Name: John Robert Beckley Date of Degree: May, 1967

Institution: Oklahoma State University Location: Stillwater, Oklahoma

Title of Study: THE BENEFICIAL EFFECTS OF BACTERIA ON VASCULAR PLANTS

Pages in Study: 34 Candidate for Degree of Master of Science

Major Field: Natural Science

Scope and Method of Study: Of the various relationships which bacteria have with other organisms, the ones which have received the least attention in high school biology texts are those concerning the beneficial effects which bacteria have upon higher plants. A survey of recent literature was made in order to determine what these effects are. The specific purpose of this report is to provide the high school science teacher with a brief, and, I hope, useful summary of (a) the various beneficial effects, (b) background information necessary to understand there effects, and (c) the present status of research in this area. The scope of this report is limited to include the beneficial effects which bacteria have upon the vascular plants, although some of the indirect benefits to vascular plants may involve lower plants or animals. The literature surveyed is that reported in the past ten years and, due to the broad scope of the subject matter, has been selected to be representative of the areas of the study involved.

Findings of the Study: The primary ways in which bacteria aid vascular plants were found to be involved with their actions on various mineral substances, their production of nutrients, activators, and antibiotics, their actions on soil poisons, and their control of predators. Current work is underway in all fields and progress is being made in both pure and applied areas.

ADVISER'S APPROVAL L. Herbert Bruneau

THE BENEFICIAL EFFECTS OF BACTERIA ON VASCULAR PLANTS

By

JOHN ROBERT BECKLEY

Bachelor of Arts

Yale University

New Haven, Connecticut

1947

Submitted to the faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the degree of
MASTER OF SCIENCE
May, 1967

THE BENEFICIAL EFFECTS OF BACTERIA
ON VASCULAR PLANTS

Report Approved:

L. Herbert Bruneau
Report Adviser

J. J. Crockett

Norman N. Durham
Dean of the Graduate College

Name: John Robert Beckley   Date of Degree: May, 1967

Institution: Oklahoma State University   Location: Stillwater, Oklahoma

Title of Study: THE BENEFICIAL EFFECTS OF BACTERIA ON VASCULAR PLANTS

Pages in Study: 34   Candidate for Degree of Master of Science

Major Field: Natural Science

Scope and Method of Study: Of the various relationships which bacteria have with other organisms, the ones which have received the least attention in high school biology texts are those concerning the beneficial effects which bacteria have upon higher plants. A survey of recent literature was made in order to determine what these effects are. The specific purpose of this report is to provide the high school science teacher with a brief, and, I hope, useful summary of (a) the various beneficial effects, (b) background information necessary to understand there effects, and (c) the present status of research in this area. The scope of this report is limited to include the beneficial effects which bacteria have upon the vascular plants, although some of the indirect benefits to vascular plants may involve lower plants or animals. The literature surveyed is that reported in the past ten years and, due to the broad scope of the subject matter, has been selected to be representative of the areas of the study involved.

Findings of the Study: The primary ways in which bacteria aid vascular plants were found to be involved with their actions on various mineral substances, their production of nutrients, activators, and antibiotics, their actions on soil poisons, and their control of predators. Current work is underway in all fields and progress is being made in both pure and applied areas.

ADVISER'S APPROVAL L. Herbert Bruneau

TABLE OF CONTENTS

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Man has long recognized that bacteria affect all other organisms in many ways, but the directions of investigations of these affects in the past have been toward elucidating the harmful effects and alleviating the damage done to man. The progress of research has been highly satisfactory in the areas of human and animal diseases, good in the area of plant diseases and fair in the area of food processing. The order of the areas mentioned indicates the degree of closeness to man's self interest, that is, those which affect him most personally and adversely will be studied first and most thoroughly, while those more remotely concerned with his well-being will receive a smaller amount of scrutiny or will be studied at a later time. The beneficial affects which bacteria have on other organisms seems to be at the end of the list of areas to be studied, and understandably so, for man has had no pressing personal or economic reason to search for these activities. A few bacterial heroes have been widely publicized as producers of antibiotics, as food processors, and as decay organisms but the fact is that most of their beneficial activities have been carried out smoothly and quietly and have not attracted much attention. Next to man's concern for himself he has a concern for the animals which he has domesticated, possibly because he can identify more readily with them than with plants, although economically he would be more sensible if he were to concentrate his food producing efforts on the plants. It is understandable then, that research concerning beneficial effects of bacteria on plants has not been as extensive as that dealing with either harmful effects or with animals.

Some of the first work done on beneficial bacteria dealt with soil bacteria, specifically with two phenomena: nitrification and decay. This is not surprising, since they are important factors in man's agricultural industry. Bacteria were utilized by ancient farmers who, without knowledge of their benefactors or the processes involved, derived methods with which to increase production of plants. As the physical, chemical, and biological sciences advanced, the methods and instruments necessary for close observation of bacteria became available and bacteriologists began to unravel the intricate story of the soil bacteria and their work. Today, although a large amount is known about the soil bacteria, the end of the tale is still distant. There seems to be much truth in the statement "the more we learn about a subject, the more we realize how little we know". Today the investigations have broadened to include almost every conceivable aspect of influence that bacteria have on other organisms. Bacteriological research has run the gamut from the earliest attempt to identify and classify the bacteria involved in certain processes to recent work to elucidate the enzymatic processes involved in each chemical action.

In his efforts to understand the mysteries of bacterial action the researcher has had to proceed from the practical knowledge gained by farmers through centuries of plant husbandry to the highly sophisticated chemical analyses carried out in today's laboratories. Methods in use vary according to the questions they are designed to answer, but they usually involve direct or indirect use of microscopic, cultural, and chemical analyses. Direct methods involve studying the bacteria and plants in the field while indirect methods involve the use of single species of bacteria grown on artificial media and inoculated onto or into plants grown hydroponically or in sterile soil. Indirect methods were often necessary because the natural ecosystem is so complex that the workers could not control the various environmental factors well enough to give them single variable to study. The use of various techniques using radioactive tracers has been extensive. Despite all of this, man is still just scratching the surface in the area of study concerning the beneficial bacteria.

The relationships which have received the least attention in high school biology texts and college fundamental microbiology courses are those concerning the beneficial effects of bacteria upon plants and vice versa. Because this is a rapidly growing area of research and because the potentialities for use grow as man finds it increasingly difficult to feed the "population explosion", I believe that a survey of the various ways in which bacteria act to aid plants is desirable. The specific purpose of this report is to provide the high school science teacher with a brief, and, I hope, useful summary of (a) the various beneficial effects, (b) background information necessary to understand these effects, and (c) the present status of research in this area. The scope of this report is limited to include the beneficial effects which bacteria have upon the vascular plants, although some of the indirect benefits to vascular plants may involve lower plants or animals. The literature surveyed is that reported in the past ten years and, due to the broad scope of the subject matter, has been selected to be representative of the areas of the study involved. Due to the limited bacterial population on the leaves, most of the work reported in the literature has to do with soil bacteria. Compared with the roots, the aerial parts of plants provide neither the environmental conditions nor the absorption-secretion exchange necessary for a large and varied bacterial population. There is, however, an emerging, though limited, interest in the bacteria living in the phyllosphere. (Last and Deighton, 1965)

CHAPTER II

MINERAL EFFECT

Chemical Actions

Chemical elements and compounds follow a cyclical path in nature which makes them alternately, available to living organisms, and unavailable to them. When available, the materials are usually in simple soluble compound form and when unavailable are present as large, usually complex organic molecules, insoluble compounds or elements, or are present in areas not conducive to life. In any case, the mineral effect may be classified as primarily a chemical action of the bacteria.

Bacteria exert their chemical influences on plants both directly and indirectly. In the former, the plant is able to utilize bacterial products without intermediate reactions taking place, as in the nitrogen-fixing activities. In the latter, the bacterial activities alter the soil chemistry which, in turn, changes materials available to the plant, as in the case of pH changes freeing bound minerals.

Alexander (1964) placed the bacterial contributions to soil chemistry in six categories; (a) they increase the chemical complexity of the soil, (b) they decrease the chemical complexity of the soil, (c) they cause the oxidation of various elements, (d) they cause the reduction of various elements, (e) they bring about the solubilization or precipitation of materials, and (f) they change the amount of an element in the ecosystem.

The first of these contributions refers to processes such as biosynthesis and humus production. The bacteria utilize relatively simple chemical compounds or elements in their metabolism, producing larger, more complex organic materials, some of which are excreted or secreted into the soil. When bacteria die, their bodies become part of the soil and when membranes break down, all of the large organic compounds are released to the soil. Many of the compounds are slow to break down and will provide the soil with a long-lasting chemical reservoir.

The second involves the bacterial action upon large molecules of organic material. The decomposition of plant and animal remains by microorganisms results in compounds such as cellulose being degraded to simpler compounds such as glucose and fructose.

In the third and fourth categories the oxidations of hydrogen and carbon during energy metabolism and the accompanying reduction of oxygen to form water and carbon dioxide are typical reactions.

Contribution (e) is often the result of another chemical action. The sulfur bacteria oxidize elemental sulfur to obtain energy and the insoluble sulfur is converted to soluble sulfates. Precipitation occurs in the action of bacteria on soluble iron salts to produce insoluble ferric hydroxide.

The last contribution listed may be characterized by the fixation of atmospheric nitrogen. The element now becomes a part of the soil ecosystem.

Studies of minerals in the soil and their various effects are complicated by the complexity of the environment. Gases, water, minerals, and living organisms are held in closer proximity than in any other type of environment, and along with a more constant temperature, these factors cause a bacterial population which may reach twenty million per gram as an average. The numbers, as well as the species, vary greatly with the depth of the soil, proximity to plant roots, and other factors. Pelczar and Reid (1958) stated the factors responsible for this variation as follows; the amount and type of nutrients present, available moisture, degree of aeration, temperature, pH, and occurrences which contribute large numbers of organisms to the soil. Every physical, chemical, or biotic change is bound to produce a multiplicity of other changes and effects. Little wonder that the soil microbiologists, ecologists, biochemists, etc. collaborate to try to produce some order in this area of study.

Plants require at least sixteen elements but, because some either have not had their bacterial relationships studied or have cycles similar to others, the following descriptions of mineral effects are limited to the three which have been studied most extensively; nitrogen, carbon, and sulfur. A brief resume of other minerals is included in the subdivision entitled Other Minerals.

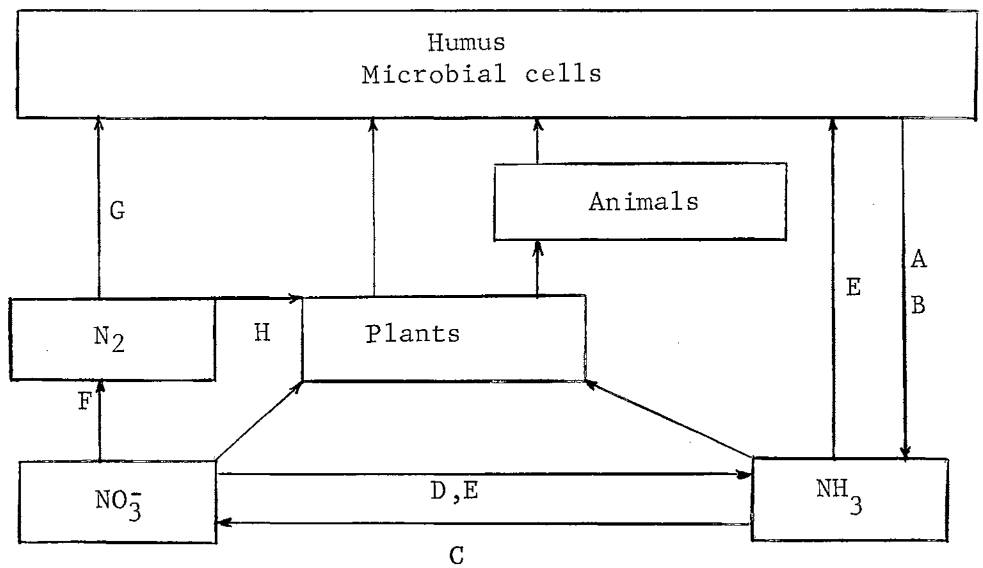
Nitrogen

The cycle of nitrogen in nature is fairly typical of most minerals in that it occurs as unavailable material when it occurs in organic compounds or as a free element, then it is converted by microorganisms to soluble forms which higher organisms can use. Proteins and nucleic acids represent the forms in which living things usually hold nitrogen. Atmospheric free nitrogen is not available to vascular plants simply because they have no enzymatic system to catalyze its use. The useful forms of nitrogen are the ammonium and nitrate compounds. Figure 1 summarizes not only the nitrogen cycle but also the various bacterial processes involved. The processes D, E, and F result in the loss or delay of nitrogen movement to plants and are therefore considered harmful and will not be discussed. The processes to be discussed are proteolysis (which includes B. mineralization), ammonification, nitrification, and nitrogen fixation.

Proteolysis is a process which results in amino acids and other simple compounds being produced from proteins, polypeptides, nucleoproteins, nucleic acids, and aromatic compounds. The large and complex molecules cannot be taken in by living cells but the smaller ones which constitute them can be used directly by vascular plants or acted upon by other bacteria to produce even simpler compounds. Since the complex compounds cannot be taken into the bacterial cell, it is obviously an extracellular enzymatic mechanism which is responsible for the degredation. Various species of Proteus, Pseudomonas, Bacillus, Clostridium, Serratia, and Micrococcus have been identified as agents.

Two steps are involved in the break down of protein. Step one results in the proteins being degraded to peptides by means of proteinases and step two results in the peptides being degraded to amino acids by means of peptidases. The present state of knowledge concerning proteolysis indicates that several substages are necessary to explain the exact nature of the chemical break down.

Ammonification results in ammonia being produced from amino acids by means of deaminases. Plants may use the ammonia or further action by bacteria might modify it to introduce nitrates to the soil.



A. Ammonification
B. Mineralization
C. Nitrification
D. Nitrate reduction
E. Immobilization
F. Denitrification
G. N_2 fixation. Non-symbiotic
H. N_2 fixation. Symbiotic

Figure 1. The Nitrogen Cycle
Alexander (1965)

Nitrification starts with the oxidation of ammonia to form nitrites. This reaction is mostly the result of the work of the Nitrosomonas bacteria which are obligate autotrophs and strict aerobes. Nitrobacters are primarily responsible for the oxidation of nitrites to nitrates and are also obligate autotrophs and strict aerobes. Vascular plants are able to absorb and assimilate the nitrates at a rapid rate.

The various nitrifying bacteria seem to be restricted to areas according to temperature. Even within a particular species there are different varieties which work best in one climatic region or another. Mahendrappa, et al, (1966) believe that natural selection has caused varieties or species to develop in response to local climate. They used soils from various parts of western United States and found that northern soils contained bacteria which nitrified most rapidly at 20°C and 25°C while the southern soil bacteria reacted best at 35°C.

There is a leak in the nitrogen cycle which would result in the ultimate depletion of this element in the soil were it not for a counter-balancing process. Available nitrogen is changed to unavailable atmospheric nitrogen during denitrification. Fortunately, there are a few types of bacteria which can change free nitrogen to nitrates which are available to higher plants. These microorganisms are called the nitrogen fixation bacteria and may be separated into non-symbiotic and symbiotic types, with the former occurring outside of plant tissue and the latter occurring within plant tissue. Exactly how nitrogen is fixed and whether or not the nitrogenous products are the only aid given the plant by these bacteria are not well known, but are being studied. The first stage work of identifying species of bacteria which fix nitrogen is still going on as evidenced by the isolation in

1964 by Centifanto and Silver of a species not previously reported.

The nonsymbiotic fixation of nitrogen has been found to occur at rates from 20 to 50 pounds per acre per year and is the work of species of Azotobacter, an aerobe, and clostridium, an anaerobe. About fifteen bacteria have been shown useful for this purpose.

Species of Rhizobium, living in nodules on the roots of legumes, are the bacteria best known as symbiotic fixers of nitrogen but there are others, including some on non-leguminous plants. The various species seem specific for a certain host plant group and have been classified according to the following categories: alfalfa, clover, peas, cowpeas, beans, lupines, and soybeans. An inoculum containing species capable of causing nodulation on clover will be worthless on soybeans, but it can be used successfully on almost all other clovers. Graham and Parker (1964), in their assessment of diagnostic features, reduce the useful groups to three because of large numbers of interactions shown among groups such as the cowpeas, soybeans and lupines or the peas and clovers. They also believe that, although infective properties are useful for diagnostic purposes, various other characteristics are better, e.g. their requirement for thiamine and calcium pantothenate or their growth at pH 4.5 and 9.5.

The use of the electron microscope was brought into the study of the nodulating process by Barbara Mosse (1964). Her excellent photographs trace the infection route of the bacteria and the reaction of plant tissue to this invasion. Goodchild and Bergerson (1966) agree with Mosse on the production of infection threads but were not able to detect the connections between bacteria and hose cell nuclei.

Their investigation did disclose some facts concerning the plant membranes which the earlier work had not shown, and they are, that the plant-produced membranes enclose the bacteria early in the infection and also stay with the bacteria through detachment from the host cell membrane.

Soil bacteria are affected by many factors in the soil environment and, in the case of symbiotic forms, by biotic factors produced by their host. Tewari (1965) and Ershow (1966) studied the effects of minerals on the soil bacteria. Tewari's (1965) work indicates that mineral balance, especially the nitrogen, is as important to the nodulating bacteria as it is to the host plant. Ershov's (1966) investigation showed an increase in nitrifying bacteria with an increase in mineral fertilizers, but he also found significant differences in the results in two different soil types. Not much practical work has been done to determine fertilization practices which would result in the maximum benefit to both bacteria and host plant. It is possible that the net amount of nitrogen available to crop plants might be increased three fold by using a two fold application of fertilizer, the extra quantity due to bacterial action. Conversely, too much nitrogen, although it might not hurt the plant, is known to diminish bacterial populations, so a farmer might decrease his fertilizing and increase the nitrifying bacteria.

Trace elements seem to have importance to the nitrogen bacteria as well as to the crop plants. Il'ina (1966) discovered that in some soils molybdenum is needed by nitrogen fixing bacteria. The absence of a type of bacteria in a soil type is often difficult to account for on the basis of macroelement and organic matter deficiencies but this study suggests an explanation. It might also help us to understand why inoculating bacteria do not survive from one growing season to the next in certain soil types.

Gibson (1965) studied the effects of root temperature on the nitrogen fixing activity of Rhizobium trifolii and concluded that this species shows much variation. He found that eight strains were present and three had optimum ranges of temperature below that of the other five. Evidently a legume inoculant for clover might be very successful in Texas but be a failure in North Dakota.

The aerial parts of plants have not been extensively studied but some facts have been found concerning the relationships between bacteria and leaves. It has been determined that nitrogen fixation takes place on the leaves as well as in the soil, and Ruinen (1961) identified five genera of nitrogen fixing bacteria on leaf surfaces. How much of the fixed nitrogen is absorbed by the leaves is not reported, but Boynton (1954) found that plants can absorb various nutrients through their leaves. Centifanto and Silver (1964) classified a leaf-nodulating, nitrogen-fixing bacterium as a Klebsiella. This would indicate that the leaf and root use some different as well as some similar types of bacteria for nitrogen fixing.

Some recent work regarding nitrogen-involved bacteria has indicated that they are long-lived and resistant to various environmental factors. An example of the former is the work of Abd-el-Malek and Ishac (1965) on Azotobacter. Viable bacteria were recovered from the interior of bricks taken from a wall built approximately 2300 years ago! Their methods seem careful enough to prevent the entry of more modern bacteria but such longevity does appear incredible. Vela (1964) found that

Azotobacter species in their natural soil environment are highly resistant to gamma radiation.

Carbon

Carbon is so often tied up in organic soil compounds that without bacterial action, photosynthesis, and life of all kinds would cease to exist. This element is stated by Alexander (1964) as being the chief limiting factor in plant metabolism. Bacteria decompose organic compounds as tough as cellulose (Alexander 1965), lignin (Sundman 1964), and chitin (Okafor 1966) and as resistant as fats, freeing the carbon for use by higher plants. As shown in the carbon cycle diagram, Figure 2, carbon, in the form of carbon dioxide, is removed from the atmoshere by photosynthetic plants and returned to the atmosphere in the same form by bacterial decomposition.

The exact chemical nature of the degradation has not been fully elucidated but a plan of stepwise reduction of molecular complexity, involving many enzymes, has been the emerging pattern. The breakdown of chitin in the soil was studied by Okafor (1966) who found that both the polysaccharide and protein portions were acted upon by species of Pseudomonas. Environmental factors influencing the decomposition of cellulose were studied by Went and deJong (1966). The rates at which Eubacteria and Cytophaga acted were dependent upon soil type, plants growing in close proximity, presence of litter, and interaction between soil organisms.

The beneficial effects of the synthesis or organic carbon compounds by bacteria are included in Chapters III and IV.

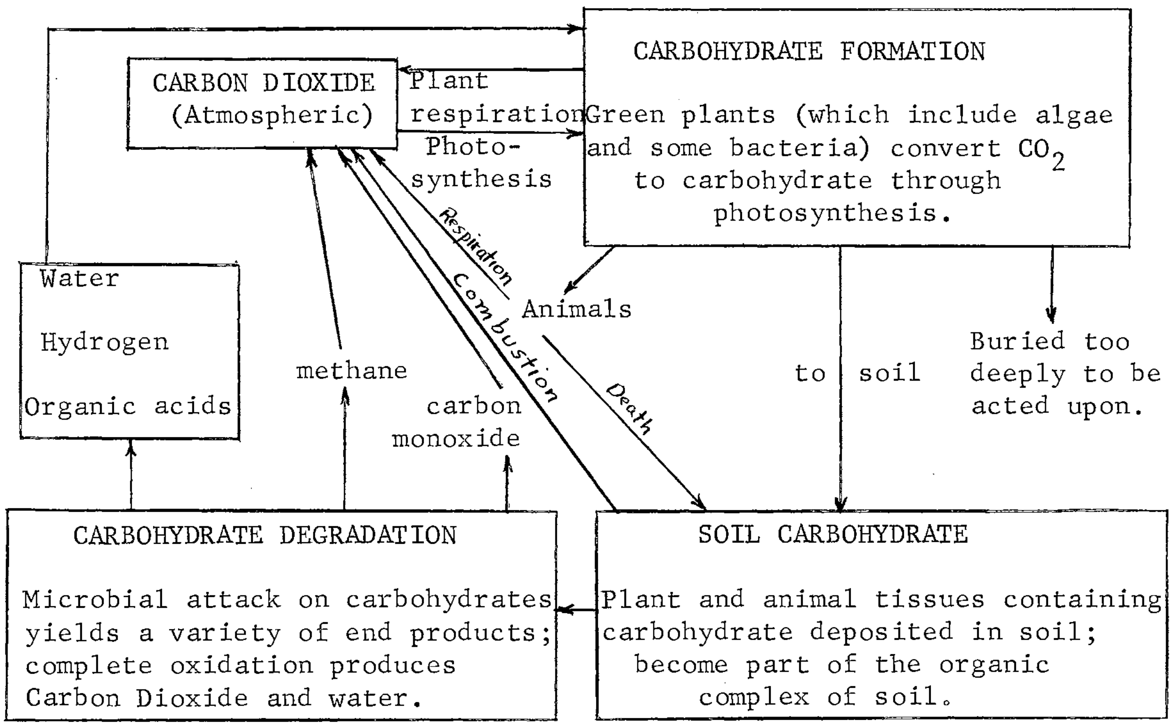


Figure 2. The Carbon Cycle
(Modified from Pelczar and Reid (1958)

Sulfur

This essential element is present in all of the useful soils of the world but often in unavailable forms such as organic residues (proteins or other compounds in oil or coal) or elemental sulfur (sulfur domes). Although we know much about the sulfur cycle, we have not been able to maintain soil sulfur quantities at a proper level in many parts of the world. Coleman (1966) believes that the increased frequency of crop deficiencies of sulfur are due to several factors; (a) the increased use of sulfur free fertilizers (due to changes in methods of production), (b) decreased use of sulfur as an insecticide, (c) increased yield of crops, and (d) less production of sulfur in combustion processes (due to improved air pollution devices and decreasing use of coal and wood among rural populations). Bacteria are capable of acting upon either form of sulfur in various ways to produce sulfates which plants can use.

That various pathways are open to use, is due to the number of oxidation states of the element. Figure 3 illustrates the groups of transformations which bacteria can produce and the state of sulfur in various stages of the cycle. The stable condition of sulfur under aerobic conditions is the sulfate and under anaerobic conditions is hydrogen sulfide, due to the necessity for oxygen in the oxidation of hydrogen sulfide. Since the reduction of sulfates and the assimilation of sulfates are not beneficial effects, they will not be discussed. The bacteria involved in the oxidation process are the _Thiobacilli_, _Beggiotoa_, _Chromatium_, and _Chlorobium_. The decomposition types are basically the same as for nitrogen since the breakdown of protein is

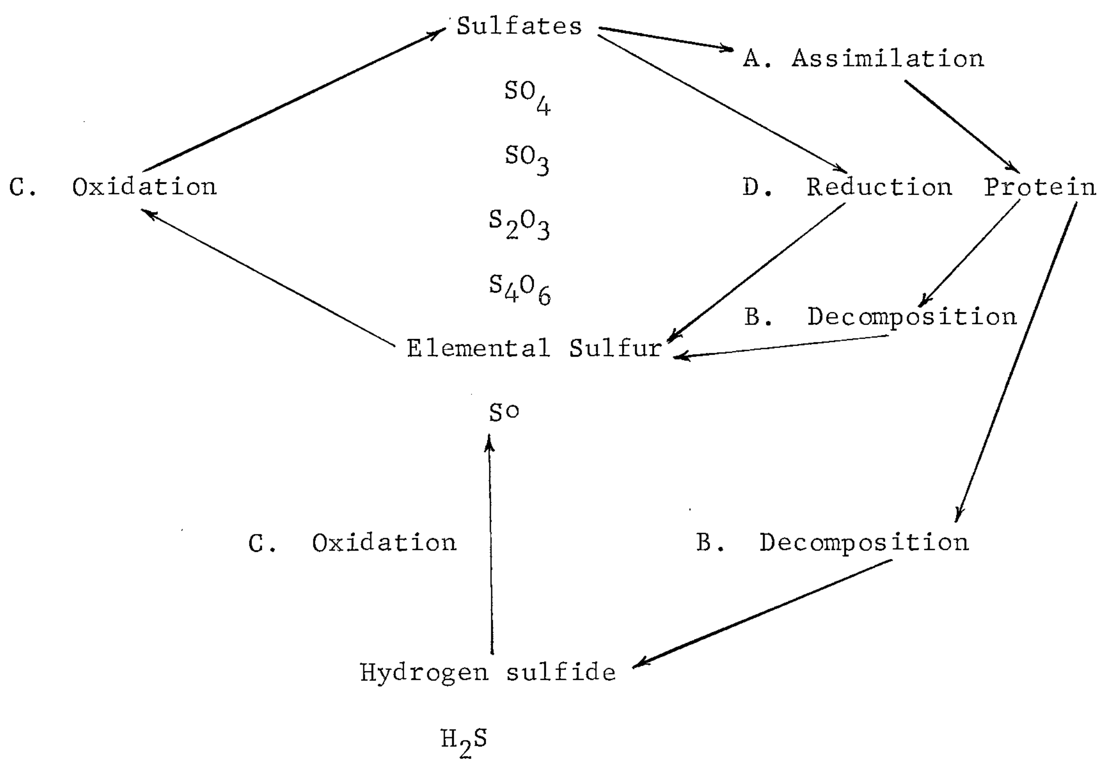


Figure 3. Sulfur Cycle

(Based upon simpler diagram by Peck (1962) involved in both cases.

The rate at which soil sulfur bacteria act has been studied by Attoe and Olson (1966) using Thiobacillus. They discovered that the soil moisture was very important, with the optimum at field capacity and sharp declines above or below. The rate of oxidation was shown to be directly related to soil temperature and pH and inversely related to the particle size of sulfur and rock phosphate-sulfur fusions and to the ratio of rock phosphate to sulfur within the fusions. The addition of lime caused an increase in sulfur oxidation.

Other Materials

In addition to nitrogen, carbon, and sulfur, plants require hydrogen, oxygen, phosphorus, potassium, calcium, iron, magnesium, boron, manganese, copper, zinc, molybdenum, and chlorine. Hydrogen is involved in all other cycles as a constituent of organic compounds and water, but there is one genus of bacteria, the Hydrogenomonas, which uses hydrogen directly for energy. Oxygen is also universally involved in other mineral cycles. Iron is precipitated by certain bacteria and solubilized by the acids produced by other bacteria. Calcium, potassium, phosphate and magnesium are also solubilized by the pH drop associated with bacterial action. All of the essential elements have been shown to be affected by bacterial actions of some nature. Phosphorus has been studied extensively but the cycle and the bacterial actions so closely resemble those of nitrogen that no further mention seems necessary.

pH Changes

Almost all of the bacterial chemical reactions previously mentioned cause some change in the pH of the environment, and this, in turn, causes a multitude of secondary effects. Among these effects are changes in the rate or direction of chemical reactions, changes in solubility of materials, and changes in the colloidal properties of soil particles. The net effect all of the individual pH changes is determined by the balance between changes tending to raise and lower it and by the buffering effect of certain compounds.

CHAPTER III

BIOTIC EFFECTS

Nutritive

There is a large degree of overlapping between this category and the minerals but here the organic compounds are given to the plants in some form more complex than the lowest degradable product. In many cases glucose, amino acids, and fatty acids can be taken in by the plant, thereby eliminating several steps of bacterial action and several steps of plant action. Various plant parts have been shown to absorb molecules of these substances. Boynton (1954) found that plants can absorb nutrients such as urea through the leaf surfaces and Miettinen (1950) found that plants can take in amino acids without further decomposition by the plant root. It is possible that the direct intake of nutrients might explain some of the growth increases noted in plants which have been inoculated with bacterial types which do not have nitrifying abilities. Notice of this effect was delayed until recently by the lack of methods necessary to detect the nutrient flow. Materials such as amino acids are produced by various organisms and used by others so rapidly that until rapid chemical analyses became possible one could not trace them. A recent report by Gilbert and Altman (1966) outlines a new, rapid method used to analyze for amino acids using ethanol.

Activators

Another effect which might account for some of the unexplained growth benefits due to bacteria is the result of production of growth promoting substances. Krasil'nikov (1958) described many experiments which showed that bacteria can produce vitamins, auxins, giberellins, amino acids, and other activators. Activators are described as those substances which alter the growth and biochemical process of the plant but are not nutrient substances. Voznyakovskaya (1959) isolated a new species of *Pseudomonas* which proved to have root growth promoting properties.

Some effects once ascribed to nitrogen fixation are now suspected to be caused by growth factors. Nodulating bacteria were observed by Brown and Burlingham (1964) to benefit non-leguminous plants despite the fact that nitrogen fixation in this case was negligible. Searching for an explanation, Jackson, *et al* (1964) noticed that the growth patterns of the plants resembled the effects which giberellins produced. They inoculated some tomato plants with bacteria and others with giberellins and noted similar effects. A major contribution was made by Sobieszcanski (1966). He showed that filtered extracts of bacterial cultures stimulated the growth of lettuce and wheat. The extracts proved to be similar to auxins and giberellins. Centrifanto and Silver (1964) stated that a tropical plant, *Psychotria bacteriophila*, showed dwarfism if deprived of nodulating bacteria. The dwarfism was shown to be independent of nitrogen produced since plants provided with nitrogen did not exhibit the trait. It is apparent that some biotic effect is present, but what kind of activating material is present has not been shown.

In Rovira's (1965) review of interactions between microorganisms and plant roots, he outlined some of the effects that have been studied in connection with activators. Those listed were; root morphology, root and shoot weight ratio, uptake of calcium and rubidium, uptake of phosphorus and sulfur, mineral content, rate of development and onset of flowering, crop yields, and various physiological processes.

Detoxification

Various substances poisonous to plants move into the soil or are produced there by indigenous organisms. Bacteria have been given credit for the removal of many of these toxic substances. In his studies of the action of *Bacillus* spp. in the soil, Rovira & Bowen (1966) encountered a reduction in phytotoxins which had been produced during heat sterilization. The heat resistant spores of four species of *Bacillus* has survived the sterilization and had proved to be good detoxifiers. They caution observers to be careful lest they misplace the credit for plant growth increases, since careful examination might show that at least a part of the benefit might derive from detoxification effects.

A type of detoxification which is not misunderstood is the reduction of herbicides in the soil. Eight types of bacteria were described by Kauffman (1964) as capable of decomposing dalapon in five different soils, although rates differed. To find out why, he probed the factors involved and found that soil type was the most important. Specifically, the pH, cation exchange capacity, organic matter present, and aeration were important.

Antagonistic

Plants receive a large degree of protection from pathogens as a result of the action of bacteria. These actions may be nothing more than normal competition for space, water, nutrients, minerals, etc. or they may be due to the production of materials which interfere with the metabolism of the pathogen. Specific antibiotic action is a well known interference phenomena.

Newton (1965) lists nine mechanisms by which antibiotics act. Antibiotics; affect cell wall synthesis, affect cell wall and membrane synthesis, affect cell membrane permeability, act as uncoupling agents and inhibitors of electron transport, act in the chelation and inhibition of metallo-protein synthesis, inhibit purine and purine nucleotide synthesis, inhibit DNA synthesis, affect the transcription of genetic information, and inhibit protein synthesis. Although all of these have not been proven for bacterial antibiotics, they should serve to show the possibilities since many antibiotic effects are unexplained and, of course, some antibiotics are still to be discovered.

Asante and Neal (1963), working with the Dutch elm disease, found that the normal metabolic production and secretion of low molecular weight acids by a species of Bacillus was responsible for the antagonism. As this serious disease becomes more widespread, this type of control might become useful. The lysis of the mycelia of Pythium debaryum by Arthrobacter sp. was the method of action observed by Mitchell and Hurwitz (1964). They protected tomato plants from the damping-off organism by inoculating either the seed or the soil. The protection lasted from 14 days to 21 days depending on soil type. Huber and

Anderson (1966) noted agglutination of hyphae and pigmentation of constituents in the bean rot organisms, *Fusarium solani* f. phaeseoli and *Rhizoctonia solani*. The bacteria responsible, *Xanthomonas*, were observed to have invaded the hyphae.

Bacillus pumilis acts in two ways against the agents of root rot and leaf spot of graminaceous plants. Interference with spore germination and an inhibiting action of unknown mechanism were observed by Gayed (1966). Inhibition of an unknown nature and origin was described by Crosse (1965) in his work with bacterial canker disease on cherry trees. He determined that it was caused by an unknown saprophytic phyllophyte. Another unknown method of action was operating when Naim and El-Esawy (1966) discovered that they could select bacteria for soil inoculation to protect cotton seedlings from *Rhizoctonia solani* Kuhn.

Trees might have to get their "shots" if Goodman's (1967) technique proves successful. He inoculated apple shoots with *Pseudomonas tabaci* prior to infecting the trees with *Erwinia amylovora* and succeeded in obtaining short term protection. This is interesting because direct injection of orchard trees might be economically feasible. Some problems have to be overcome to obtain long term protection. Bacteria used so far are not able to establish a continuing population in the tree and other strains might show undesirable effects on the tree or might not provide full protection. The action is unknown and could be either direct bacterial antagonism or a host-produced antigen.

An antibiotic substance was shown to be the substance causing a reduction of pigeon-pea wilt for a period of seven weeks. *Bacillus subtilis* was the agent used by Singh, *et al* (1964) to produce the antibiotic which became systemic in the plant. A highly desirable product of the systemic movement of the antibiotic was the creation of a protective zone around the roots of the host. In an earlier paper, Teliz-Ortiz and Burkholder (1959) assumed that an antibiotic was involved in the action of Pseudomonas fluorescens against various plant pathogens. They based their conclusion on the movement of the inhibiting material through the plant although the bacteria remained external. This movement of antibiotics within a plant is a highly desirable method of fighting diseases. Dekker (1963) lists the advantages as: pathogens can be eradicated even when they are located deep within the plant; the antibiotics cannot be washed off by rain; protection of all parts of the plant can be obtained, including new growth; ordinary chemicals may produce injurious side effects on the crop or may be toxic to humans or animals while antibiotics are less likely to be harmful. He also lists various antibiotics and their target organisms.

Predator Control

Predators of plants can be classified as beneficial to man's interests or harmful to man's interests. Man, himself, and his domestic animals fall into the first category while insects are probably the primary group in the second category. Insects harm plants by acting directly against them, as they eat plant parts, and by acting indirectly against them, as they carry plant pathogens and open plant tissues for the pathogens. Man's control methods have been general, costly, short term, and largely controversial. If biological control, using bacteria, could be perfected, we might solve most of those problems.

Bacterial controls are usually specific whereas the insecticides are usually general. Bacteria, at the present time, are still rather expensive to use, but either the bacteria or their secretions will provide the effect desired, so future investigations will probably reduce the cost. Since bacteria can be passed from insect to insect and plant to insect or in some cases can survive as spores, there is a long lasting effect possible. The controversy concerning insecticides probably will not be solved by bacterial controls, because there is little likelihood that man will find useful diseases for all plant destroying insects, and also because the nature of man is such that, if controversy is possible, it will occur. We'll not have to think seriously about the question for some time unless some of the cost and application problems are solved.

Some of the problems associated with this type of control can be appreciated by observing the effects of Bucher's (1961) experiments with the tent caterpillar, *Malacosoma americanum* (Fabricius). He obtained control of the caterpillars by spraying them with spores of two species of *Clostridia*. Although six years have passed since he proved that control is possible, we still have no widespread use of this method because of the unsolved cost and application problems. The first problem, the cost of production and application, is difficult to solve because the colonies are widely scattered, are often on trees of little or no commercial value, and are protected by the foliage of the tree or by the silky tent. Application problems arise from the need to spray when the larvae are younger than the third instar and to spray frequently.

The uses of this control on commercial crops are more successful and have been used around the world. Burges (1964) lists *Bacillus poppilliae* (milky disease of Japanese beetles) and *Bacillus thuringiensis* as outstanding examples of effective control organisms. The literature abounds in studies involving Bacillus thuringiensis and most are reporting success in its use. Begg (1964) used it to control hornworms attacking tobacco in Ontario and found it to be as successful as insecticides. A problem which often appears when using bacterial sprays is the lack of adhesion which makes the application to the undersides of leaves difficult and also allows rain to wash the spray from plant parts. Substances were found which are called "stickers" and, as the name implies, have solved the problem.

Occasionally an introduced species of insect becomes a problem because there are no natural predatory controls in their new environment. Such was the case with the European skipper, Thymelicus lineola, which feeds on timothy. Arthur and Angus (1965) used Bacillus thuringiensis to affect a control and this would suggest that various other introduced insect pests might be removed early in their invasion by this method. The same bacteria was used by Chatterjee (1965) and Wolfenbarger (1965). Chatterjee used it to control a moth which parasitized jute, a very important commerical plant in the Far East. Wolfenbarger completed a preliminary study which indicates that the cabbage-looper might be susceptible. This bacterium has been used by the Russians for a number of years. Pristovko (1965) lists several formulations of both Bacillus thuringiensis and Bacillus cereus which are in commercial use in that country.

CHAPTER IV

PHYSICAL EFFECTS

The physical effects which bacteria have upon higher plants are mostly indirect ones concerned with the tilth of the soil. Previously described chemical changes alter the soil's ability to hold water, ability to move gases, texture, structure, color and temperature. Since plants respond to all of these factors, bacterial effects of this type must be considered important.

Soils are generally improved by bacterial action. The decomposition of litter, bacterial cells, both deal and alive, and the organic secretions and excretions of bacteria cause the soil to become more porous and spongy. Aggregation of soil particles is enhanced by the bacterial chemical changes as well as by the glue-like quality of some bacterial exudates. The strongly glued soil aggregates are very important in determining soil quality. Various solid portions of the soil are rendered soluble by the acid condition produced by bacteria.

A benefit not often considered is the removal of plant waste materials from the root area. This creates physical space for useful water, nutrients and more plant waste. The modification of soil water by bacteria was well covered by Kuznetsov, et al in 1963.

CHAPTER V

CONCLUSIONS

The study of the beneficial effects which bacteria have upon vascular plants is expanding at a tremendous rate now that the necessary physical and chemical methods have been developed. Physiological processes which could not have been understood twenty years ago are now being investigated successfully. Rapid world-wide communication has been a factor in the increased rate of knowledge concerning these processes and in science we have one of the few areas in which communication is really worldwide. Faced with a food shortage, man must increase crop yields, find new crop areas, and reduce losses of crop plants. In order to do these things he must have a better understanding of bacteria-plant interrelationships. The degree of complexity of these interrelationships is beginning to become apparent, and this, I believe, is one of the most important outcomes gained by man from these studies. If we alter one facet of a community we affect all other parts of that community, whether we are concerned with wildlife in the Kaibab or with bacteria in the rhizosphere. We can't make proper use of the beneficial effects of bacteria until we understand the full impact of any alterations we make in their environment.

The research encountered in the area of mineral effects indicates that the status of work concerning the major elements has gone beyond the description of effects and identification of organisms and is now moving in the area of biochemical processes and ecological relationships. The minor elements have not received the same attention regarding bacterial effects and the current work appears to be mostly descriptive.

The biotic effects are under intensive study at the present time after a period of very little investigation. The practical uses of the biotic effects and the biochemical aspects are the major areas of current concern. This is potentially the most promising group of effects for use in agriculture.

The physical effects are not usually studied individually, but are most often recorded as observations encountered in other investigations. This situation is not likely to change.

It should be obvious that without the bacteria, vascular plants could not survive. Although some of the effects such as decomposition of animal and plant remains are obviously necessary, many others not as well known may be just as important. Without the continuous protection of bacterial antagonists, continuous production of activators, and the continuous complex chemical changes it is probable that plants would soon disappear.

SELECTED REFERENCES

1. Abd-el-Malek, Y. and Y. Z. Ishac. 1965. Longevity of Azotobacter. Plant and Soil 24: 325-327.

2. Alexander, M. 1964. Biochemical ecology of soil microorganisms. Ann. Rev. of Microb. 18: 217-252.

3. Alexander, M. 1965. Introduction to Soil Microbiology. John Wiley & Sons, Inc. New York. Pages 3-18, 127-350.

4. Arthur, A. P., and T. A. Angus. 1965. Control of a field population of the introduced European skipper, Thymelicus lineola (Ochsenheimer) (Lepidoptera: Hesperiidae) with Bacillus thuringiensis Berliner. J. Invert. Path. 7: 180-183.

5. Asante, G. S., and A. L. Neal. 1963. Characterization of fungistatic substances produced by a Bacillus antagonistic to Ceratocystis ulmi. Phytopathology 54(2): 819-822.

6. Attoe, O. J., and R. A. Olson. 1966. Factors affecting rate of oxidation in soils of elemental sulfur and that added in rock phosphate sulfur fusions. Soil Sci. 101(4): 317-325.

7. Begg, J. A. 1964. Microbial and chemical control of hornworms attacking tobacco in Ontario. J. Econ. Ent. 57: 646-649.

8. Boynton, D. 1954. Nutrition by foliar application. Ann. Rev. Pl. Phys. 5: 31-54.

9. Brown, Margaret E., Susan K. Burlingham, and R. M. Jackson. 1964. Studies on Azotobacter species in soil. III. Effects of artificial inoculation on crop yields. Plant and Soil 20(2): 194-214.

10. Bucher, G. E. 1961. Control of the Eastern tent caterpillar, Malacosoma americanum (Fabricius) by distribution of the spores of two species of Clostridium. J. Insect Path. 3: 439-445.

11. Burges, H. D. 1964. Control of insects with bacteria. World Crops. 16(3): 70-76.

12. Centifanto, Ysolina M., and Warren S. Silver. 1964. Leaf-nodule symbiosis. I Endophyte of Psychotria bacteriophila. J. Bact. 88(3): 776-781.

13. Chatterjee, P. B. 1965. The utilization of Bacillus thuringiensis Berliner in the control of Anomis sabulifera Guenee (Lepidoptera, Noctuidae) on jute plant (Corchorus olitorius Linnaeus) J. Invert. Path. Note 7: 512-513.

14. Coleman, Russell. 1966. The importance of sulfur as a plant nutrient in world crop production. Soil Science 101(4): 230-239.

15. Crosse, J. E. 1965. Bacterial canker of stone fruits. Ann. Appl. Biol. 56: 149-160.

16. Dekker, Johannes. 1963. Antibiotic in the control of plant diseases. Ann. Rev. Microb. 17: 243-262.

17. Ershov, V. V. 1966. Effect of mineral fertilizers on the microbiological processes in meadow soils in Kareliza. Microbiology 35(1): 168-173.

18. Gayed, S. K. 1966. Bacillus pumilis and its mycolytic action against Helminthosporium sativum. Plant and Soil 24: 178-180.

19. Gibson, A. H. 1965. Physical environment and symbiotic nitrogen fixation. II Root temperature effects on the relative nitrogen assimilation rate. 18(2): 295-310.

20. Gilbert, R. G. and J. Altman. 1966. Ethanol extraction of free amino acids from soil. Plant and Soil 24(2): 229-238.

21. Goodchild, D. J. and F. J. Bergerson. 1966. Electron microscopy of the infection and subsequent development of soybean nodule cells. J. of Bacteriology. 92(1): 204-213.

22. Goodman, R. N. 1967. Protection of apple stem tissue against Erwinia amylovora infection by avirulent strains and three other bacterial species. Phytopathology 57(1): 22-24.

23. Graham, P. H. and C. A. Parker. 1964. Diagnostic features in the characterization of the root-nodule bacteria of legumes. Plant and Soil. 20(3): 383-396.

24. Huber, Don M. and Axel L. Anderson. 1966. Necrosis of hyphae of Fusarium solani f phoeseoli and Rhizoctonia solani induced by a soil-borne bacterium. Phytopathology 56: 1416-1417.

25. Il'ina, T. K. 1966. Effect of molybdenum on nitrogen fixation by mycobacteria. Microbiology 35(1): 155-161.

26. Jackson, R. M., M. E. Brown, and S. K. Burlingham. 1964. Similar effects on tomato plants of Azotobacter inoculation and application of gibberellins. Nature 203: 851-852.

27. Kauffman, D. D. 1964. Microbial degradation of 2, 2-dichloropropionic acid in five soils. Can. J. Microbiol. 10: 843-852.

28. Krasil'nikov, N. A. 1958. Soil Microorganisms and higher plants. Academy of Science of the U.S.S.R. Moscow. Pages 313-341, 356-412.

29. Kuznetsov, Sergey I., Mikhail V. Ivanov, Natal'ya N. Lyalikova. 1963. Introduction to Geological Microbiology. McGraw Hill, New York. Pages 165-189.

30. Last, F. T., and F. C. Deighton. 1965. The non-parasitic microflora on the surfaces of living leaves. British Mycological Society Transactions. 48: 83-99.

31. Mahendrappa, M. K., R. L. Smith and A. T. Christiansen. 1966. Nitrifying organisms affected by climatic region in western U. S. Soil Sci. Soc. Am., Proc. 30(1): 60-62.

32. Miettinen, J. K. 1950. Assimilation of amino acids in higher plants. Soc. Exp. Biol. Sympos. 13: 210-229. (Quoted by Gilbert and Altman 1966).

33. Mitchell, R., and Esther Hurwitz. 1964. Suppression of Pythium debaryanum by lytic rhizosphere bacteria. Phytopathology 55(1): 156-158.

34. Mosse, Barbara. 1964. Electron-microscope studies of nodule development in some clover species. J. Gen. Microbiol. 36(1) 49-66.

35. Naim, M. S. and A. A. El-Esawy. 1966. Growth vigor of some Egyptian cotton variety seedlings in relation to selected rhizospheric microflora antagonistic to Rhizoctonia solani Kuhn. Plant and Soil 24(2): 222-228.

36. Newton, B. A. 1965. Mechanisms of antibiotic action. Ann. Rev. Microb. 19: 209-240.

37. Okafor, N. 1966. The ecology of micro-organisms on, and the decomposition of, insect wings in the soil. Plant and Soil 25(2): 211-237.

38. Peck, H. D., Jr. 1962. Symposium on metabolism of inorganic compounds. Bact. Rev. 26: 67-94.

39. Pelczar, M. J., and R. D. Reid. 1958. Microbiology. McGraw-Hill Book Co. Pages 96-109, 473-489.

40. Pristavko, V. P. 1965. Role of intestinal entomogenic bacteria in the microbiological control of harmful insects. MICROBIOLOGY, 34(5): 806-808.

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

42. Rovira, A. D., and G D. Bowen. 1966. The effects of microorganisms upon plant growth. Plant and Soil. 25(1): 129-142.

43. Ruinen, Jakoba. 1961. The Phyllosphere. Plant and Soil. 15 (2): 81-109.

44. Singh, Pritam, R. S. Vasudeva, and B. S. Bajaj. 1964. Seed bacterization and Biological activity of bulbiformin. Ann. Appl. Biol. 55: 89-97.

45. Sobieszcanski, Jerzy. 1966. Studies on role of microorganisms in the life of cultivated plants: III. Origin of the bacterial substances stimulating the growth of plants. Acta Microbiol. Pol. 15(1): 67-84. Biol. Abstr. 47: 98499.

46. Sundman, Veronica. 1964. The ability of conidendrin-decomposing Agrobacterium strains to utilize other lignans and lignin-related compounds. J. Gen. Microbiol. 26(2): 185-201.

47. Teliz-Ortiz, Moises, and Walter H. Burkholder. 1959. A strain of Pseudomonas fluorescens antagonistic to Pseudomonas phaesiolicola and other plant pathogens. Phytopathology 50: 119-123.

48. Tewari, G. P. 1965. Effects of nitrogen, phosphorus, and potassium on nodulation in cowpea. Exp. Agr. 1(4): 257-259.

49. Vela, G. R. 1964. Radiation resistance of soil bacteria in their natural state. Diss. Abstr. 25(1): 35.

50. Voznyakovskaya, Y. M. 1959. A new species of epiphytic microorganism, Pseudomonas epiphytica n.sp. Microbiology 28: 840-843.

51. Went, Johanna C., and Frankje de Jong. 1966. Decomposition of cellulose in soils. Antonie van Leeuwenhoeck 32: 39-56.

52. Wolfenbarger, Dan A. 1965. Polyhedrosis-virus-surfactant and-insecticide combination, and Bacillus thuringiensis - surfactant combination for cabbage-looper control. J. Invert. Path. 7: 33-38.

VITA

John Robert Beckley

Candidate for the Degree of

Master of Science

Report: THE BENEFICIAL EFFECTS OF BACTERIA ON VASCULAR PLANTS

Major Field: Natural Science

Biographical:

Personal Data: Born in Lebanon, Pennsylvania, February 4, 1925, the son of John L. and Alice E. Beckley.

Education: Graduated from Lebanon High School, Lebanon, Pennsylvania; received the Bachelor of Arts degree from Yale University in 1947.

Professional experience: Taught high school science and mathematics at Red Lion, Pennsylvania from 1948 to the present.