be expected in the rhizosphere. Clark (10) and Clark and Thom (13) claim that organic amendments applied to soil have no apparent effect on the root surface microflora. It was later (103) suggested that "once the root parasite reaches the susceptible plant, factors of nutrition, host resistance or parasite virulence are probably more important than microbial antagonisms". Katznelson (41) obtained definite evidence of stimulation of certain groups of organisms in the rhizosphere of mangels growing in manured soil, although it was not entirely clear whether this effect was due directly to the treatment or attributable indirectly to its influence on rate of growth and vigor of the plant. The effect of soil treatment on the qualitative nature of the soil microflora on plant roots has been brought out by Hildebrand and West (29) working with strawberries. Steam sterilization of soil or the repeated incorporation of soybean into root rot soil completely controlled the disease, whereas use of red clover did not. By means of the qualitative-nutritional approach discussed earlier they showed that these treatments had altered the bacterial equilibrium in both soil and rhizosphere so that untreated root rot soil gave bacterial balance index values of -46 and $+10$ for rhizosphere soil and 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 (114). 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 and Richardson (43) pointed out that it is possible that these organisms are 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. In this connection it should be pointed out in general that qualitative or quantitative rhizosphere studies of plants with diseased roots are complicated by the fact that once the root has been penetrated by the parasite, other organisms usually gain entry, decompose the root tissue and multiply, thus giving an abnormal and possibly a false picture of the rhizosphere microflora (10, 107). West and Hildebrand (130) 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 (*i.e.*, higher bacterial balance index) 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). Katznelson and Richardson (43) in general obtained very similar results, particularly with fungi. The results with bacterial groups in the rhizosphere were not very consistent, due perhaps to the fact that the treatments used were not applied as frequently as they were by Hildebrand and West (29). Of particular interest was the fact that dried blood when added to root rot soil also induced healthy root formation. It was also noted that fungi with pathogenic propensities were more susceptible to acetic acid than normal saprophytic forms and that some of the former produced substances in culture which induced rapid wilting and drying up of strawberry seedlings (43). This type of work offers a promising line of approach to an understanding of the many problems involved in root rot diseases of plants, especially those of complex or unknown etiology. In this connection consideration should also be given to the influence of soil treatment on the physiology, vigor and resistance of the plant itself (21, 63, 125, 135) as well as on the pathogen. According to Walker (125), "there is reason to believe that studies of host nutrition in relation to disease development may yield information on the basis of which modifications in soil practices may often be used to reduce the acuteness of disease losses".

Seed Inoculation (*Bacterization*). Although soil and seed inoculation are artificial means of enriching soil, especially soil in the root zone, with specific organisms capable of establishing intimate associations with some plants, no review of the rhizosphere would be complete without some mention of this practice. The benefit derived by legumes from transfer of soil containing root nodule bacteria or by treatment of seed with pure cultures has been long recognized, and the latter procedure has become accepted agricultural practice. Inoculation of nursery beds with soil containing mycorrhizal fungi has also been shown to be an important step in raising planting stock in new nurseries and as an aid to the establishment of exotic species of forest trees (81, 82, 120). Bacterial treatment of seeds of non-legumes (bacterization), especially with *Azotobacter,* has often been attempted, but the results have been disappointing. However, there has been a recent revival of interest in this field, especially in Russia, where bacterization is being applied on a large scale and is considered of great agricultural and scientific importance. Claims of increases in yield of a large variety of crops, such as potatoes, sugar beet, oats, barley, flax, cabbage, wheat and tomatoes, have been made (38, 86, 87, 137); wheat yields, for example, increased from 123 to 484 pounds per acre; cabbage, 5,368 to 15,480; tomatoes, 2,904 to 11,600; and potatoes, 1,672 to 7,920. However, not all workers in Russia have reported such favorable results (38). Turchin (116) obtained no significant benefit from "Azotogen" or another similar preparation applied to a large number of crops in pot and field experiments. Nitrogen fertilizers gave large responses under similar conditions. Jensen (37) has recently assembled and critically reviewed the pertinent Russian literature.

Use of *Azotobacter* is based upon the premise that organic root excretions of plants are able to support sufficient growth and fixation of nitrogen by this organism to satisfy the nitrogen requirements of the plants. This presupposes that *Azotobacter* is an important rhizosphere organism, a theory not supported by the present weight of evidence.

Starc (92) inoculated sand growing maize with *Azotobacter* but obtained no increase in yield of plants; he suggested that these nitrogen-fixing bacteria use products of living and dead root portions only through the activity of other organisms. In addition, he observed that other bacteria and molds, especially *B. fluorescens*, rapidly developed on the root hairs so that *Azotobacter* was suppressed in the "inner" rhizosphere. Most recently Clark (12), in attempting to establish *Azotobacter* on roots of tomato seedlings by dipping them into heavy suspensions of *Azotobacter* and adding cultures to the roots on transferring from flats to soil in pots, observed that the organisms did not persist on the root but diminished in numbers rapidly over a period of weeks. In addition, Jensen (37) states that "no evidence has been produced that the quantities of organic matter secreted by the roots are upon the whole sufficient for nitrogen fixation of any significance in comparison with the nitrogen requirements of the plant". He inclines somewhat more to the suggestion of Isakova, Berezova, Krassilnikov and others that the effect of bacterization is due to stimulation of plants by growth factors elaborated by *Azotobacter* and other bacteria used for seed treatment, such as *Pseudomonas* spp., root nodule bacteria, tyrosin-decomposing bacteria and crude bacterial mixtures of rhizosphere organisms from different plants. Synthesis of various growth factors by *Azotobacter* and other organisms has been demonstrated by many investigators (40, 45, 109). However, Jensen (37) contends that "stimulation of intact plants by extraneously supplied growth-compounds seems to be the exception rather than the rule" and that "no proof has been given that bacterization results in a permanent modification of the rhizospheral microflora of such a character that organisms with particular aptitude for synthesis of growth-compounds become more prominent than otherwise". In this connection it should be pointed out, as already indicated, that organisms of the *Pseudomonas* type have been found in great abundance in the rhizosphere and that these bacteria are richest in growth substances, followed by *Azotobacter* and *Achromobacter* types with spore-formers and mycobacteria-producing none (52). More recent work (7, 101) casts some doubt on the importance of an external source of vitamins for higher plants, although the possibility that they may benefit at least to some extent from specific growth factors in soil, and particularly from those synthesized by the multitude of organisms in direct contact with the root (63, 101, 133) can not be entirely dismissed. Perhaps of greater significance are the claim of Isakova (35) that the rate and percentage of germination of seed can be increased by bacterization and the claim of a number of Russian workers that bacterial treatment of seed may exert a protective effect against infection of seedlings by parasitic fungi, such as *Fusarium* and *Colletotrichum* spp. (37). These investigators found that *A. chroococcum, P. fluorescens* and certain mycolytic fungi were active in this respect. Although it is difficult to consider *A. chroococcum* in the light of an antagonist, the use of specific antagonists for controlling soil borne parasites has been attempted by many investigators with varying success (122, 127). In regard to the influence of bacterization on seeds Jensen (37) agrees that favorable effects may be obtained, at least with seed of low germination capacity, as a result of the presence of bacteria capable of synthesizing growth-accessory compounds. However, Bawden (4) observed no stimulation of barley germination by plant hormones such as indole-3-acetic acid, α-naphthalene acetic acid and related compounds. Von Euler and Perje (19) also failed to note any effect of para-aminobenzoic acid, nicotinic acid, nicotinamide or adenosinephosphoric acid on seed germination; in fact, the first of these compounds was very inhibitory at certain concentrations. Recent reports from Russia and eastern Europe indicate very wide use of bacterial fertilizers, even to the extent of two and a half million hectares in the U.S.S.R. Emphasis appears to have been placed on selection of strains of *Azotobacter* specifically adapted for symbiosis with an individual species of plant (2). Again the effect of inoculation is claimed to be threefold: delivery of growth accessory substances, increase in the nitrogen content of the plant, and marked increase in the harvest yield of the crop. Apparently in no case was it possible for the individuals returning with these reports to assess the value of this work, and consequently much careful and critical research is required to substantiate the claims made.

DISCUSSION

Although adequate explanations for the congregation of organisms on root surfaces and for the preferential stimulation of specific types or groups are, in most cases, not available, these phenomena are unquestionably intimately related to the supply of inorganic and organic nutrients at this root-soil interface (94). Plant excretion of inorganic substances (70, 79, 93), accessory growth factors (68, 129, 133), amino nitrogen (68, 133, 134) and a variety of other substances (68, 93, 96, 99) have been generally accepted. Nor should the food materials supplied by "sloughed-off" root caps, root hairs, cortical and epidermal cells be overlooked, since they are rapidly attacked by soil microorganisms and supply considerable quantities of organic and inorganic nutrients (106). In fact, one of the most difficult technical problems in the study of the influence of higher plants on soil microorganisms concerns the distinction between actual excretory products of the root and the products of decomposition or autolysis of "sloughed-off" root fragments. It is not unlikely that some instances of observed "excretion" by plants should be attributed merely to breakdown of portions of the root.

As a result of this increased supply of nutrients in the rhizosphere the organisms normally present in soil, including fungi, bacteria and actinomycetes, and especially those capable of decomposing fresh organic matter (106, 120, 124) or of utilizing inorganic and simple organic nutrients directly, begin to multiply. Accompanying this increased biological activity are various microbial interactions such as symbiosis, mutualism, stimulation and inhibition with their resultant effect on the microbiological equilibrium, as well as on the plants (21, 63, 67, 122). It is quite conceivable that the observed increases or decreases in numbers of specific groups of organisms in the rhizosphere are due as much to associative or antagonistic effects as to the influence of the root itself (67). Excretion of inorganic elements such as potassium or phosphorus by plants may favor the development of bacteria with simple nutritional requirements (42, 43, 126, 131) in the rhizosphere, and liberation of amino acids may stimulate those requiring these compounds. Breakdown of root parts may also yield such substances and result in relative increases of these groups. On the other hand, the decreased relative incidence in the rhizosphere of bacteria requiring complex unidentified substances in soil extract (42, 43, 67, 126) may be explicable on the assumption that these organisms are more closely associated with breakdown of humus residues (121) than of the fresh organic material present at the root surface, or on the hypothesis that they are relatively depressed by the rapid development of the microorganisms (bacteria, fungi and actinomycetes) capable of utilizing the latter substances.

Bacteria oxidizing ammonia-nitrogen might be expected to be stimulated in the root zone as a result of the liberation of ammonia, due to the extensive development and activity of ammonifying organisms in the rhizosphere. Furthermore, as a result of the presence of both nitrate-nitrogen and appreciable quantities of carbon dioxide, denitrifying bacteria, as well as other facultative or even obligate anaerobes, might be stimulated. Accompanying the decomposition of readily available organic materials in the rhizosphere or subsequent to it, cellulosic substances are attacked, resulting in large numbers of cellulose-decomposing bacteria (and likely of fungi as well). Anaerobic bacteria can develop at the expense of the products of excretion, decomposition or cellular autolysis. With such extensive bacterial proliferation protozoa may be expected to find this zone a rich storehouse, provided antagonistic phenomena do not intervene to suppress them. The failure of such forms as *Azotobacter* and aerobic spore-forming bacteria to thrive in this environment may be ascribed to competition for food, susceptibility to antibiotics (which appear to be on the whole more toxic to Gram-positive bacteria) or to the fact that these organisms are less adapted to the facultative conditions obtaining in this zone. There are suggestions that different plant species or even varieties may favor the development of a specific rhizosphere microflora, but much more work is required before this can be regarded as established. It is of course recognized that the rhizosphere of certain groups of plants is characterized by the presence of specific organisms such as rhizobia and mycorrhizal fungi. This phenomenon is, however, distinct from the preferential stimulation by plant roots of soil microorganisms not regarded as symbionts or pathogens.

It is evident that 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. Environmental factors may exert their influence directly on the soil and the rhizosphere organisms or indirectly by stimulating or retarding plant development. Increased knowledge of the specific effects involved in this zone of interaction between roots and microorganisms may provide a basis for a better understanding of various phenomena related to plant feeding and growth, cropping systems and root disease control. The fields of investigation which such problems open are of great interest both academically and practically.

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ADDENDUM

Since the submission of this paper for publication several articles have appeared which have a direct bearing on the subject matter included in this review and which, therefore, should be noted.

ALLISON, F. E. *Azotobacter* inoculation of crops: I. Historical. Soil Sci. 64: 413–429. 1947.
———— *et al.* *Azotobacter* inoculation of crops: II. Effect on crops under greenhouse conditions. Soil Sci. 64: 489–497. 1947.
CLARK, F. E. *Azotobacter* inoculation of crops: III. Recovery of *Azotobacter* from the rhizosphere. Soil Sci. 65: 193-202. 1948.

Allison *et al* observed no significant effect of inoculation on growth or nitrogen content of barley, Sudan grass, kale, rape, rye