MICROBIOLOGICAL STUDIES ON THE PHYSIOLOGICAL DISEASE OF RICE IN TAIWAN

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Ming-huei Wu Sheng

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A THESIS

Submitted to
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ABSTRACT

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Studies were conducted on the microbial populations in root environments of healthy rice and of rice exhibiting symptoms of "suffocation disease," a physiological disorder associated with reductive soil conditions in eastern Taiwan.

Numbers of total and anaerobic bacteria, fungi, and aerobic and anaerobic cellulose decomposers reached seasonal maxima during the tillering period. This was also the period during which disease symptoms appeared and developed to maximum intensity. These groups were much more numerous on root surfaces than in the rhizosphere or edaphosphere, and they responded to incorporated additions of straw or green manure.

Actinomycetes, sulfate reducers and ammonifiers were influenced to a much lesser degree by either organic amendments or proximity to root surfaces.

Numbers of denitrifying bacteria showed relatively little effect of organic amendments but appeared to be very sensitive to plant influences. Extreme seasonal fluctuations in numbers of denitrifiers, principally on root surfaces,

appeared to reflect changes in physiology of the rice plant.

Disease symptoms were successfully transmitted to healthy rice seedlings in nutrient culture by root washings from diseased plants, indicating the probable involvement of microbial toxins. However, it appeared that a primary factor affecting the nutrition and physiology of rice was most likely the strongly reduced soil conditions observed: E_h = -400 mv at tiller initiation, -250 mv at panicle initiation and -150 to -100 mv at maturity.

A review of the literature revealed numerous possibilities for toxin production by denitrifying species. Preliminary pure culture studies gave promise of useful nutritional discrimination within this group for further studies on rice rhizosphere physiology.

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INTRODUCTION

Taiwan is a large semitropical island lying about 100 miles off the China mainland in the same latitude as the Hawaiian Islands. It has a land area of 35,760 square kilometers. Average January temperatures in lowlands range from 15°C in the north to 20°C in the south. The summer season lasts for six to seven months with temperatures averaging 23-28°C.

Rice is the most important crop of Taiwan. The basic cropping pattern on Taiwan's paddy fields is to grow two rice crops a year, one in the spring (the first crop of rice) the other in the fall (the second crop rice). In recent years, farmers of central and southern Taiwan have gone into the growing of as many as four crops a year on the same piece of land, because of year round warm temperatures and especially good irrigation systems.

Organic matter content of tropical soils is generally low. So is that of soils in Taiwan. It may be as high as 5% or as low as 1% but generally between 2-3%. Therefore, maintenance of organic matter in soils, especially in paddy fields, is considered essential. Unfortunately, the application of green manure or straw to paddy soil aggravates the seriousness of a major rice disorder which has

been known in eastern Taiwan for more than 30 years. Until recently this problem has been restricted to an area of about 200 hectares in the eastern district of Ilan. However, during the present decade a similar unthrifty condition of paddy rice has been recognized on extensive areas in central and southern Taiwan. About 15,500 hectares were affected in 1961, 25,635 hectares in 1962 and 3,570 hectares in 1963 (162).

On the basis of field observations and other related information, it is generally agreed that abnormal development, poor growth and root rot observed in the problem area in Ilan is a physiological disorder associated with poor natural drainage and the resulting reductive soil condition. Whether the same causes account for the newly arising rice problem in the central and southern areas of Taiwan is more controversial.

The apparent physiological disorder of rice in Ilan is locally called "suffocation disease." It is one example of a number of physiological diseases associated with poor drainage. These may have different symptoms and are known by various local names in other countries.

It is generally believed that the so-called "suffocation disease" of rice is caused by chemically reduced toxic products which interfere with nutrient uptake from the soil. However, the root zone is also the area of the greatest activity of microorganisms. Any product of microbial

metabolism which is formed at the root surface, whether it be harmful or beneficial, can have an immediate effect upon the root cells. Conversely, changes in root physiology, such as a decline or loss of oxygenating ability of rice roots or changes in composition of root exudates or sloughed off cellular debris, can drastically alter microbial populations in the rhizosphere.

Since chemical methods seem to be unavailable to define the physical and chemical status of the rhizosphere, resort must be made to biological means of definition. Changes in the microbial population in the root zone during the growth of the plant will serve to indicate changes in root physiology. Knowledge of the biological activities of the microflora of root surfaces would be helpful in predicting the nature of possible toxic compounds.

OBJECTIVES

Microbial populations and activities in the rhizosphere have been studied extensively for many upland crops. Very little is known in the case of rice, especially with regard to harmful effects of microbial activities in poorly drained paddy soil with the application of organic matter. In order to reach a better understanding of the nature of physiological disorders associated with these conditions and to develop amelioration practices for rice fields made unproductive by them, it seems an urgent matter to clarify the relation between rice roots and the soil population. A systematic study of rhizosphere physiology in its relationship to development of disease symptoms is called for.

Such a study has been initiated in the investigation reported here. The following objectives were undertaken:

1. To enumerate major taxonomic and physiological groups of microorganisms in the rhizospheres of healthy and diseased rice plants.
2. To correlate enumeration data with disease development and with physiological age of rice.
3. To investigate effects of soil type and organic amendments on disease development and microbial numbers.

4. To demonstrate transfer of the disease by root washings from diseased to healthy plants.
5. To develop media and procedures for more detailed characterization of predominant physiological groups found in the rhizosphere flora of diseased rice.

LITERATURE REVIEW

Physiological disorders of rice occur in association with flooding in many countries of Asia and America. Symptoms and the stage of growth at which they occur are different in different localities. In all cases, however, an excessively reduced condition of the soil is an associated phenomenon.

The corresponding diseased condition of rice is called by different local names (131). In Burma, three distinct patterns of symptomology are distinguished: "Amiyit-po," "Miyit-po" and "yellow leaf." In Japan, the names "Akagare," "Akiochi," "Aodachi," "Aogare" and "Hideri-Aodachi" are applied to five distinctly different sets of symptoms observed in rice under different soil, management or climatic conditions conducive to stagnation of impounded water in rice paddies. No distinction is made in the name "Penyakit Merah" which is applied to two distinct types of symptoms in Malaya. Names applied to rice disorders associated with reductive soil conditions in other countries include "Bronzing"in Ceylon, "Mentek" in Indonesia, "Pansuk" in Pakistan, "Straight Head" in the U.S.A. and "Suffocation Disease" in Taiwan.

Under tropical and subtropical climates, maintenance of organic matter in soils, especially in paddy fields, is

considered essential. However, the application of green manures or straw in some soils aggravates the seriousness of the physiological disease. The role of organic matter in the growth of plants has been a subject of much investigation and controversy since the sixteenth century. Adverse effects on plant growth of straw and other mature plant residues low in nitrogen were studied extensively prior to 1925 (31, 50). Since that time many workers (35, 50, 101, 115, 116, 136) have found that various crops suffer some injury during the decomposition period of green manure.

Allison pointed out that the plant disease problem cannot be viewed in only the narrow parasitic sense (6). Associated saprophytic organisms may also reduce plant vigor through the production of toxins or undesirable products of organic matter decomposition, through competition for major and minor plant nutrients or through depletion of the oxygen supply.

According to the discussion by Cochrane (30) there are at least four general types of mechanisms which may be responsible for adverse effects of readily decomposable organic materials when they are added to soil:

1. Stimulated growth and activity of pathogenic organisms.
2. Effects on the availability of nitrogen and phosphorus in the soil.
3. An unfavorable microbiological balance in the soil or the rhizosphere that leads eventually to plant injury.

4. Chemical substances may cause root injury during the microbial decomposition of the residue.

Research by many workers (10, 29, 30, 34, 44, 86, 87, 113, 116, 136, 137, 140, 151, 174) has served to focus attention on several specific effects of added organic matter on the soil environment and the soil microbial population. These are summarized in the following sections.

Effects of Organic Matter

Soil Aeration

It was assumed by many workers that the composition of the soil air is changed unfavorably for the young plants during the decomposition of organic amendments (13, 63, 101, 110, 113, 114, 168, 172). The incorporation of fresh organic matter into the soil greatly affects aeration by depressing the oxygen content and increasing the carbon dioxide content. The extent of poor aeration may be sufficient to reduce plant development (1) and crop yield (34). Russell (113) indicated that green manuring will increase the content of carbon dioxide and it may inhibit germination or harm the very young root system of the seedlings. Such results have also been reported by others (2, 108, 113, 116, 139, 154). When oxygen content falls below 1 per cent, there is a rapid and appreciable decrease in potassium content of rice due to outward movement of potassium from the roots (154). The injurious

effects on plants of low oxygen tension may be due more to competition between the higher plants and the microorganisms for the limited supply of oxygen (101) or nutrients and oxygen (63) than any other single factor. Epstein and Kohnke (34) suggested that planting of crops sensitive to low oxygen and high carbon dioxide content should take place about three weeks after the organic residues have been incorporated into the soil. In heavy, poorly drained soils, it may be advisable to delay planting even longer.

Toxic Concentration of Gases

Ammonia (2), methane (4, 108, 115), hydrogen (108, 115) hydrogen sulphide (33, 104, 108, 111, 116, 154), methyl mercaptan (133) may accumulate in concentrations which are toxic to plants.

Takai, Koyama and Kamyra (134) have described the following sequence of gas formation after soil amended with fresh organic matter is flooded: oxygen is rapidly consumed, accompanied by vigorous evolution of carbondioxide. Within one day, hydrogen begins to evolve. The initially vigorous production of carbon dioxide then decreases, with a reciprocal rapid increase in evolution of methane. Nitrate disappears quickly, and ammonia is liberated as reduction progresses. In the early stage of incubation, oxidation reduction potential (E_h) drops rapidly, and ferric iron is reduced. Active production of H_2S and sulfides is not observed until after a considerable period of incubation.

Accumulation of Active Ferrous Iron and Reduced Manganese

The addition of organic matter is very effective in promoting the formation of ferrous iron in soil (13, 25, 29, 33, 94, 108, 148, 154). Organic matter decomposition and high moisture also favor the reduction of manganese (108). Clark (29) pointed out that the concentrations of these two elements in the soil solution have been observed to change markedly with prolonged soil submergence and added organic matter. Ponnamperuma, Bradfield and Peech (106) suggested that the unthrifty growth of rice in poorly drained or poorly aerated soils is due largely to the accumulation of ferrous iron in the soil solution. According to Somers and Shive (123), an unbalanced ratio of iron to manganese produces specific types of chlorosis. Takai reported that ferric iron is reduced vigorously in the early stages of decomposition of organic matter in soil because of limited oxygen supply from the surface water (134).

Availability of Nitrogen and Phosphorus

Waksman (158) demonstrated that the decomposition of younger plants results in liberation of some of the nitrogen from protein as ammonia which may be reassimilated by the microorganisms. In the presence of an excess of available organic matter, the fungi, actinomycetes, and various heterotrophic bacteria synthesize an extensive protoplasm. For this purpose, they assimilate nitrate and ammonium

present in the soil and thus compete with higher plants. According to Alexander (2), as carbon is assimilated for the generation of new protoplasm by microflora, there is a concomitant uptake of nitrogen, phosphorus, potassium, and sulfur. Thus, in the presence of decomposable organic matter, microorganisms reduce the quantity of nitrogen (8, 30, 42, 95, 141, 142, 174), phosphorus (30, 141) and other plant-available nutrients in soil.

Phytotoxic By-products from Plants or Microorganisms

Many studies have been made on phytotoxic by-products from decomposition of plant residue (2, 10, 50, 75, 86, 94, 117, 118, 119, 137, 139, 162), living plants (117) and microorganisms (2, 87).

Takijima (138) reported that the growth of paddy roots was poor even at the earlier period of flooding due to the harmful effects of major metabolites, such as carbon dioxide, organic acids and unknown inhibitory substances. He also pointed out that the physiological functions, especially nutrient absorption and oxygenation of soil by roots, therefore were retarded. He concluded that organic acids and some organic metabolites harmful to plants should play a key role in the occurrence of root damage in the ill-drained paddy soil (135).

Takijima (139) found that organic acids (predominantly formic, acetic, butyric, lactic, and succinic acids)

present in Japanese paddy fields where the soil was humic, or ill-drained, or with heavy application of green manure, exerted inhibitory effects on rice production.

Takijima and Sokuma (137) reported that water-logged paddy soil treated with green manure--Chinese milk vetch--caused the most extensive rice root injury and inhibited the growth of rice. Growth inhibition due to some organic acid in soil treated with vetch reached a maximum about one week after water-logging and then decreased rapidly to the same level as that in the controls.

A number of studies have been made on the physiological basis of root rot which develops during plant growth. The view that organic acids accumulating in rice paddy soil may be one of the factors impeding the function of roots has been expressed only recently in connection with poorly productive paddy fields. Organic acids, especially lower fatty acids present in soils of the paddy fields in Japan, have been considered to be of great importance in their inhibitory effects on rice production. About ten fatty acids were found in paddy soil (including formic, acetic, butyric, lactic, oxalic, propionic and succinic) by Takijima (139). Collison (31) and many German scientists (10) have reported in the early days that phenolic compounds (ferulic acid, p-coumaric acid, vanillic acid and p-hydroxybenzoic acid) were liberated from plant residues and affected or inhibited plant growth directly.

Schreiner and Reed (117) reported many organic compounds other than organic acids, which are ordinarily regarded as by-products of vegetable metabolism, to be highly toxic to seedlings when present in sufficient quantities. Recently, Wang (160) found that lower aliphatic alcohols which were formed in soils under waterlogged and anaerobic condition may exert an influence upon plant growth on many occasions in the soil. Studies on the effect of phenolic acids upon the growth of paddy rice seedlings have also been done by Wang. According to Wang's experiments (161) the depressive effect of p-hydroxybenzoic, vanillic, and syringic acids on the growth of paddy rice seedlings would have become significant at 75 ppm, that of p-coumaric acid at 50 ppm. Wang also found that paddy rice seedlings might be more tolerant to lower fatty acids than young sugar cane.

Some microorganisms also produce many organic substances which are toxic to plant growth. Krassilnikov (66) investigated more than 300 cultures of non-sporeforming bacteria for the production of plant growth inhibitors. About 100 of the cultures suppressed plant growth and germination to some degree. Bacillus mesentericus, B. subtilis, B. cereus, B. brevis, accounted for most of the inhibitory spore formers. McCalla and Haskins (87) found that products from certain strains of Pseudomonas fluorescens, P. pyocyanea, and Bacterium spp. possess strongly herbicidal properties. Alexander (2) noted that products of microbial metabolism often have a detrimental effect upon higher plants, and

common non-pathogenic bacteria produce harmful effects through the release of soluble toxic factors. He mentioned that several antibiotics are assimilated by higher plants through the root systems and then are translocated to above-ground portions, that antibiotics are also known to affect the physiology of the plant entirely apart from any anti-microbial action they might have. Livingston (74, 75, 178), working on the harmful effect of bog soil, showed that waters obtained by leaching a bog soil contain materials which are toxic to the growth of numerous plant species. It was noted that the substances were in some cases volatile, and in some cases apparently of colloidal nature.

Sulfate Reduction and Denitrification

Alexander (2) indicated that many bacteria, fungi, and actinomycetes can convert sulfate and partially reduced sulfur compounds to sulfide. In water-saturated soils (under prolonged flooding) or in excessively compacted soils, anaerobic decomposition of sulfates or proteins may result in the formation of hydrogen sulfide which, even in low concentrations, is toxic to plants (87). Vamos (152) reported that one of the physiological rice diseases--Brusone--found in Hungary is caused by the toxic effect of hydrogen sulfide. He also suggested that the disease was more likely to occur on the heavier soils at lower elevations and having higher contents of organic matter and total nitrogen (150). As Takai, Koyama and Kamura (99)

explained, the hydrogen formed during the decomposition of cellulose is used by the sulphate reducing bacteria (Desulphovibrio desulfuricans) as a source of energy. These bacteria obtain oxygen for utilization of the hydrogen by reduction of the sulfates which are abundantly present in the flood water and soil. Vamos pointed out that protein-decomposing bacteria also take part in sulphate reduction. The application of organic matter to paddy soil promotes production of toxic hydrogen sulfide, as mentioned above. Hydrogen sulfide inhibits not only photosynthetic processes in the leaf, but also cytochrome oxidase systems in the rice plant and nutrient uptake (20, 92).

It is widely known that denitrification requires a goodly supply of readily oxidizable organic compounds (15, 156), high nitrate levels (167), low oxygen tension (5, 7, 20, 60, 99, 167) and poor drainage (3, 53, 99). Bremner and Shaw (11) found that denitrification is much more rapid in soil saturated with water than in soil at lower moisture levels. The supply of oxygen in water-saturated soil is not adequate to meet the requirement of the soil microorganisms and, in consequence, the denitrifying microorganisms utilize nitrate instead of oxygen as a hydrogen accepter and so cause denitrification. The rate of denitrification increases with the amount of straw added. If at the same time the water content of the soil is high, intensely anaerobic conditions may easily arise,

favouring denitrification as well as the reduction of nitrous oxide to molecular nitrogen or the reduction of nitrate to ammonia.

Nommik (99) reported that one of the many factors that influence the rate and course of denitrification is the content of easily oxidizable organic material to serve as energy substrate. Supplying organic material as a rule brings an increase in the microbial activity of the soil which in turn means an increase in the consumption of oxygen. High water content of the soil proved to be of decisive importance to denitrification.

Rhizosphere Relationships

Investigations cited above provide ample evidence that many inorganic or organic toxic compounds are associated with microbiological activities in the rhizosphere. Numerous workers have reported on phenomena associated with activities of microbes in the rhizospheres (28, 56, 64, 65, 127) of upland crops.

Starkey (127, 129) reported that each kind of plant has a typical rhizosphere population. He found that organisms were many times more numerous on root surfaces than in the soil close to the roots. The greater the distance from the region of extensive root development the smaller the number of bacterial inhabitants found in the soil. He also pointed out that the greatest rhizosphere effect occurred during periods of active plant development and the effect

disappeared promptly on death of the plant. He believed that the rhizosphere effect is associated with normal growth. The majority of the rhizosphere microorganisms are saprophytic and convert both organic and inorganic materials in the rhizosphere. The products of these transformations may be beneficial or injurious to plants.

MacCalla (87) states that some of the organic material produced by microbes in the rhizosphere is absorbed by the plants, but the kind and amounts are not known.

Timonin (143) reported that different varieties of plants affected the activities of the various groups of soil microbes differently. Ishizawa et al. (51) noted that the effect of organic substances upon microflora differed according to the type of organic matter. Among microorganisms, the increase of actinomycetes was smallest, among organic materials (glucose, alfalfa, rice straw and manure) the effect of manure on the increase of microbes was smallest. Bacteria were always higher than other microorganisms and did not show a rapid decrease for longer periods. Ishizawa and Toyoda (52) found that total bacteria and anaerobes were more and actinomycetes and fungi were less abundant in paddy than in upland soil. Sulfate reducers and denitrifiers were more numerous in paddy than other soils in Japan, especially under conditions of abundant supply of available organic matter. The latter two groups of organisms were present not only in the surface but also

in the substratum. The authors observed that a high moisture condition and a supply of available organic matter were necessary for the maintenance of a high bacteria count.

Numerous rhizosphere studies indicate that the abundance of microbial cells is affected by the kind of plant and its stage of development and vigor. The maximal number of bacteria is observed during the period of the most active growth of the plant. Observations show that an abundant growth of microorganisms takes place in the early stages of plant growth, however the most vigorous growth of microbes ensues during the period of flowering and in the period directly preceding it (66). The abundance of mycelial groups such as fungi and actinomycetes is greater on old root surfaces (52).

It is suggested that the roots of plants may excrete significant amounts of stimulative substances which can promote bacterial growth (42, 164). Krassilnikov (79) reported that small non-sporing bacteria (B. denitrificans, B. fluorescens) were found to multiply extensively in rhizospheres, presumably under the stimulation of organic root excretions. It is generally accepted that the main factors for the stimulation of microorganisms in the rhizosphere are the excretion of organic substances and the sloughing off of root hair and epidermal cells (112, 128). Mucoid materials (59), nucleotides, flavones and other reducing substances (81), pentose sugars or a closely allied substance, D-xyloketone (16), acetaldehyde (97) were found

by many workers in studying plant-microbial interactions in the rhizosphere. Numbers of workers have found that root excretions stimulated the maximum growth of various groups of organisms, particularly these requiring amino acids (58, 80, 109, 159, 165). Katznelson and Richardson (57) found a higher production of bacteria stimulated by amino acids in the rhizosphere of tomato plants than in the control soil. The rhizosphere of diseased plants may contain greater concentrations of microorganisms than that of healthy plants (57).

There is still very little information about the kinds of microorganisms in the rhizosphere of different plants and their significance in development of the plants. Very little is known about rhizosphere microbial activities associated with physiological disorders of paddy rice.

Denitrifying Bacteria

Denitrifying species or denitrifying bacteria as a group are frequently implicated in the above cited investigations of rhizosphere phenomena and effects of incorporating organic matter in paddy soils of poor drainage. It appears that this physiological group might be usefully studied in greater detail in relation to physiological disorders of rice.

All denitrifiers can produce nitrous oxide (96). Most of the denitrifying bacteria reduce nitrate to nitrogen gas and nitrous oxide in varying proportions, B. nitroxus being

particularly active (158). It was found that non-spore-forming organisms of the genera Pseudomonas, Micrococcus and Spirillum, aerobic spore formers (bacilli) and a number of facultative anaerobes can reduce nitrate (170). More than forty organisms have been reported as denitrifiers (88). Denitrifying bacteria are summarized as follows:

Nitrate Reducers	Denitrifiers	Nitrate Reduction Catalyzers(2)
E. coli (36)	Spirillum itersonii (88)	Thiobacillus denitrificans
Micrococcus denitrificans (36, 62)	*Microc. denitrificans (170)	Chromabacterium mycoplana
Streptoccocus faceaim (47, 69)	B. denitrificans (88)	Serratia or Vibrio species
Clostridium spp. (170)	B. licheniformis (149)	
Achromobacter nephridii (Corynebacterium nephridii) (47)	*B. subtilis (96, 153, 170)	
**A. hartlebii (149)	*Pseudomonas stutzeri (88)	
A. arcticum (88)	Ps. aeruginosa (71)	
A. liquefaciens (153, 170)	Ps. putida (88)	
*Bacillus cereus (68)	Ps. indigofera (89)	
**B. licheniformis (36)	Ps. fluorescens (88)	
B. proteus (88)	Ps. denitrificans (88)	
B. macerans (170)	Ps. perfectomarinus (107)	
B. prodigiosus (88)	Ps. pseudionallei (124)	
	*Achromobacter denitrificans (149)	

Nitrate Reducers	Denitrifiers	Nitrate Reduction Catalyzers (2)
*B. polymyxa (68)		
B. laterosporus (170)		
B. coagulans (170)		

*Need amino acids
**Require ammonium

A comparison of the denitrifying population of grassland sods with and without nitrate addition was made by Woldendorp (170). It revealed that under normal field conditions without nitrate the population consisted mainly of Bacillus species (B. cereus, B. circulans, B. macerans, B. coagulans, B. laterosporus) of which B. cereus was by far the most numerous. These organisms did not give rise to gaseous products but formed large quantities of nitrite and some ammonia. Pseudomonas and Achromobacter were dominant genera in soil to which nitrate fertilizer had been added. Valera and Alexander (149) reported, as did Woldendorp, that the most rapid gas evolution was brought about by Ps. aeruginosa.

During the past decade, experiments have shown that the producers of antibiotic substances are widespread in soils. It has been determined that the plants absorb through the roots different antibiotic substances produced by microorganisms in the soil. It is known that mycerin, subtilin, glyotoxin and others induce strong poisoning in plants by

comparatively small doses. Clavacin suppresses the growth of cereal roots at a dilution of 1:1,000,000; subtilin depresses the germination of wheat and pea seed at a dilution of 1:100,000-1,000,000 (67).

Knight (68) listed a number of species of interest as producers of antibiotics, including B. subtilis, B. brevis, B. polymyxa, B. licheniformis, B. circulans. B. polymyxa was originally detected as a producer of an antibiotic by Stansly, Shepherd and White (125).

Pseudomonas pseudomallei is essentially an accidental pathogen (124). Ps. tabaci, can produce a true toxin which is the cause of tobacco wildfire disease and causes several leafspot necroses. The toxin appears to interfere with the metabolism of a normal amino acid, methionine (124).

The most important denitrifying bacteria--Ps. aeruginosa--is widely known for two distinctive properties; one is production of the blue pigment, pyocyanin, the other is its ability to lyse or to inhibit the growth of other bacteria, especially Gram positive bacteria (176).

Pyocyanin is easily oxidized and reduced in cultures and acts in conjunction with the cytochrome system of the cells to increase respiration (37, 38). Chemically, pyocyanin is a phenazine and is similar in structure to iodinine, a pigment produced by Chromobacterium iodinum and to chloraphin, a green pigment formed by Ps. chlororaphis (43).

Some Ps. aeruginosa strains can produce a red diffusible pigment--pyrorubrin--after 16 hours of incubation

at 37°C and an additional 24 hours at room temperature. Strains producing pyrorubrin did not appear to produce pyocyanin and vice versa. Four types of diffusible pigment are produced by Ps. aeruginosa (157):

Pigment	Percent of strains
Blue-green or brownish green	68.2%
Light-brown	10.5%
Red pyrorubrin	3.5%
No pigment	17.8%

Morihara reported recently that Ps. aeruginosa can produce proteinase on non-carbohydrate carbon sources (93).

The study of nutritive differences among microorganisms found in the rhizosphere offers a means for increasing knowledge of the physiological activities of the root system of plants. Many contributions have been made by numerous investigators. They have found that nutrient requirements are very specific, not only for the growth of denitrifying bacteria but also for the formation of pigments. Burton found that phosphate, sulfate, magnesium, potassium and iron ions were essential for growth of Ps. aeruginosa. Magnesium promoted the synthesis of pyocyanin (43). Glycerol and tartrate appeared to yield a greater population than citrate (149). Glutamic acid appeared to be superior to sugars or fatty acids as hydrogen donor in respiration (170) and also for pyocyanin synthesis (43). Glucose over 1% may inhibit the production of pyocyanin by Ps. aeruginosa (176).

The presence of amino acids stimulated many denitrifying bacteria (55, 176) as well as the entire denitrification process (170). Nitrate served as an extremely effective electron acceptor (124).

The reaction of media is also a critical factor. Not only does the optimum pH range vary with the bacterium, but the specific activity in gas evolution is greatly different at different pH's for several microorganisms. Wijler and Delwiche (167) point out that total denitrification rates were quite constant above pH 6.0, but the proportion of nitrous oxide and nitrogen were pH dependent. Above pH 7.0, nitrous oxide could be readily reduced to nitrogen. Below pH 7.0, the reduction of nitrous oxide was strongly inhibited. Below pH 6.0, nitrous oxide became predominant, and the rate of denitrification was decreased. Karlsen found that denitrification would occur at pH 5.8 to 9.2, with an optimal range between pH 7.0 and 8.2. Young (176) reported that, in nutrient broth incubated two weeks at 37°C, only pyocyanin and a fluorescent pigment were formed. Acid cultures of Ps. aeruginosa produced no pigments, formed no antibiotic substances of any kind (176).

Various synthetic and semisynthetic media have been used in studies of pyocyanin production by Ps. aeruginosa. A synthetic medium consisting of d,l-alanine or glycine at 0.4% concentration, combined with 0,8% l-leucine, 1.0% glycerol and salt mixture has been shown to be the most

suitable medium for pyocyanin production by five representative strains of Ps. aeruginosa by Burton (19).

Liu (73) found that hemolysin, lecithinase and protease of Ps. aeruginosa were produced with cellophane covered plates and completely synthetic agar of simple composition containing l-alanine, aspartic acid, glutamic acid, glucose and salts. He also observed that glucose and phosphate played a unique role in production of these extracellular toxins. Glucose appeared to be more important than just an energy source. It was suggested that products of anaerobic metabolism of glucose are needed in the production of these toxins. The most critical factor in the production of these extracellular toxins appeared to be the concentration of phosphate. The best medium for the production of these toxins appeared to be 0.45% l-alanine, 0.45% glutamic acid, 1.2% glucose and 0.003% phosphate (KH_2PO_4).

Other pigments produced by Pseudomonas species include fluorescin and pyroverdin (163). Lenhoff found that the oxygen tension in the growth medium influenced the formation of pyroverdin. Large amounts of the pigment were produced in well-aerated cultures with high oxygen tension (71).

Knight and Proom (68) found the following nutritional patterns to be characteristic of Bacillus spp.:

1. B. subtilis, B. licheniformis and B. megatherium grew with ammonia as sole nitrogen source and in the

absence of added growth factors.

2. B. cereus and B. brevis grew in the absence of added growth factors but required mixtures of amino acids instead of ammonia only as sources of nitrogen.
3. B. pumilus and B. macerans grew with ammonia only in the presence of biotin and aneurin.
4. B. alvei required amino acids and aneurin; B. circulans and B. coagulans had more complex requirements.

Lochhead and Chase (78) described seven cultural media, ranging from a simple basal medium to complex media, for qualitative studies of rhizosphere bacteria in relation to their amino acid nutrition:

Medium B	Basal medium	
" A	"	plus amino acid
" C	"	" growth factors
" A G	"	" amino acids plus growth factors
" Y	"	" yeast extract
" S	"	" soil extract
" Y S	"	" yeast extract and soil extract

Rovira (111) found that, for some organisms, yeast extract exhibited similar growth promoting properties as did pea root exudate, but for others, the exudate stimulated growth in the presence of yeast extract. The root exudate

could not be completely replaced by glucose, soil extract, vitamin-free casamino acids or a synthetic mixture of known growth factors.

Wasserman used either tryptone-glucose-yeast extract agar or Difco Pseudomonas Agar F to maintain stock cultures of Pseudomonas spp. (163).

EXPERIMENTAL METHODS

Experiments were conducted during the fall of 1964, which is the normal season for the second crop of rice in Taiwan, and again in the spring of 1965, the season during which the first crop is normally grown under a two-crop system. Greenhouse experiments and field plantings were used as sources of soil and rhizosphere samples for studying microbial populations associated with diseased and healthy rice plants.

Greenhouse Experiments

Greenhouse experiments were designed to investigate effects of organic amendments on microbial populations and disease development in rice growing on normal and problem soils.

First Trial

In the first trial (fall, 1964), four soils were used: (1) Lateritic paddy soil from Chungli, (2) alluvial rice soil from Taichung, (3) soil from a problem area in Pingtung and (4) Lotung silty clay loam from a problem area in Ilan.

The Lotung soil was taken in two ways. In one case, a profile sample, including top soil and subsoil, was taken

without disturbance of its structure to insure the occurrence of the disease. In the other, surface soil was composited, as was done in the case of the other soils.

Organic amendments are outlined in Table 1. Freshly chopped green Sesbania at the rate of 20 tons per hectare was used for green manure. Chopped rice straw was used at the rate of 5 tons per hectare. Organic amendments and soils were mixed thoroughly before dispensing into four replicate boxes of each treatment. In the case of the Lotung profile, the organic materials were incorporated only into the top soil, as in field practice.

Table 1. Treatments in the first greenhouse experiment.

Soil Samples	C Chungli			T Taichung		
Treatment	No Manure	Green Manure	Straw	No Manure	Green Manure	Straw
Treatment Code	C_o	C_g	C_s	T_o	T_g	T_s

Soil Samples	P Pintung			L_1 Lotung		
Treatment	No Manure	Green Manure	Straw	No Manure	Green Manure	Straw
Treatment Code	P_o	P_g	P_s	Ll_o	Ll_g	Ll_s

Soil Samples	L_2 Lotung (Undisturbed profile)		
Treatment	No Manure	Green Manure	Straw
Treatment Code	$L2_o$	$L2_g$	$L2_s$

The boxes containing the Lotung profiles (L_2) were flooded 2 days before transplanting rice (not usually done in the field). All other soils and treatments were flooded 14 days before transplanting. The water level was maintained at 4 to 10 mm above the soil surface continuously until the 25th day after transplanting. At that time, water was drained off to observe effects on rice development of improved soil aeration.

Seedlings of a susceptible strain of rice, Taichung 65, were grown on Taichung soil in the field to an age of 23 days before transplanting into the experimental boxes. On August 6, 1964, five seedlings were planted per hill and two hills per box (30 x 30 x 30 cm), except that four hills per box (60 x 30 x 30 cm) were planted on the Lotung profiles.

The rates of applied fertilizer were 80, 60, and 60 kg per hectare of N, P_2O_5 and K_2O, respectively. Ammonium sulfate, superphosphate and potassium chloride applications were split, half two days before transplanting and half 30 days later.

All experimental units, except those involving Taichung soil, were grown to maturity in the greenhouse. The rice cultures on Taichung soil were grown outdoors on the Chung-Hsing University farm.

Second Trial

In the first rrial, disease symptoms developed only on the Lotung profile (L_2). Accordingly, only Lotung

profile cultures (L_3) were used in the second trial. Organic amendments were incorporated at two different times, two days and fourteen days before transplanting (Table 2). Because Sesbania grows poorly during the winter months, it was necessary to use lupines for green manure. The rate of nitrogen fertilizer was increased from 80 to 120 kg per hectare. The soil was flooded at the time of organic matter addition and was not drained at any time.

Table 2. Treatments in the second greenhouse experiment.

Check	Green manure		Straw	
	Incorporated before transplanting			
(No organic amendment)	2 days	2 weeks	2 days	2 weeks

Forty-one-day-old seedlings of the Taichung 65 rice strain were transplanted on March 10, 1965. Five seedlings per hill and four hills per box (60 x 30 x 30 cm) were planted, as in the first trial, and the rice was grown to maturity in the greenhouse.

Soil pH and oxidation-reduction potential (E_h) were measured between plants at a depth of 10-15 cm daily up to the time of maximum tillering and weekly in later stages of growth (26, 105, 171).

Inoculation Experiment

An inoculation experiment was conducted to investigate, in a preliminary manner, the etiology of disease symptoms

observed during the first greenhouse trial.

Healthy rice seedlings were grown in 1X Hoagland's solution (49) from seeds sterilized before germination by immersion for one hour in 1:500 Uspulum solution. These seedlings were germinated and grown in 100 ml bottles covered with black paper.

Boxes of the Lotung profile which had produced severe disease symptoms during the first greenhouse trial were used to cultivate diseased rice again in the same way, with incorporated rice straw and green manure. When severe symptoms of the disease appeared about one week after transplanting rice to these boxes, soil leachates and root washings were collected from them.

Soil leachates and root washings from diseased rice cultures were mixed with equal volumes of fresh 2X Hoagland's solution in large bottles. Healthy seedlings grown on 1X Hoagland's solution were then transferred to these bottles.

When plants inoculated in this way reproduced symptoms of the disease, a 1 ml aliquot of the nutrient solution was withdrawn from each bottle for enumeration of total bacteria and sulfate reducers. Nutrient agar was used for bacteria, and yeast extract-sodium sulfate agar for sulfate reducers.

Field Locations

Soil and rhizosphere samples of second crop rice were taken in the fall of 1964 from farm paddies at two locations where the disease was known to occur. One

location on Lotung soil in Ilan was chosen because the disease occurs regularly in that area. The other location was in Pingtung where the development of disease symptoms is less predictable.

With first crop rice in the spring of 1965, only one field location was sampled. This was on Lotung soil in Ilan.

Microflora Studies

Soil and rhizosphere samples for enumeration of microbial populations were taken from both greenhouse experiments and field location. Times of sampling were based on the stage of physiological development of the rice:

(1) Seedling stage (prior to or at transplanting time)
(2) Tillering stage (when first detectable tiller appeared)
(3) Time of maximum tiller number
(4) Panicle development (panicle primordium first detectable by naked eye--usually 1-2 mm long)
(5) Anthesis (first visible anther)

The first symptoms of physiological derangement, when observed, usually appeared at the tillering stage and reached maximum intensity about the time of maximum tiller number.

Samples of non-rhizosphere soils (S) were taken mid-way between plants in the field or between hills in the greenhouse box cultures. Core samples 10 cm in

diameter and 9 to 15 cm deep (depending on age of rice) were taken.

Similar cores were taken for estimation of root (R) and rhizosphere (Rh) populations. During the early stages of development, these cores included the entire root system of a single hill. In later stages of growth, only a part of the root system would have been included. In the greenhouse experiments, one hill per treatment was sacrificed for this purpose on each sampling date. In treatments where no disease symptoms developed, the sampled hill was selected at random from one of the four replicate boxes of each treatment. In treatments where disease symptoms appeared, the most severely injured hill for that treatment was taken. The corresponding non-rhizosphere (S) sample for each treatment was taken from an inter-hill area adjacent to the hill taken for the root and rhizosphere sample.

Samples were placed in individual plastic bags for transport to the laboratory. Aliquots of non-rhizosphere soil samples (S) were suspended immediately in physiological saline solution and quantitative dilutions made for inoculation of enumeration media. Root and rhizosphere cores were immersed in water, with gentle kneading and agitation, to remove the bulk of the soil. They were then transferred to physiological saline solution and agitated for 5 minutes on a flask shaker. Rhizosphere (Rh) populations were estimated on serial dilutions of the suspension so obtained and calculated on the basis of the dry weight of

soil remaining after evaporation of the saline solution.

After removal of the rhizosphere soil, the roots were placed in fresh saline solution and shaken for 20 minutes on a flask shaker. Microbial numbers estimated on serial dilutions of this suspension were calculated on the basis of the oven dry weight of root tissue and are designated as root (R) populations (84, 110, 130).

Emphasis was placed upon enumerating physiological rather than taxonomic groupings. Selective media were used to differentiate between microorganisms with specific biochemical activities. However, counts were made also of the major taxonomic groups to give further insight into the biochemical capabilities of dominant microflora.

The following media were used:

(1) Sulfate reducers

Sodium lactate-asparagine (Van Delden, 9)

(2) Denitrifiers

Asparagine-nitrate-citrate broth (Timonin, 145)

(3) Ammonifiers

Urea broth (Viehoeven, 4)

(4) Cellulose digesters (aerobic)

Cellulose dextrin agar (Fuller and Norman, 39)

(5) Cellulose digesters (anaerobic)

Cellulose dextrin agar (Fuller and Norman, 39)

(Incubated in N_2 atmosphere)

(6) Anaerobes

Sodium thioglycollate agar (Brewer, 12)

(7) Bacteria

Soil and yeast extract agar (Bunt and Rovira, 17)

(8) Actinomycetes

Dextrose-casein agar (Jensen, 54)

(9) Fungi

Rose Bengal agar (Smith and Dawson, 122)

After inoculation, all media were incubated for appropriate periods at 28°C.

With broth media and the sodium lactate-asparagine agar for H_2S production, numbers were estimated from a table of most probable numbers based on a 10-fold dilution series and 5 tubes per dilution (85). With agar media numbers were calculated from the mean of 3 replicates of a significant dilution in a dilution plate series.

Nitrate Reducing Bacteria--Pure Culture Studies

In the microflora studies, it was found that numbers of cellulose digesters, sulfate reducers, nitrate reducers and ammonifiers were frequently more numerous in rhizospheres of diseased than of healthy rice. It appeared that each of these groups might be studied in greater detail in its relationship to development of disease symptoms. To this end, a nutritional study of nitrate reducing bacteria in pure culture was undertaken. The objective was to find differences in nutritional requirements of representative species which might be used to develop solid media for enumeration and isolation of more restricted physiological

types among the larger group of nitrate reducers.

Eighteen species and strains of facultatively anaerobic bacteria with known capability for reducing nitrate were used (Table 3). Stock cultures were maintained on nutrient agar, with periodic transfer at 30°C followed by storage in the log phase at 10°C.

Eleven experimental media were employed (Table 4). Media 103 through 112 are modifications of media described by Woldendorp (170) and by Valera and Alexander (149). Stock solutions listed in Table 4, when combined in the proportions shown, give the following concentrations of minerals in the final medium: 123 mg $Na_2H\ PO_4$, 724 mg KH_2PO_4, 89 mg $MgSO_4 \cdot 7H_2O$, 53 mg $CaCl_2 \cdot 2H_2O$, 380 ug $CuSO_4 \cdot 5H_2O$, 440 ug $ZnSO_4 \cdot 7H_2O$, 310 ug $MnSO_4 \cdot H_2O$, 250 ug H_3BO_4, 5 ug $NaMoO_4 \cdot 2H_2O$ and 1 mg Fe (as FeEDTA) per liter.

In preparing these media, buffer and mineral salts solutions and agar were combined and sterilized by autoclaving at 121°C for 15 minutes. Energy sources, amino acids, vitamins and iron chelate were added through a Millipore filter. The pH of the hot medium was adjusted just before pouring the plates at 45 to 50°C.

Plates for anaerobic incubation were allowed to solidify and then were placed immediately into an H_2 atmosphere for storage until ready to use.

For aerobic incubation, plates were stored in sealed glass jars containing a few ml of 10 per cent glycerol to maintain humidity.

Table 3. Nitrate reducing bacteria investigated in pure culture.

Laboratory number	Organism	Source identification
15	Achromobacter hartlebii	ATCC[1] 365
9	Bacillus cereus	ATCC 6464
10	Bacillus cereus	ATCC 14579
13	Bacillus circulans	ATCC 4513
7	Bacillus coagulans	Woldendorp[2] 1963 II
5	Bacillus laterosporus	Woldendorp 468B
8	Bacillus licheniformis	Woldendorp P_1
6	Bacillus licheniformis	Woldendorp 430
14	Bacillus licheniformis	ATCC 14580
11	Bacillus macerans	ATCC 843
12	Bacillus macerans	ATCC 8244
16	Micrococcus denitrificans	ATCC 13543
1	Pseudomonas aeruginosa	ATCC 10145
2	Pseudomonas aeruginosa	Woldendorp
18	Pseudomonas denitrificans	ATCC 13867
17	Pseudomonas fluorescens	ATCC 11250
3	Pseudomonas stutzeri	Woldendorp
4	Serratia marcescens	MSU MPH[3]

[1]American Type Culture Collection, Rockville, Maryland.

[2]J. W. Woldendorp, Laboratory of Microbiology, Agricultural University, Wageningen, Netherlands.

[3]Department of Microbiology, Michigan State University.

Table 4. Experimental media.

Component	Medium number			
	103	104	105	106
Stock sol'n. A[1]	510 ml	510 ml	510 ml	510 ml
" " B[2]	90 ml	90 ml	90 ml	90 ml
" " C[3]	180 ml	180 ml	180 ml	180 ml
" " D[4]	10 ml	10 ml	10 ml	10 ml
" " E[5]	10 ml	10 ml	10 ml	10 ml
KNO_3	2.5 g	2.5 g	2.5 g	---
Asparagine	---	---	---	2.5 g
Glucose	2.5 g	2.5 g	2.5 g	2.5 g
Vitamins[6]	---	---	20 ml	20 ml
Amino acids[7]	---	20 ml	20 ml	20 ml
Na thioglycollate	---	---	---	---
Yeast extract	---	---	---	---
Na citrate	---	---	---	---
K H_2PO_4	---	---	---	---
Mg $SO_4 \cdot 7H_2O$	---	---	---	---
$CaCl_2 \cdot 6H_2O$	---	---	---	---
$FeCl_3 \cdot 6H_2O$	---	---	---	---
Agar	15 g	15 g	15 g	15 g
H_2O to:	1 liter	1 liter	1 liter	1 liter
pH	7.3	7.3	7.3	7.3

[1]Stock sol'n. A: 1.42 g Na_2HPO_4 per liter (.01 M).

[2]Stock Sol'n. B: 1.36 g KH_2PO_4 per liter (.01 M).

[3]Stock Sol'n. C: 493 mg $MgSO_4 \cdot 7H_2O$ (.002 M) plus 294 mg $CaCl_2 \cdot 2H_2O$ (.002 M) per liter.

[4]Stock Sol'n. D: 38 mg $CuSO_4 \cdot 5H_2O$, 44 mg $ZnSO_4 \cdot 7H_2O$, 31 mg $MnSO_4 \cdot H_2O$, 25 mg H_3BO_3, and 5 mg $Na_2MoO_4 \cdot 2H_2O$ per liter.

[5]Stock Sol'n. E: 769 mg FeEDTA per liter.

[6]Vitamin stock solution to provide 1 µg biotin, 2 µg vitamin B_{12}, 2 µg folic acid, 100 µg riboflavin, 500 µg thiamine, 500 µg nicotinic acid, 500 µg pyridoxine - HCl, 500 µg ca-pantothenate and 5 mg inositol per liter in the final medium.

[7]Amino acid stock solution to provide 950 mg vitamin-free casamino acids, 50 mg tryptophane and 10 mg cysteine per liter of final medium.

Medium number						
107	108	109	110	111	112	113
510 ml	510 ml	510 ml	510 ml	510 ml	510 ml	---
90 ml	90 ml	90 ml	90 ml	90 ml	90 ml	---
180 ml	180 ml	180 ml	180 ml	180 ml	180 ml	---
10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	---
10 ml	10 ml	10 ml	10 ml	10 ml	10 ml	---
2.5 g	2.5 g	2.5 g	---	1.25 g	1.25 g	1.0 g
---	---	---	2.5 g	1.0 g	1.0 g	1.0 g
2.5 g	2.5 g	2.5 g	2.5 g	2.5 g	2.5 g	---
---	---	20 ml	20 ml	20 ml	---	---
---	20 ml	20 ml	20 ml	20 ml	---	---
500 mg	500 mg	500 mg	500 mg	500 mg	500 mg	500 mg
---	---	---	---	---	10. g	---
---	---	---	---	---	---	8.5 g
---	---	---	---	---	---	1.0 g
---	---	---	---	---	---	1.0 g
---	---	---	---	---	---	0.2 g
---	---	---	---	---	---	0.03 g
15 g	15 g	15 g	15 g	15 g	15 g	15 g
1 liter	1 liter	1 liter	1 liter	1 liter	1 liter	1 liter
7.3	7.3	7.3	7.3	7.3	7.3	7.1

Streak inoculations were made, using 12 to 18 hour cultures in nutrient broth as inoculum. With slow growers, 34 to 42 hour cultures were found to be more reliable (*Achromobacter hartlebii*, *Bacillus macerans*, *B. laterosporus*).

For anaerobic incubations, atmospheres of H_2 and/or CO_2 were used in two cabinet type anaerobic incubators. The cabinets were evacuated to 5 cm Hg and filled with H_2 twice to exhaust O_2 to very low concentrations. Palladium-impregnated asbestos was used as a catalyst to reduce remaining traces of O_2. Small tubes of methylene blue agar, visible through the glass door panel, were used to indicate the establishment of reducing conditions. Partial or complete replacement of H_2 with CO_2 was effected only after reducing conditions had been established.

Aerobic incubations were accomplished in loosely covered glass jars containing a few ml of 10 per cent glycerol to maintain humidity.

Both aerobic and anaerobic incubations were carried out at 28 to 30°C. After various incubation periods, growth was estimated visually on a scale of 0 to 6 and pigmentation on a scale of 0 to 4. In some experiments, cross-streak inoculations were made and appropriate notations for synergism or antagonism were used.

RESULTS

Symptomology and Developmental Behavior of Rice

First Greenhouse Trial

Typical symptoms of suffocation disease were not observed in rice growing in the Chungli, Pingtung or Lotung (L_1) surface soils (Figs. 5, 6).

Rice in the Lotung profile (L_2) cultures with Sesbania green manure started to wilt just 2 days after transplanting. As the disease progressed, the leaves rolled inwards, beginning with older, basal leaves. Severely affected plants were severely stunted (Fig. 7). At 10 days, lower leaves were yellowing from the margin and apex toward the midrib and base. Roots of these plants were poorly developed, coarse in texture, with short lateral roots, very little lateral branching and no root hairs (Fig. 8). The roots were dark brown to black in color, and their lower portions were decaying, with a putrid odor.

After maximum tillering, brown spots appeared on the older leaves of both diseased and non-diseased plants in check and amended cultures.

The first symptoms did not begin to appear until 5 days after transplanting in the Lotung profile cultures

where straw was incorporated. At 22 days, however, the injury with the straw treatment was more severe than with green manure. When the flood water was drained from the boxes after 25 days, plants in the green manured profile cultures gradually recovered their green color and grew normally again, whereas the plants where straw had been used recovered very little.

These symptoms are the same as are regularly observed in the field on Lotung soil (70). From the observed production of gas bubbles on the surface of the flood water, it appeared that the symptoms developed during the height of decomposition of the added organic matter. With reference to stage of development of the rice itself, symptoms began to appear at about the same time as the first tillers and became progressively more severe through the tillering period. The severely affected plants were markedly stunted, tillered poorly (Fig. 1), headed late and produced thin, narrow panicles with light weight, poorly filled kernels (Fig. 4). Yields of straw and grain were severely reduced (Figs. 2, 3).

Yields of straw and grain were severely reduced by the straw amendment on the Pingtung and Lotung (L_1) surface soils, even though no symptoms of disease were observed. This was likely due to microbial competition for nitrogen. This explanation is supported by the stimulating effect on growth and yields of the Sesbania green manure. This

effect was expressed on all four soils. Even in the severely injured Lotung profile (L_2) cultures, rice following green manure recovered after drainage to produce larger yields of straw than the check and equivalent yields of grain.

Panicle development and maturity of rice were delayed 20 days in all soils by amendment with either straw or green manure, which suggests a delayed release of nitrogen from soil sources in the presence of microbial competition during earlier stages of decomposition of these materials.

Since it appeared that nitrogen at the rate of 80 kg per hectare was inadequate for maximum yields in the first trial, the rate was increased to 120 kg in the second trial.

Second Greenhouse Trial

From Tables 5 and 6 it can be seen that, in the second trial, vegetative development was again retarded by both straw and green manure in Lotung profile cultures of rice, as it was in the first trial. The retardation was greater when the amendments were incorporated two days before transplanting than when they were allowed to decompose in the flooded soil for two weeks before transplanting (Figs. 12, 14, 16, 18). This difference gradually diminished during later stages of growth (Figs. 13, 15, 17, 19). Final yields of grain and straw showed little benefit from the earlier incorporation (Table 7). Yields of grain

and straw were drastically reduced by both organic amendments, as compared with the unmanured check cultures.

Table 5. Height of rice plants in second greenhouse trial (first crop rice, Lotung profile).

Age of rice plant (days after transplanting)	No manure	Green Manure		Straw		$LSD_{0.5}$
		Incorporated before planting				
		2 days	2 weeks	2 days	2 weeks	
		cm.	cm	cm	cm	
25	34.9	26.4*	27.4*	23.8*	30.1*	3.01
50	65.1	56.1*	59.0*	51.7*	57.4*	7.58
75	73.4	69.5	70.0	65.2	65.6	6.59

*Significantly less than for no manure.

Table 6. Number of tillers per hill in second greenhouse trial (first crop rice, Lotung profile).

Age of rice plant (days after transplanting)	No manure	Green Manure		Straw		$LSD_{0.5}$
		Incorporated before planting				
		2 days	2 weeks	2 days	2 weeks	
		cm	cm	cm	cm	
25	7.8	5.1*	5.2*	5.0*	5.7*	1.17
50	16.6	16.0	15.9	13.1	13.4	N.S.
75	19.2	18.6	16.9	13.7*	13.7*	4.39
Efficient tillers	14.2	9.7*	10.1*	8.0*	9.0*	3.34

*Significantly less than for no manure.

Comparing yields in the second trial (Table 7) with those in the first (Figs. 2, 3), it is apparent that much more straw was produced in the second trial (for the unmanured check, 83 g vs. 24 g) and less grain (9 g vs. 18 g, again for the check treatment). The dominantly vegetative character of growth during the second trial may have been partly a seasonal effect. However, more nitrogen was applied and the cultures were grown to maturity under flooded conditions in the second trial, instead of being drained at the maximum tiller stage as in the first trial. It is likely that the continuously flooded condition was responsible for the fact that rice in green manured cultures failed to recover from the early retardation as completely as in the first trial.

Table 7. Weight of straw and number and weight of rice grains per hill in second greenhouse trial (first crop rice, Lotung profile).

		Green Manure		Straw		
		Incorporated before planting				
Measurement	No Manure	2 days	2 weeks	2 days	2 weeks	$LSD_{0.5}$
Grains per hill	322.33	278.77*	197.69*	289.95*	210.20*	5.23
Grains (gm)	8.83	4.54*	4.77*	2.56*	3.07*	3.39
Straw (gm)	82.75	65.65	67.36	45.69*	52.08*	21.50

*Significantly less than for no manure.

In the second trial, the symptoms of young seedlings were the same as occurred in the first trial and began to appear 2 days after transplanting in lupine-amended soil and 5 days after transplanting with straw. No symptoms developed in check soils.

In amended cultures, root injury and rolling and chlorosis of leaves became progressively worse through the stage of maximum tiller number. After that, leaves regained their green color and plants developed more or less normally, except that they remained stunted in comparison with check plants, and panicle formation, anthesis and maturity were greatly delayed.

Brown spots began to appear on the older leaves, spreading along the edges from the tip toward the base, after 43 days after transplanting (maximum tiller number stage). The brown spots gradually turned reddish and increased greatly after anthesis (84 days after transplanting). At this time, most of the older leaves were dead. The most severely affected plants were in the straw-treated boxes.

Soil oxidation-reduction measurements failed to show any differences between healthy unmanured cultures and those which were injured by straw or lupine green manure treatments. In the early stages of growth, the redox potential was below -400 mv. It increased as the rice grew older, reaching -250 mv at the time of panicle development and -150 to -100 mv at maturity. These potentials are well below the critical threshhold range of -6 to -100 mv found in association with "Akagare," a similar disorder of paddy rice in Japan (131).

FIGURES

Effects of Organic Amendments on Growth Characteristics and Yields

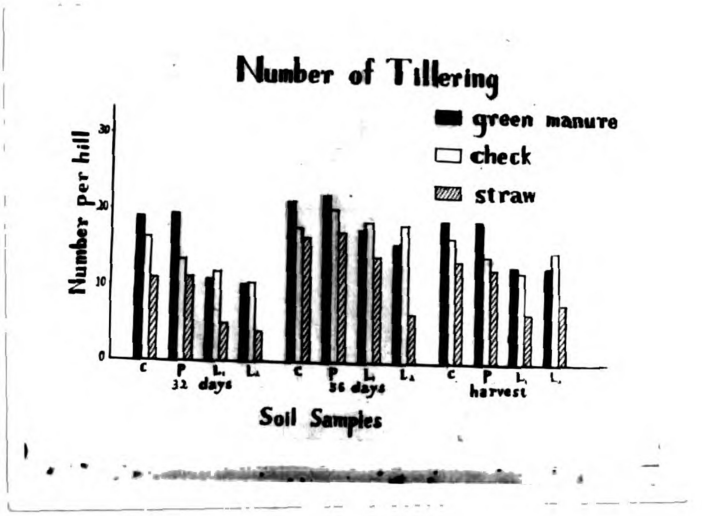

Fig. 1. Number of rice tillers on Chungli (c), Pingtung (P) and Lotung (L_1) surface soils and on the Lotung profile (L_2) at various times after transplanting. (Fall crop rice, greenhouse, 1964.)

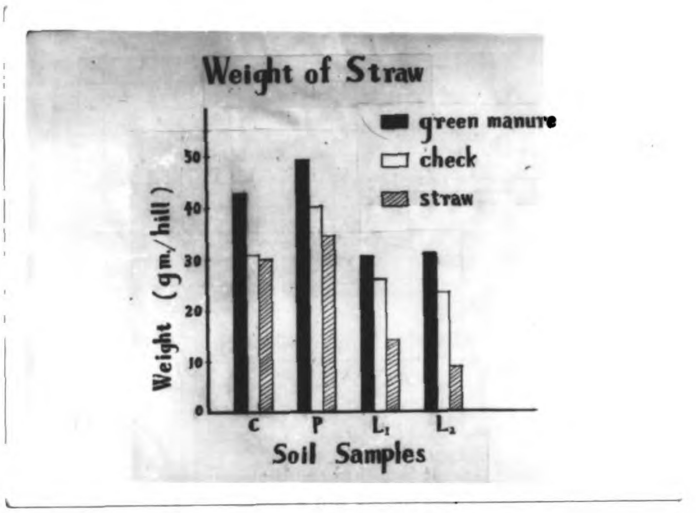

Fig. 2. Weight of rice straw on Chungli (c), Pingtung (P) and Lotung (L_1) surface soils and on the Lotung profile (L_2). (Fall crop rice, greenhouse, 1964.)

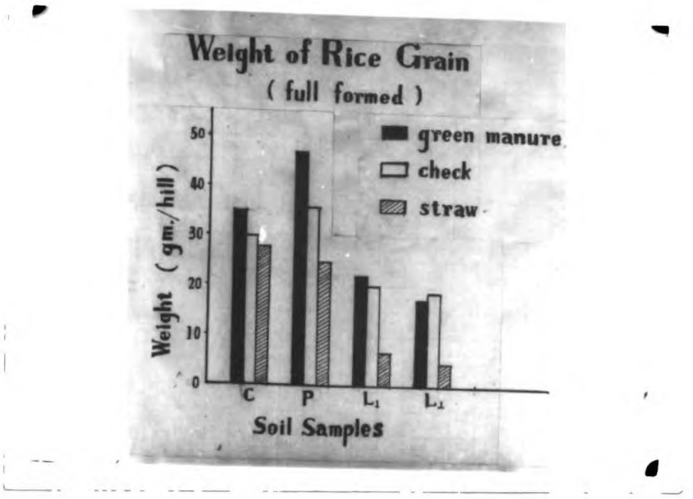

Fig. 3. Weight of rice grain on Chungli (c), Pingtung (P) and Lotung (L_1) surface soils and on the Lotung profile (L_2) at various times after transplanting. (Fall crop rice, greenhouse, 1964.)

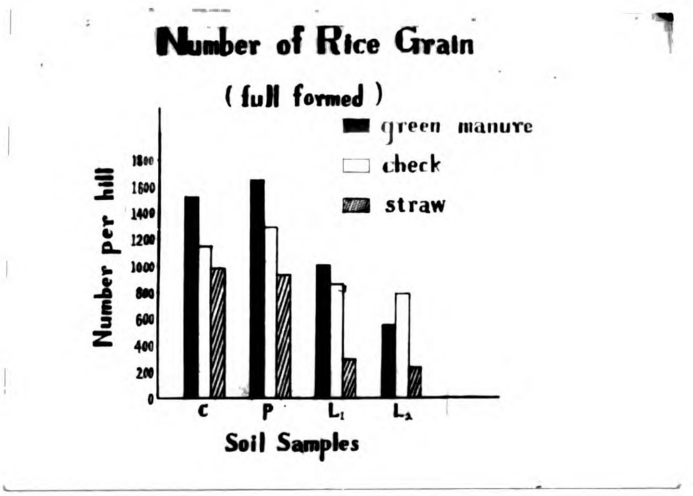

Fig. 4. Number of rice grains on Chungli (c), Pingtung (P) and Lotung (L_1), surface soils and on the Lotung profile (L_2). (Fall crop rice, greenhouse, 1964.)

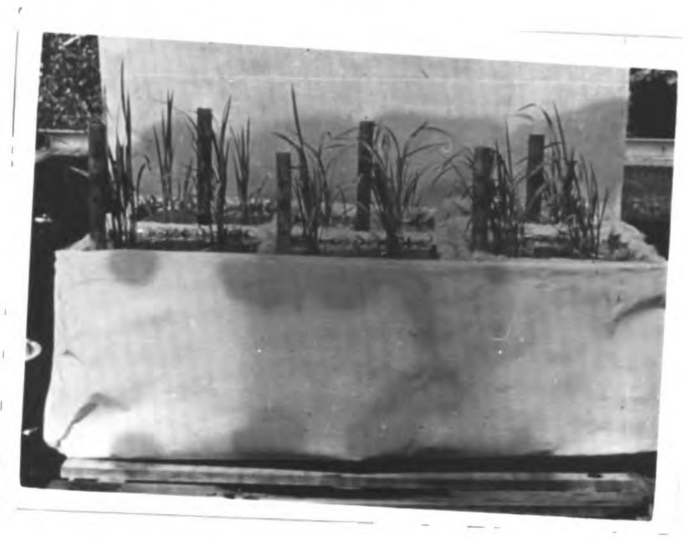

Fig. 5. Chungli surface soil, fall crop rice, 1964, 10 days after transplanting. Left, straw; right, green manure: center check.

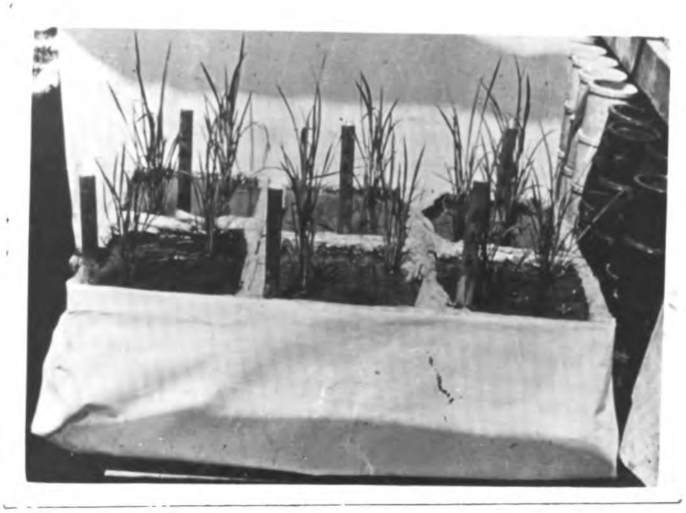

Fig. 6. Pingtung surface soil, fall crop rice, 1964, 10 days after transplanting. Front row: left, straw; right, green manure; center check.

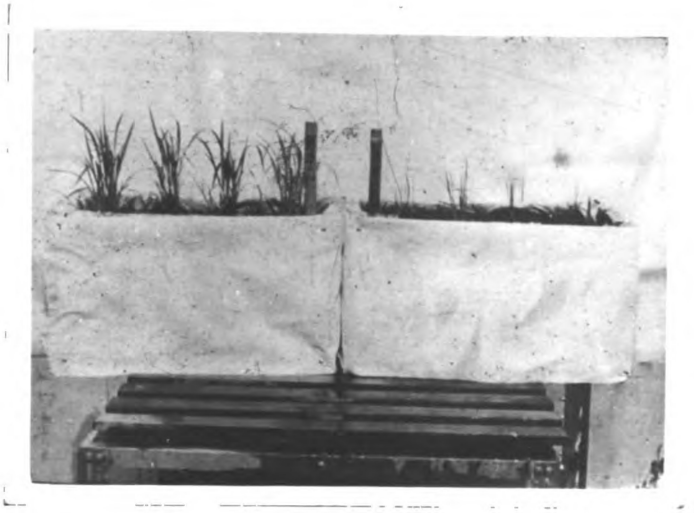

Fig. 7. Lotung profile, fall crop rice, 1964, 10 days after transplanting. Left, straw; right, green manure.

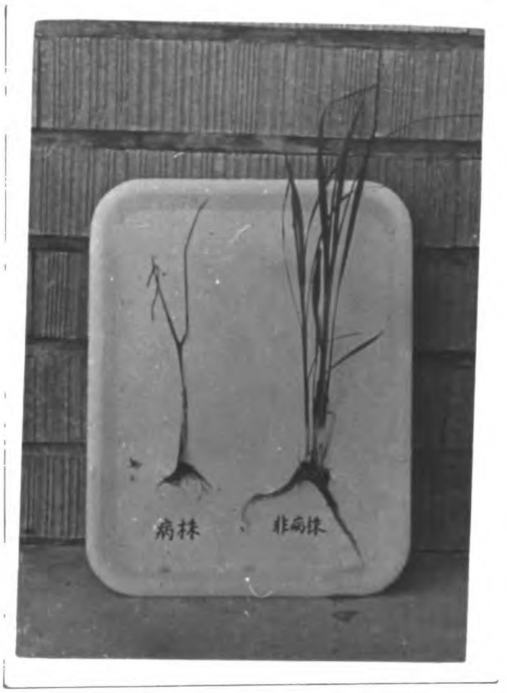

Fig. 8. Lotung profile, fall crop rice, 1964, 10 days after transplanting. Left, diseased plant; right, healthy plant.

Fig. 9. Lotung profile, fall crop rice, 1964, at harvest. Left, straw; right, green manure.

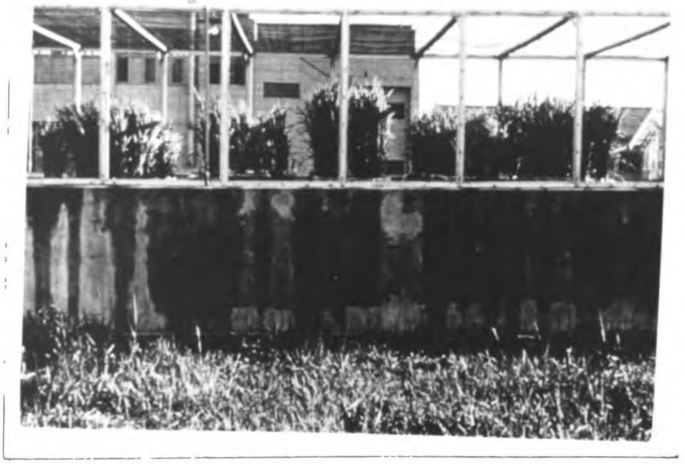

Fig. 10. Overall view, fall crop rice, 1964, at flowering. From left to right: Lotung surface, Lotung profile, Pingtung surface (check), Pingtung surface (manured), Chungli surface soil.

Fig. 11. Lotung profile, spring crop rice, 1965, 15 days after transplanting.

Fig. 12. Lotung profile, spring crop rice, 1965, 15 days after transplanting. Amended 2 days before transplanting with straw (left) and green manure (right). Unmanured check in center.

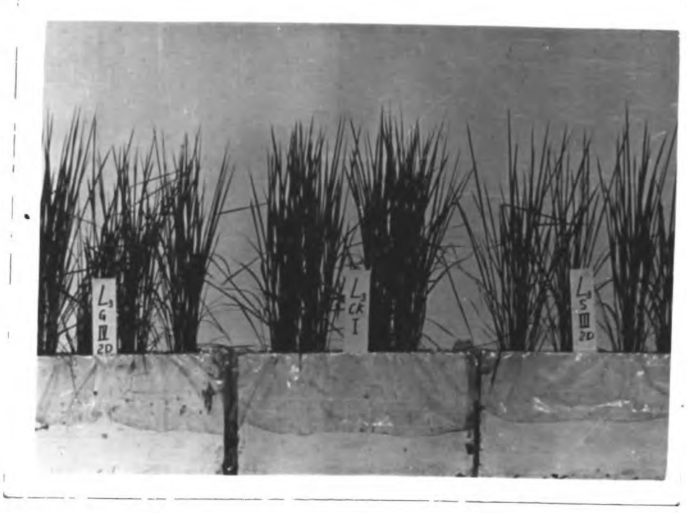

Fig. 13. Lotung profile, spring crop rice, 1965, 55 days after transplanting. Amended 2 days before transplanting with green manure (left) and straw (right). Unmanured check in center.

Fig. 14. Lotung profile, spring crop rice, 1965, 15 days after transplanting. Amended 2 weeks before transplanting with straw (left) and green manure (right). Unmanured check in center.

Fig. 15. Lotung profile, spring crop rice, 1965, 55 days after transplanting. Amended 2 weeks before transplanting with green manure (left) and straw (right). Unmanured check in center.

Fig. 16. Lotung profile, spring crop rice, 1965, 15 days after transplanting. Straw incorporated into surface soil 2 days (left) and 2 weeks (right) before transplanting. Unmanured check in center.

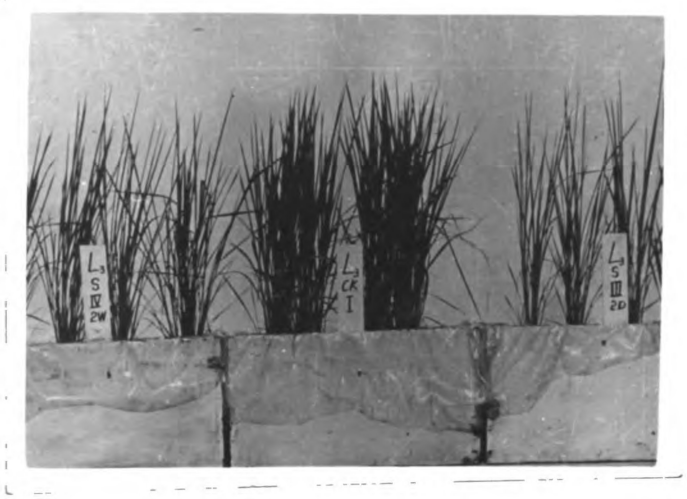

Fig. 17. Lotung profile, spring crop rice, 1965, 55 days after transplanting. Straw incorporated into surface soil 2 weeks (left) and 2 days (right) before transplanting. Unmanured check in center.

Fig. 18. Lotung profile, spring crop rice, 1965, 15 days after transplanting. Green manure (Lupines) incorporated into surface soil 2 weeks (left) and 2 days (right) before transplanting. Unmanured check in center.

Fig. 19. Lotung profile, spring crop rice, 1965, 45 days after transplanting. Green manure (Lupines) incorporated into surface soil 2 days (left) and 2 weeks (right) before transplanting. Unmanured check in center.

Fig. 20. Rice in nutrient solutions 25 days after inoculation with root washings from diseased rice grown in the Lotung profile amended with straw (left) and Lupines (right). Center three plants inoculated with root washings from healthy rice grown in the unamended Lotung profile.

Inoculation Experiment

Similar symptoms of physiological disorder were found when Lotung (L_2) profiles which had been treated with straw or Sesbania in the first trial were retreated with straw or lupines, respectively. When root washings from these retreated and replanted cultures were used to inoculate rice plants growing in nutrient solution, similar symptoms again developed.

Within one week after inoculation, the leaves began to turn yellow. The height of the plants was markedly reduced, in comparison with plants inoculated from replanted cultures on the Lotung profile which had not received organic amendments either in the fall or the spring. By the 22nd day after inoculation the leaves of the affected plants were completely yellow. The relative appearance of injured and uninjured plants at this time can be judged from Fig. 20.

Numbers of total bacteria and of sulfate reducers found in the inoculated nutrient solutions at the time when distinct disease symptoms were apparent are shown in Table 8.

Table 8. Numbers of total bacteria and sulfate reducers in nutrient solutions 10 days after inoculation with root washings from retreated and replanted rice cultures in Lotung profiles from the first greenhouse trial.

Microbial group	Source of inoculum		
	No Manure	Green Manure	Straw
Total bacteria x 10^6 per ml	11.9	20.4	23.0
Sulfate reducers x 10^3 per ml	23.0	54.0	35.0

Numbers of total bacteria were two-fold greater in nutrient solutions inoculated with root washings from amended cultures than from unamended checks. Sulfate reducers were also greater where inocula from amended cultures were used, but maximum numbers were associated with the green manure treatment.

When soil leachates from these same replanted Lotung profiles were used as inoculum, only mild symptoms developed in nutrient solution cultures and no estimates of microbial numbers were attempted.

Microflora Studies

Microbial numbers as determined for root surfaces (R) and for non-rhizosphere (S) samples are recorded for the two greenhouse trials and the three field locations in Tables 22 to 27 in the Appendix. Differences between edaphosphere soils (S) counts and rhizosphere soil (Rh) counts were not very great or very consistent. The rhizosphere counts (Rh) have been recorded only for the two field locations on the fall crop in 1964 (Tables 24, 25).

Microbial numbers on root surfaces (R) were frequently much greater than in either the rhizosphere or the edaphosphere. Fluctuations in numbers on root surfaces were frequently large and frequently appeared to be directly related to organic amendments in the greenhouse or the diseased condition of the plant in the field. For this reason, the root counts have been summarized in Tables 9 through 17.

Table 9. Numbers of bacteria on rice roots in fall crop (1964) and spring crop (1965).

Soil	Location	Year	Treatment or Plant Condition	Developmental Stage of Rice				
				Tiller Initiation	Maximum Tillers	Panicle Initiation	Anthesis	Maturity
				Numbers/g dry roots x 10^7				
Lotung profile	Green-house	1964	Check	34.0	39.8	20.8	17.5	15.9
			Gr. man.	103.7	65.0	43.8	21.8	19.0
			Straw	--	100.0	27.6	31.8	26.8
		1965	Check	9.7	21.0	2.2	2.0	2.1
			Gr. man.	80.9	12.0	1.4	1.4	1.3
			Straw	48.8	70.0	4.8	4.8	4.7
Lotung	Field	1964	Healthy	95.6	30.9	81.0	22.5	9.9
			Diseased	--	43.5	172.2	23.7	23.1
		1965	Healthy	2.9	47.2	6.6	5.3	4.8
			Diseased	2.3	14.9	14.1	13.6	12.5
Pingtung	Field	1964	Healthy	55.4	108.8	16.0	21.7	18.9
			Diseased	12.5	113.7	19.8	13.4	12.0

Table 10. Numbers of actinomycetes on rice roots in fall crop (1964) and spring crop (1965).

Soil	Location	Year	Treatment or Plant Condition	Stage of Development of Rice				
				Tiller Initiation	Maximum Tillers	Panicle Initiation	Anthesis	Maturity
				Numbers/g dry roots x 10^5				
Lotung profile	Green-house	1964	Check	13.3	72.0	6.4	17.1	16.3
			Gr. man.	10.1	60.2	12.6	5.2	11.2
			Straw	--	40.2	11.3	16.1	17.1
		1965	Check	82.7	60.1	6.5	6.0	5.6
			Gr. man.	251.5	18.6	7.6	7.2	7.6
			Straw	229.9	65.2	6.8	7.8	6.5
Lotung	Field	1964	Healthy	63.9	5.5	25.1	11.5	14.8
			Diseased	--	25.9	26.5	18.3	7.8
		1965	Healthy	99.3	21.2	8.5	7.6	6.8
			Diseased	42.8	33.8	15.0	15.3	14.2
Pingtung	Field	1964	Healthy	53.1	27.8	16.2	37.2	29.3
			Diseased	137.8	38.4	59.1	28.1	21.2

Table 11. Numbers of fungi on rice roots in fall crop (1964) and spring crop (1965).

Soil	Location	Year	Treatment or Plant Condition	Stage of Development of Rice				
				Tiller Initiation	Maximum Tillers	Panicle Initiation	Anthesis	Maturity
				Numbers/g dry roots x 10^3				
Lotung profile	Green-house	1964	Check	21.3	5.9	58.7	49.9	38.6
			Gr. man.	33.0	45.6	27.8	41.2	32.5
			Straw	--	195.3	9.4	25.8	25.8
		1965	Check	146.2	358.9	9.6	9.5	7.6
			Gr. man.	537.0	149.3	5.8	6.7	6.2
			Straw	486.0	495.6	4.3	5.3	4.7
Lotung	Field	1964	Healthy	175.9	3.9	5.0	355.6	4.9
			Diseased	--	--	11.8	84.0	5.8
		1965	Healthy	114.5	141.7	7.1	7.0	12.7
			Diseased	89.9	75.1	13.7	6.9	10.3
Pingtung	Field	1964	Healthy	17.7	85.0	54.0	49.0	32.0
			Diseased	37.4	63.1	6.7	31.4	28.7

Table 12. Numbers of anaerobic bacteria on roots of rice in fall crop (1964) and spring crop (1965).

Soil	Location	Year	Treatment or Plant Condition	Stage of Development of Rice				
				Tiller Initiation	Maximum Tillers	Panicle Initiation	Anthesis	Maturity
				Numbers/g dry roots x 10^5				
Lotung profile	Green-house	1964	Check	588.3	66.2	5.9	39.5	36.4
			Gr. man.	282.9	113.0	17.7	131.0	89.7
			Straw	--	114.8	6.6	39.5	78.6
		1965	Check	214.3	46.7	4.3	4.7	4.0
			Gr. man.	233.4	14.9	5.1	5.6	4.6
			Straw	383.0	41.7	8.2	8.9	5.6
Lotung	Field	1964	Healthy	454.2	25.3	136.7	13.1	13.4
			Diseased	--	131.3	537.9	7.2	20.4
		1965	Healthy	23.3	19.8	8.5	7.6	6.9
			Diseased	74.4	8.7	26.0	23.8	22.6
Pingtung	Field	1964	Healthy	17.7	29.4	62.0	28.2	25.0
			Diseased	8.7	90.6	18.9	14.7	13.2

Table 13. Numbers of sulfate reducing bacteria on rice roots in the spring crop (1965).

Soil	Location	Year	Treatment or Plant Condition	Developmental Stage of Rice				
				Tiller Initiation	Maximum Tillers	Panicle Initiation	Anthesis	Maturity
				Numbers/g dry root x 10^3				
Lotung profile	Green-house	1965	Check	--	7.9	7.0	6.8	6.0
			Gr. man.	1.7	70.0	22.0	17.0	17.0
			Straw	21.0	14.0	22.0	16.0	17.0
Lotung	Field	1965	Healthy	3.3	4.6	7.0	6.8	6.2
			Diseased	14.0	4.8	24.0	23.0	17.0

Table 14. Numbers of aerobic cellulose decomposers on rice roots in fall crop (1964) and spring crop (1965).

Soil	Location	Year	Treatment or Plant Condition	Stage of Development of Rice				
				Tiller Initiation	Maximum Tillers	Panicle Initiation	Anthesis	Maturity
				Numbers/g dry roots x 10^6				
Lotung profile	Green-house	1964	Check	512.3	74.7	11.2	17.5	16.5
			Gr. man.	1090.0	66.7	35.8	30.3	25.4
			Straw	--	229.9	15.6	23.6	21.3
		1965	Check	3.6	5.3	2.4	2.1	2.2
			Gr. man	82.6	5.5	3.0	2.9	2.9
			Straw	84.3	25.8	4.3	4.3	4.0
Lotung	Field	1964	Healthy	268.4	220.0	529.4	45.4	9.1
			Diseased	--	316.4	884.7	8.8	15.1
		1965	Healthy	36.6	7.5	3.4	3.6	3.4
			Diseased	54.8	5.6	21.8	20.8	19.9
Pingtung	Field	1964	Healthy	28.0	110.8	42.5	13.7	12.7
			Diseased	12.9	332.2	27.7	11.6	9.9

Table 15. Numbers of anaerobic cellulose decomposers on rice roots in fall crop (1964) and spring crop (1965).

Soil	Location	Year	Treatment or Plant Condition	Stage of Development of Rice				
				Tiller Initiation	Maximum Tillers	Panicle Initiation	Anthesis	Maturity
				Numbers/g dry roots x 10^6				
Lotung profile	Green-house	1964	Check	42.7	39.1	2.1	10.1	9.0
			Gr. man.	65.0	24.1	5.3	4.7	4.6
			Straw	--	105.7	2.6	6.3	5.9
		1965	Check	38.5	3.1	5.8	5.7	5.3
			Gr. man.	60.2	1.5	1.4	1.0	1.1
			Straw	72.8	27.7	4.1	3.7	3.8
Lotung	Field	1964	Healthy	183.9	10.8	28.1	7.6	2.6
			Diseased	--	25.4	33.5	6.9	2.8
		1965	Healthy	17.2	12.7	4.5	4.4	4.2
			Diseased	17.4	8.7	10.6	10.0	7.9
Pingtung	Field	1964	Healthy	22.4	47.3	30.7	6.2	5.9
			Diseased	97.7	102.6	34.0	1.7	2.7

Table 16. Numbers of denitrifying bacteria on rice roots in fall crop (1964) and spring crop (1965).

Soil	Location	Year	Treatment or Plant Condition	Developmental Stage of Rice				
				Tiller Initiation	Maximum Tillers	Panicle Initiation	Anthesis	Maturity
				Numbers/g dry roots x 10^6				
Lotung profile	Green-house	1964	Check	4.3	5.4	9.5	16000.0	16000.0
			Gr. man.	16.0	11.0	5.4	16000.0	16000.0
			Straw	--	3.5	9.5	69.0	16000.0
		1965	Check	1.3	8.0	11.1	9.5	3.7
			Gr. man.	1.4	1600.0	16.0	46.0	16.0
			Straw	5.4	180.0	3.7	31.0	5.6
Lotung	Field	1964	Healthy	0.1	9.2	9.2	5.8	11.0
			Diseased	--	9.2	16.0	28.0	22.0
		1965	Healthy	0.5	1.7	--	16.0	9.5
			Diseased	3.5	2.1	--	46.0	12.0
Pingtung	Field	1964	Healthy	0.2	7.0	9.5	7.5	2.1
			Diseased	0.3	1.6	16.0	320.0	2.8

Table 17. Numbers of ammonifiers on rice roots in fall crop (1964) and spring crop (1965).

Soil	Location	Year	Treatment or Plant Condition	Developmental Stage of Rice				
				Tiller Initiation	Maximum Tillers	Panicle Initiation	Anthesis	Maturity
				Numbers/g dry roots x 10^5				
Lotung profile	Green-house	1964	Check	24.0	9.2	13.0	40.0	54.0
			Gr. man.	7.0	13.0	17.0	32.0	43.0
			Straw	--	2.4	7.9	19.2	17.0
		1965	Check	1.4	11.0	18.0	11.0	1.7
			Gr.man.	2.6	28.0	35.0	27.0	16.0
			Straw	7.9	49.0	22.0	22.0	17.0
Lotung	Field	1964	Healthy	0.7	13.0	14.0	54.0	5.4
			Diseased	--	28.0	4.0	14.0	17.0
		1965	Healthy	1.7	2.5	2.4	1.7	1.7
			Diseased	1.7	9.5	3.3	3.1	2.5
Pingtung	Field	1964	Healthy	1.1	35.0	16.0	0.9	14.0
			Diseased	1.4	9.2	54.0	7.0	13.0

Major Taxonomic Groups

Bacteria and actinomycetes on root surfaces were frequently high during early vegetative growth through maximum tiller number and then tended to decline to stable low numbers (Tables 9, 10). In the greenhouse experiments, numbers shortly after transplanting were usually distinctly higher where energy materials had been added as green manure or straw. In the field samples, there appeared to be no consistent relationship to diseased or healthy plants.

Fungi also increased in response to organic amendments during the period from tiller initiation to maximum tiller number (Table 11). Later, numbers were consistently lower in amended cultures than in the checks. In the field, fungi were frequently more numerous on roots of healthy than diseased plants.

Physiological Groups

1. Anaerobic bacteria

Variations in numbers of anaerobic bacteria (Table 12) showed a similar tendency to decline from high numbers early to lower numbers late in the season as was seen with total bacteria (Table 9). However, in both fall and spring crops at the Lotung field locations, numbers were consistently higher on diseased roots. In the 1965 greenhouse experiment, it appeared that the anaerobes may have responded to the straw amendment at the time of the first

sampling. In general, however, the anaerobes did not reflect effects of added energy materials as clearly as the three major aerobic groups above.

2. Sulfate reducers

Sulfate reducers were counted only on the spring crop (Table 13). In both the greenhouse and the field, numbers were consistently higher on roots of plants showing disease symptoms than of check or healthy plants, although the differences were not great.

3. Cellulose decomposers

Both aerobic and anaerobic cellulose decomposers (Tables 14, 15) responded to additions of green manure or straw. Except for one field location (Lotung 1965), numbers were rather consistently higher on roots of diseased plants in the field and of amended cultures in the greenhouse during the early vegetative period through maximum tiller number or panicle initiation.

4. Denitrifying bacteria

Of all microbial groups studied, the denitrifiers showed the most extreme fluctuations in numbers, both on root surfaces (Table 16) and in the rhizosphere (Tables 24, 25). The seasonal pattern was related inversely to that observed in most groups considered in above sections. Numbers were low in the beginning and reached maximum

numbers later, usually in the late stages of anthesis or maturation.

In the 1964 greenhouse experiment, a dramatic increase in numbers at the end of the season occurred later with straw than either the checks or green manured cultures. In the 1965 experiment and at all field locations, however, this group was rather consistently higher on roots of amended cultures and diseased plants from the late vegetative or early flowering period through the remainder of the season.

5. Ammonifying bacteria

Root populations of ammonifiers (Table 17) showed only moderate fluctuations during the season. There was a tendency, as with the denitrifiers, to start from low numbers and rise to a maximum at some later stage of development. However, there was no consistent relationship to organic amendment or condition of plant, except in the 1965 greenhouse experiment where larger numbers were maintained in amended cultures from the maximum tiller stage to maturity.

Pure Culture Studies

Woldendorp (170), using broth cultures, found that his collection of denitrifying bacteria could be divided into two groups on the basis of whether they could or could not grow anaerobically in the absence of nitrate. Additional

distinctions could be made within each of these groups on the basis of whether or not amino acids or vitamins were required for growth and/or gas production. His observations were, in general, consistent with those of Valera and Alexander (149).

Pure culture studies were undertaken with 18 species and strains with a view to developing solid media for differential enumeration and isolation of physiological groups among the denitrifiers. The chemically defined media of Woldendorp were used, with slight modifications (see Table 4). Buffer concentration and the concentration of Ca and Mg were reduced to avoid precipitation of phosphates and iron. The vitamin component was augmented by addition of inositol and Vitamin B_{12} as in media described by Chase and Lochhead (78). Plates were streaked using loop inoculations taken directly from heavy log-phase suspensions in nutrient broth.

Aerobic growth

Visual estimates of growth under aerobic conditions are shown in Table 18. In the absence of growth factors (medium 103), growth of most organisms was restricted, as compared with more complete media. On the basis of Woldendorp's data, no growth would have been expected on glucose with nitrate in the absence of growth factors in the case of organisms 3, 5, 7, 9, 10, 11, 12, 13 or 15. The fact that slight, slow growth did occur with a number of

Table 18. Aerobic growth of bacterial agar streak cultures in relation to source of nitrogen and growth factors.

Organism	Medium number and composition						
Component	103		104	105	106	111	
Glucose	+		+	+	+	+	
Minerals	+		+	+	+	+	
Nitrate	+		+	+	-	+	
Asparagine	-		-	-	+	+	
Am. acids	-		+	+	+	+	
Vitamins	-		-	+	+	+	
Growth period	1 day	5 days	7 days	7 days	7 days	1 day	5 days
1. Ps. aeruginosa ATCC 10145 *	3	4	3	3	4	4	5
2. " " Woldendorp	3	4	3	2	4	4	5
3. " stutzeri "	0	2	3	3	4	2	4
4. S. marcescens MSU-MPH	0	3	3	3	4	4	5
5. B. laterosporus 468 B(W)	1/2	1	3	3	4	2	3
6. " licheniformis 430(W)	1/2	1	---	---	---	3	3
7. " coagulans 1963 II(W)	2	3	3	3	4	4	4
8. " licheniformis P_1(W)	0	2	3	3	4	3	3
9. " cereus ATCC 6464	0	1/2	---	---	---	4	4
10. " " " 14579	0	1/2	---	---	---	4	4
11. " macerans ATCC 843	0	1/2	---	---	---	0	1
12. " " ATCC 8244	0	1	---	---	---	0	2
13. " circulans ATCC 4513	0	1	---	---	---	1	2
14. " licheniformis ATCC 14580	1	2	---	---	---	4	4
15. A. hartlebii ATCC 365	0	1/2	---	---	---	0	2
16. M. denitrificans ATCC 13543	2	4	---	---	---	2	4
17. Ps. fluorescens ATCC 11250	2	3	---	---	---	3	3
18. " denitrificans ATCC 13867	1	2	---	---	---	2	3

*Growth at 28°C estimated visually on a scale of 0 to 5.

Table 19. Anaerobic growth of bacterial agar streak cultures in relation to source of nitrogen, growth factors and CO_2.

Organisms	Medium composition and number					
Component	107		109		110	
Glucose	+		+		+	
Minerals	+		+		+	
Thioglycollate	+		+		+	
Nitrate	+		+		+	
Asparagine	-		-		+	
Am. acids	-		+		+	
Vitamins	-		+		+	
Atmosphere	H_2	H_2 plus 10% CO_2	H_2	H_2 plus 10% CO_2	H_2	H_2 plus 10% CO_2
	*	*	*	*	*	*
1. Ps. aeruginosa ATCC 10145	3—4	3—4	4—4	4—4	2—3	2—3
2. " " Woldendorp	3—4	3—4	4—4	4—4	2—4	2—4
3. " stutzeri "	1—3	1—3	4—4	2—3	2—3	1—1
4. S. marcescens MSU-MPH	3—4	2—4	4—4	4—4	4—4	2—4
5. B. laterosporus 468 B(W)	1/2-4	1/2-4	2—4	2—4	1—2	1—2
6. " licheniformis 430(W)	1—2	1—2	3—4	3—0	2—2	2—3
7. " coagulans 1963 II(W)	3—3	2—4	4—4	4—3	2—2	3—3
8. " licheniformis P_1(W)	1—3	1—4	3—4	3—4	2—3	3—3
9. " cereus ATCC 6464	1—1	1—2	3—4	2—4	2—2	2—3
10. " " ATCC 14579	1—2	1—2	2—4	2—3	1—2	2—2
11. " macerans ATCC 843	0—2	0—1	0—2	0—1	0—2	0—1
12. " " ATCC 8244	1—3	1—2	2—3	2—2	1—3	1—3
13. " circulans ATCC 4513	1—3	1—2	2—3	2—2	2—2	2—1
14. " licheniformis ATCC 14580	2—3	1—3	2—4	3—4	2—3	3—4
15. A. hartlebii ATCC 365	0-1/2	0—0	0—1	0—0	0—0	0—0
16. M. denitrificans ATCC 13543	4—4	3—4	4—4	4—4	2—3	2—2
17. Ps. fluorescens ATCC 11250	0—0	1-1/2	1-1/2	2-1/2	1—1	2—2
18. " dinitrificans ATCC 13867	1—2	1—3	3—2	3—3	2—3	3—4

*Growth in 3 — 13 days at 28°C estimated visually on a scale of 0 to 4.

Table 20. Anaerobic growth of bacterial agar streak cultures in relation to source of energy, nitrate, growth factors and CO_2.

Organism	Component	110		111		112		113	
	Glucose	+		+		+		-	
	Citrate	-		-		-		+	
	Minerals	+		+		+		+	
	Thioglycollate	+		+		+	=	+	
	Nitrate	-		+		+		+	
	Asparagine	+		+		+		+	
	Am. acids	+		+		-		-	
	Vitamins	+		+		-		-	
	Yeast extract	-		-		+		+	
	Atmosphere*	H_2	CO_2	H_2	CO_2	H_2	CO_2	H_2	CO_2
		**	**	**	**	**	**	**	**
1.	Ps. aeruginosa ATCC 10145	1	3	2	3	3	4	2	2
2.	" " Woldendorp	1	5	2	4	3	6	2	3
3.	" stutzeri "	1	0	2	0	3	2	1	1
4.	S. marcescens MSU-MPH	1	2	3	2	3	3	2	1
5.	B. laterosporus 468B(W)	1	1	3	1	3	2	0	0
6.	" licheniformis 430(W)	2	3	3	2	3	3	2	0
7.	" coagulans 1963 II(W)	3	4	3	3	3	4	2	1
8.	" licheniformis P_1(W)	3	4	3	2	3	4	2	0
9.	" cereus ATCC 6464	2	2	3	2	3	2	2	0
10.	" " ATCC 14579	2	2	3	3	4	4	2	1
11.	" macerans ATCC 843	-	-	-	-	-	-	-	-
12.	" " ATCC 8244	3	2	2	2	2	3	2	2
13.	" circulans ATCC 4513	2	3	2	2	2	3	1	0
14.	" licheniformis ATCC 14580	3	4	4	3	5	5	2	1
15.	A. hartlebii ATCC 365	0	0	0	0	2	0	0	0
16.	M. denitrificans ATCC 13543	3	0	3	0	4	0	4	0
17.	Ps. fluorescens ATCC 11250	0	3	3	0	4	0	0	0
18.	" denitrificans ATCC 13867	3	5	3	3	4	6	3	2

*100 per cent H_2 or 100 per cent CO_2.

**Growth in 15 days at 28°C, estimated visually on a scale of 0 to 6.

these probably reflects a carry-over of growth factors in the inoculum.

The rather extensive growth of organism 7 is unexplained. Physiological tests on milk and starch and for production of indole and acetylmethylcarbinol (Vosges-Proskaur test) corresponded to the Bergey's Manual description of Bacillus coagulans.

Anaerobic growth

A major difficulty in anaerobic studies was the complete elimination of traces of oxygen at the beginning of incubation. Traces of oxygen can permit initiation of growth and adaptation to anaerobic conditions in the case of some facultative anaerobes (149). Media were reduced chemically by storage in H_2 prior to streaking. Sodium thioglycollate was added to minimize oxidation during the time that plates were necessarily exposed to air while being streaked.

Data in Table 19 indicate that these precautions were inadequate. In the absence of nitrate (medium 110), no growth would have been expected for organisms 1, 2, 3, 16 or 18 (170). In the absence of growth factors (medium 107), no growth would have been expected for organisms 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15. Limited growth was observed in all of these, and rather extensive growth in some, especially after 13 days. Plates were exposed to air when the observations were made on the

third day, and oxygen absorbed during the observation period may have stimulated growth during the following 10-day incubation under anaerobic conditions. Rapid growth of the two Pseudomonas aeruginosa strains was actually observed during the 90-minute observation period.

As can be seen in Table 19, the introduction of 10 per cent CO_2 into the H_2 atmosphere had little effect on growth of most cultures. In the case of organism 6, an apparent lysis of cells occurred with CO_2 on medium 109, resulting in completely transparent zones after 13 days where vigorous growth had been observed at 3 days.

In a subsequent experiment (Table 20), additional precautions were taken to minimize the length of time that media were exposed to air during inoculation. The incubators were not opened during a 15-day incubation period. In one incubator, after reducing conditions had been established under H_2, the H_2 was completely replaced with CO_2.

Under these conditions, complete suppression of growth of several cultures was achieved. However, unexpected responses to CO_2 were expressed.

The two Ps. aeruginosa strains (1 and 2) were restricted in growth in the absence of nitrate (medium 110) in the H_2 atmosphere but grew profusely in CO_2. With strain 2, this response to CO_2 was expressed also in the presence of nitrate, without regard to source of energy or growth factors (media 111, 112, 113). In the absence of nitrate,

growth of organisms 17 and 18 was also enhanced by CO_2.

Growth of Ps. stutzeri was completely inhibited by CO_2 in the completely defined media 110 and 111, but not when yeast extract was used as a source of growth factors (medium 112). Micrococcus denitrificans (# 16) was completely inhibited by CO_2 on all media. With Ps. fluorescens (# 17) a similar CO_2 inhibition occurred only in media containing nitrate (media 111 or 112).

Yeast extract was a better source of growth factors for several organisms growing on glucose (medium 112) than the defined vitamin and amino acid mixture (medium 111). For most organisms, citrate supplied as energy source in medium 113 was used less readily than glucose (medium 112).

Antagonistic relationships

Woldendorp (170) found that Ps. aeruginosa strains were the dominant denitrifying organisms in rhizospheres of forage grasses. It appeared of interest to know whether this might be due to antagonistic effects on other denitrifying species. Accordingly, cross-streak inoculations were made using organism # 1 as the test organism. Other organisms were streaked at right angles to the test organism, taking care to avoid direct contamination of the streaked inocula.

Under aerobic conditions (Table 21), a number of organisms were markedly inhibited in the vicinity of the test strain when no growth factors were present (medium 103).

Table 21. Growth inhibition by Pseudomonas aeruginosa ATCC 10145 under aerobic conditions on cross-streaked agar plates.

Organism	Medium composition and number	
	Component	103	111
	Glucose	+	+
	Minerals	+	+
	Nitrate	+	+
	Asparagine	-	+
	Am. acids	-	+
	Vitamins	-	+
		mm*	mm*
2.	Ps. aeruginosa Woldendorp	0	0
3.	" stutzeri "	4	3
4.	S. marcescens MSU-MPH	8	0
5.	B. laterosporus 468B(W)	0	8
6.	" licheniformis 430(W)	8	0
7.	" coagulans 1963II(W)	8	0
8.	" licheniformis P_1(W)	8	0
9.	" cereus ATCC 6464	0	0
10.	" " ATCC 14579	0	0
11.	" macerans ATCC 843	4	4
12.	" " ATCC 8244	0	0
13.	" circulans ATCC 4513	0	0
14.	" licheniformis ATCC 14580	8	0
15.	A. hartlebii ATCC 365	0	0
16.	M. denitrificans ATCC 13543	6	2
17.	Ps. fluorescens ATCC 11250	2	2
18.	" denitrificans ATCC 13867	10	2

*Length of inhibition zone.

This suppression of growth was less frequently expressed in the complete medium 111. In the case of B. laterosporus (strain # 5), growth was inhibited only in the complete medium.

Antagonistic growth inhibition by Ps. aeruginosa under aerobic conditions was associated with the production of a blue-green pigment in the absence of growth factors and asparagine. When growth factors were present, a yellow-green pigment was produced. The production of the yellow-green pigment became apparent with the addition of amino acids, was increased when vitamins were also added, and was at a maximum in complete media containing asparagine and growth factors supplied either as yeast extract or as amino acid plus vitamin mixtures.

Under anaerobic conditions no pigments were produced by Ps. aeruginosa strains. However, they appeared within a few hours after cultures were removed from anaerobic conditions into the air. This post-incubation development of pigment was greater in media supplying glucose, rather than citrate, as carbon source.

Under anaerobic conditions, Ps. aeruginosa (strain 1) markedly inhibited the growth of B. macerans (strains 11 and 12) and of M. denitrificans (strain 16). Under anaerobic conditions, also, the ATCC strain (strain 1) showed a consistent, though mild, dominance over Woldendorp's strain (strain 2). No other antagonisms were observed under

anaerobic conditions.

The characteristic bright red pigment of Serratia marcescens was also produced only under aerobic conditions. This was true also of a dull reddish brown pigment produced by the B. licheniformis strains (numbers 6, 8, 14) on nutritionally rich media. Neither of these genera, however, showed any antagonistic effects on other strains when they were used as test organisms, instead of Ps. aeruginosa.

DISCUSSION

The present investigations were undertaken on the proposition that the study of rhizosphere microorganisms is one useful means of approach in obtaining a better knowledge of the physiological activity of the root system of rice plants and of factors concerned with such practical problems as evaluating a given soil treatment.

Research by numerous investigators has developed the view that, in a constant soil environment, a restricted but dynamic equilibrium is established among components of the microbial population. Any change in this environment--produced by soil treatments, season or growing crop--will shift the microbial balance.

Seasonal Behavior of Microbial Groups

The present biological studies have shown that there can be great divergences in seasonal behavior of different microbial groups associated with the development of rice and/or the decomposition of added organic materials. Numbers of fungi and of total and anaerobic bacteria were usually much higher on root surfaces than either the rhizosphere or the edaphosphere during the vegetative and early flowering stages of rice (Tables 22 to 27). This was

true also of both aerobic and anaerobic cellulose decomposers. Numbers in these groups were usually enhanced by organic amendments and showed a seasonal decline from large numbers during the tillering and panicle initiation stages to low numbers unrelated to organic addition at anthesis and maturity (Tables 9, 11, 12, 13, 14).

Actinomycetes usually showed no relation to organic amendments but did decline in numbers after maximum tillering or panicle initiation (Table 10). Only occasionally were there any marked differences in numbers between root surfaces and rhizosphere or edaphosphere (Tables 22 to 27).

The behavior of the above groups is consistent with results reported by others for upland crops (129, 144) and for paddy rice (52, 116). The data indicate that the size of the general microflora is largely determined by the supply of readily available energy materials. These energy sources may be in the form of residues from a previous crop, amendments from extraneous sources, or from exudates or abraded debris from roots of a growing crop.

It is of interest that both aerobic and anaerobic types were able to respond to additions of organic matter, even under flooded conditions and in the strongly reductive environment observed in the greenhouse (E_h =-100 to -400 mv). It is likely that rice roots may have helped to supply oxygen for growth of aerobes, particulary during earlier stages of growth. This is supported by the fact

that aerobic cellulose decomposers declined sharply in numbers after the tillering stage under continuous flooding in the second greenhouse trial but not when soil was drained at this time in the first greenhouse experiment (Table 13).

The distribution and seasonal behavior of the other three microbial groups was less well defined. In the case of sulfate reducers and ammonifiers,[1] there appeared to be no consistent relationship to their position in the root zone, so that differences between root surface, rhizosphere or edaphosphere were not great (Tables 22 to 27). Seasonal variations in these two groups were also small. The greatest numbers of sulfate reducers tended to come at the beginning of the growth period, whereas larger numbers of ammonifiers appeared at some later time.

With the denitrifiers, extreme seasonal fluctuations in numbers were observed. These occurred mainly in the root surface population or sometimes in the rhizosphere. Variations in the edaphosphere from sampling to sampling were relatively small.

Thus, the sulfate reducers, denitrifiers and ammonifiers appeared to be much less influenced by organic amendments than the other groups. The sulfate reducers and ammonifiers appeared to be influenced relatively little by nearness to the root surface. By contrast, the denitrifiers

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[1] A more specific term for "ammonifiers" as estimated in this study would be "urea hydrolyzers."

appeared to have been extremely sensitive to influences arising from the growing rice plants by way of their root systems. Among anaerobic groups, the denitrifiers were usually less numerous than cellulose decomposers in earlier samplings (Tables 12, 15). However, they were capable of increasing dramatically to become the dominant anaerobic group in later samplings.

Relationships between Microflora and Disease Symptoms

During the tillering period when disease symptoms were developing to peak intensity, total and anaerobic bacteria, fungi and cellulose decomposers were at their highest numbers for the season. In the greenhouse experiments, these groups were more numerous in the amended cultures. These were also the cultures in which disease symptoms developed on the Lotung profiles. At field locations also, these microbial groups were, at times, higher on diseased roots than healthy ones during the tillering period.

It is difficult to assign a cause and effect relationship to these associated phenomena. It is likely that these saprophytic types were drawing their main energy supplies from added organic materials and from residues from the previous crop. Root exudates from the growing rice would have stimulated growth of these organisms by supplying growth factors, such as amino acids. The fact that numbers were greater on diseased

roots in the field probably reflects more rapid release of growth factors, as well as energy substrates, from roots injured by other mechanisms associated with depletion of oxygen and reduction of alternate electron acceptors, such as ferric iron and manganic manganese.

There is the possibility that the above groups may have included species which produced toxins injurious to the rice. However, it appears likely that their major role was to deplete oxygen and bring about highly reduced soil conditions. When redox potentials were measured in the second greenhouse trial, E_h was less than -400 mv at the beginning of the tillering period and had increased only to -250 mv by panicle initiation. Bormer (101) and Kononova (63) have expressed the opinion that competition between higher plants and microorganisms for a limited supply of oxygen may be more important than any other factor in plant injury associated with organic manuring.

A second role of these main saprophytic groups would have been to deplete nutrients, notably nitrogen (31, 95). Nitrogen deficiency may have limited rice yields with the straw treatment in the first greenhouse trial.

A more direct role in promoting disease symptoms might be inferred for the sulfate reducers. These were somewhat more numerous in amended soil during the tillering period and throughout the season in the second greenhouse trial (Table 13). They were consistently slightly higher

on diseased than healthy roots in the field.

It is known that H_2S may inhibit cytochrome oxidase systems and photosynthetic activity of plant leaves (173). When Takahashi visited Taiwan in 1961, he noted the similarity between "suffocation disease" and "Akagare" which in Japan is found on soils in which there is insufficient iron to precipitate sulfide. Ferrous iron forms the complex $nK_2SO_4 \cdot mFeSO_4$, thereby reducing the toxicity of H_2S (22). However, the problem Lotung soil has been examined by Takahashi (132) Ponnamperuma (106) and Chang (23) and found to be high in ferrous iron. Thus, the small differences observed in numbers of sulfate reducing bacteria are probably of little significance as far as the associated disease symptoms are concerned.

Of all the groups studied, the denitrifiers appeared to be the most sensitive to the influence of the growing plant, as evidenced by extreme seasonal fluctuations in numbers on the root surface and/or rhizosphere, with relatively minor fluctuations in the edaphosphere. In both greenhouse experiments, this group was more numerous during the tillering period on roots of the green manured cultures where symptoms first appeared (Table 15). In the second trial, without drainage, higher numbers were maintained in amended cultures throughout the season. At field locations denitrifiers were rather consistently higher on diseased than healthy roots.

Again, cause and effect cannot be assigned to these associations. It must be assumed that denitrifiers were present in larger numbers on diseased roots because nutritional conditions were more favorable. Whether they contributed to the diseased condition of the plant by toxin production or some other mechanism cannot be said.

It is known that a number of nitrate reducing bacteria produce antibiotics and lytic enzymes. These may have toxic effects on plants, as in the case of tobacco "wildfire," which is caused by a toxin produced by Pseudomonas tabaci (124).

In the nutritional studies reported here, it was observed that the color of pigments produced by Pseudomonas aeruginosa were different in media with and without growth factors, and that the spectrum of antagonism was also different. Thus, a change in composition of root exudates associated with plant nutrition might bring about a change from a toxic to a non-toxic product of the root surface microflora.

The denitrifiers were enumerated on the basis of gas production in asparagine-nitrate-citrate broth. It was assumed that the gas was N_2 or N_2O, and that the organisms responsible were capable of denitrification. It cannot be assumed that, in the soil environment, these organisms were actually maintained on a nitrate respiration. However, if they were, a new problem arises as to where the nitrate came from.

It has been shown that rice has a root structure which permits oxygen to diffuse to the roots and to oxygenate the environment in the immediate vicinity of the roots (131). This would permit a nitrifying population to remain active under flooded conditions on the root surface or in the rhizosphere. An active nitrifying population would be necessary to maintain an actively denitrifying population. The nitrifying flora of flooded rice rhizospheres does not appear to have been studied.

Although a specific microbial agent responsible for the symptoms of the "suffocation disease" has not been identified, the transfer of symptoms from diseased to healthy rice by root washings was shown in the inoculation experiment. This experiment makes it quite clear that microbial agents are involved in some way.

Even if a microbial agent, or agents, are found, it is unlikely that they will be primary agents in a pathogenic sense. Rather, their presence will reflect a degree of nutritional imbalance in the plant arising from highly reductive soil conditions. According to Yanagiswawa and Takahashi (173), low productivity in paddy soils is frequently attributable to deficiencies of nitrogen, potassium, phosphorus, or silica and, at later stages of growth, to toxic substances produced in the soil. Chiu (26) found that diseased rice on the problem Lotung soil in eastern Taiwan contained more nitrogen and less potassium

and phosphorus than healthy plants. Lee (27) found that the diseased rice on the same soil was higher in iron and manganese, but that there was no difference between soils taken from healthy and diseased areas in their content of available iron and manganese.

Nutrition of Denitrifiers

It is of interest to note that counts of anaerobic cellulose digesters and of denitrifiers frequently were very much greater than the more general count for anaerobic bacteria on Brewer's thioglycollate-dextrose-peptone agar. This serves to emphasize the importance of medium composition and cultural techniques in permitting significant groups of organisms to grow in the laboratory.

The nutritional studies reported are preliminary but show promise for developing procedures for enumeration, isolation and nutritional characterization of anaerobic organisms with the capability for reducing nitrate. The microflora studies showed that this group is very sensitive to influence by plant factors on root surfaces and in the rhizosphere. It should be a useful group to work with in further investigations of the rhizosphere physiology of rice.

SUMMARY

The rhizosphere physiology of "suffocation disease" of rice was investigated by following the seasonal distribution of nine microbial groups in root environments of healthy and diseased plants in two greenhouse experiments and at three field locations in Taiwan. Fall and spring crops of rice were studied.

In the greenhouse, disease symptoms similar to those observed in the field were induced by amendments of straw and green manure on only one of four soils tested--an undisturbed Lotung profile from eastern Taiwan.

Early symptoms included chlorosis and rolling of leaves, accompanied by darkening and dying back of roots and greatly reduced branching of secondary roots. These symptoms became more severe as vegetative development of tillers progressed. Some plants died before maximum tillering.

After the tillering stage, surviving plants recovered their green color but remained stunted and were delayed in panicle initiation, anthesis and maturity as compared with unmanured check cultures.

Recovery was more rapid and complete following green manure treatment, even though symptoms appeared earlier and were initially more intense with this treatment

than where straw was added.

Greater recovery occurred when soil was drained after maximum tillering than with continuous flooding to maturity. A two-week delay in transplanting after green manure or straw were incorporated reduced injury and hastened recovery.

Final yields of straw and grain were drastically reduced by straw treatment, regardless of season or drainage. Without drainage in the spring crop, yields were also reduced by green manure, but not in the fall crop where cultures were drained after maximum tillering.

Total and anaerobic bacteria, fungi, and aerobic and anaerobic cellulose decomposers were most numerous during the tillering period, responded to organic amendments, and were much more numerous on root surfaces than in the rhizosphere or edaphosphere. Actinomycetes were less responsive to organic amendments or to the influence of rice roots.

It appeared that demand for oxygen and other electron acceptors by the above groups was responsible for the strongly reduced soil conditions which were observed: E_h = -400 mv at tiller initiation, -250 mv at panicle initiation and -150 to -100 mv at maturity.

Sulfate reducers, denitrifiers and ammonifiers (urea hydrolyzers) were affected relatively little by organic amendments. Sulfate reducers and ammonifiers were relatively insensitive to the proximity of the root, whereas

denitrifiers appeared to be extremely sensitive to plant influences. Explosive increases in numbers of denitrifiers, principally on root surfaces, appeared to reflect changes in physiology of the rice plant.

Disease symptoms were successfully transmitted to healthy rice seedlings in nutrient culture by root washings from diseased plants, indicating the involvement of a microbial agent.

Preliminary pure culture studies with denitrifying bacteria gave promise of useful nutritional discrimination within this group for further studies on rice rhizosphere physiology. Numerous possibilities appeared for toxin production by denitrifying species and strains.

LITERATURE CITED

1. Alberda, T. 1953. Growth and root development of lowland rice and its relation to oxygen supply. Plant and Soil 5:1-28.

2. Alexander, M. 1961. Introduction to soil microbiology. Wiley, New York, 472 p.

3. Allen, M. B., and Van Niel, C. B. 1952. Experiments on bacterial denitrification. J. Bacteriol. 64:397-402.

4. Allen, O. N. 1957. Experiments in soil bacteriology. Burgess, Minneapolis.

5. Allison, F. E., Carter, J. N., and Sterling, L. D. 1960. The effect of partial pressure of oxygen on denitrification in soil. Soil Sci. Soc. Am. Proc. 24:283-285.

6. Allison, F. E. 1961. Twenty five years of soil microbiology and a look to the future. Soil Sci. Soc. Am. Proc. 25:432-439.

7. Allison, F. E., and Carter, J. N. 1960. Investigation on denitrification in well aerated soil. Soil Sci. 90:173-177.

8. Bartholomew, W. V., and Clark, R. F. 1950. Nitrogen transformations in soil in relation to the rhizosphere microflora. Trans. Intern. Congr. Soil Sci., 4th, Amsterdam. 2:112-113.

9. Bodily, H. L. 1944. The activity of microorganisms in the transformation of plant materials in soil under various conditions. Soil Sci. 57:341-349.

10. Bormer, H. 1960. Liberation of organic substances from higher plants and their role in the soil sickness problem. Botan. Rev. 26:393-424.

11. Bremner, J. M., and Shaw, K. 1958. Denitrification in soil. II. J. Agri. Sci. 51:40-52.

12. Brewer, J. H. 1940. Clear liquid mediums for the aerobic cultivation of anaerobes. J. Am. Med. Assoc. 115:598-600.

13. Brinhart, B. 1963. Soil air essential for good plant growth. Horticulture 41:92.

14. Broadbent, F. E., and Stojonovic, B. E. 1952. The effect of partial pressure of oxygen on some soil nitrogen transformations. Soil Sci. Soc. Amer. Proc. 16:359-362.

15. Broadbent, F. E. 1951. Denitrification in some California soils. Soil Sci. 72:128-137.

16. Brown, R., Robinson, E., and Johnson, A. W. 1949. The effects of D-xyloketose and certain root exudates in extension growth. Proc. Roy. Soc. (London). B, 136:577-591.

17. Bunt, J. S., and Rovira, A. D. 1955. Microbiological studies of some subantarctic soils. J. Soil Sci. 6:119-128.

18. Burton, M. O., Eagles, B. A., and Campbell, J. J. R. 1947. The amino acid requirements for pyocyanin production. Can. J. Res., 25(C):121-128.

19. Burton, M. O., Eagles, B. A., and Campbell, J. J. R. 1948. The mineral requirement for pyocyanin production. Can. J. Res. 26(C):15-22.

20. Cady, F. B., and Bartholomew, W. V. 1961. Influence of low pO_2 on denitrification processes and products. Soil Sci. Soc. Amer. Proc. 25:362-363.

21. Callahan, W. S., Beyerlein, B., and Mull, J. D. 1965. Toxicity of Pseudomonas aeruginosa slime. J. Bacteriol. 88:805-806.

22. Chang, S. C. 1960. The physiological disease of the second crop of rice in Pingtung [in Chinese]. Newsletter of Soils and Fertilizers, Taiwan, No. 112.

23. Chang, S. C. 1962. My impressions on the physiological disease of rice in Taiwan [in Chinese]. Newsletter of Soils and Fertilizers, Taiwan, No. 139.

24. Chang, S. C. 1964. Evidence, significance and research of suffocating disease of the second crop of rice in southern Taiwan [in Chinese]. Joint Committee on Rural Reconstruction, PID 287, March 23, 1964.

25. Christensen, P. D., Toth, S. J., and Bear, F. E. 1950. The status of soil manganese as influenced, by moisture, organic matter and pH. Soil Sci. Soc. Am. Proc. 15:279-282.

26. Chiu, T. F., and Lee, L. D. 1962. Comparison by pot culture experiments between Pingtung sick and Taipei normal soil [in Chinese]. J. Agr. Assoc. China. New Series No. 38.

27. Chiu, T. F., and Lee, L. D. 1956. Studies on Chin-Sag-Tsan of Tung-San Ilan [in Chinese]. Natl. Taiwan Univ. Agr. Chem. Rep. No. 5.

28. Clark, F. E. 1940. Notes on types of bacteria associated with plant roots. Trans. Kansas Acad. Sci. 43:75-84.

29. Clark, F. E., Nearpass, D. D., and Specht, A. W. 1957. Influence of organic additions and flooding on iron and manganese uptake by rice. Agron. J. 49:586-589.

30. Cochrane, V. W. 1949. Crop residues as causative agents of root rot of vegetables. Connecticut Agr. Exp. Sta. Bull. 526:3-31.

31. Collison, R. C., and Conn, H. J. 1925. The effect of straw on plant growth. N.Y. Geneva Agr. Exp. Sta. Tech. Bull. 114.

32. Dastur, J. F. 1937. "Pan-Sukh" disease of rice in the central provinces. Agr. and Livestock, India 7:509-511.

33. De, P. K., and Mandol, L. N. 1957. Physiological diseases of rice. Soil Sci. 84:30-37.

34. Epstein, E., and Kohnke, H. 1957. Soil aeration as affected by organic matter application. Soil Sci. Soc. Am. Proc. 21:85-92.

35. Evenari, M. 1949. Germination inhibitors. Botan. Rev. 15:153-194.

36. Fewson, C. A., and Nicholas, D. J. C. 1960. Utilization of nitric oxide by micro-organisms and higher plants. Nature 188:794-796.

37. Friedheim, E. 1931. Pyocyanin, an accessory respiratory enzyme. J. Exptl. Med. 54:207-221.

38. Friedheim, E., and Michaelis, L. 1931. Potentiometric study of pyocyanin. J. Biol. Chem. 91:355-368.

39. Fuller, W. H., and Norman, A. G. 1942. A cellulose dextrin medium for identifying cellulose organisms in soil. Soil Sci. Soc. Am. Proc. 7:243-246.

40. Gato, Y., and Tai, K. 1956. On the differences of oxidizing power of paddy rice seedling roots among some varieties. Soil and Plant Food, Japan. 2:198-200.

41. Gerretsen, F. C. 1948. The influence of microorganisms on the phosphate intake by the plant. Plant and Soil 1:51-58.

42. Goring, C. A. A., and Clark, F. E. 1948. Influence of crop growth on mineralization of nitrogen in the soil. Soil Sci. Soc. Am. Proc. 13:261-266.

43. Grossowicz, N., Hayat, P., and Halpern, Y. S. 1957. Pyocyanine biosynthesis by Pseudomonas aeruginosa. J. Gen. Microbiol. 16:576-583.

44. Guenzi, W. D., and McCalla, T. M. 1962. Inhibition of germination and seedling development by crop residues. Soil Sci. Soc. Am. Proc. 26:456-458.

45. Gyllenberg, H. G. 1957. Seasonal variation in the composition of the bacterial soil flora in relation to plant development. Can. J. Microbiol. 3:131-134.

46. Halvorson, H. O., and Ziegler, N. R. 1933. A means of determining bacterial populations by the dilution method. J. Bacteriol. 25:101-121.

47. Hart, L. I., Larson, A. D., and McCloskey, C. S. 1963. Denitrification by Corynebacterium nephridii. J. Bact. 89:1104.

48. Hays, E. E., and Wells, I. C. 1945. Antibiotic substances produced by Pseudomonas aeruginosa. J. Biol. Chem. 159:725-750.

49. Hoagland, D. R., and Arnon, D. I. 1938. The water-culture method for growing plants without soil. Calif. Agr. Exp. Sta. Circ. 347.

50. Huber, D. M., Watron, R. D., and Steiner, G. W. 1965. Crop residue, nitrogen and plant disease. Soil Sci. 100:5.

51. Ishizawa, S., Suzuki, T., and Sato, T. K. 1958. Studies on microorganisms and their activities in soil. Natl. Inst. Agr. Sci. Tokyo, Bull. 8 (Ser. B).

52. Ishizawa, S., and Toyoda, H. 1964. Microflora of Japanese soil. Natl. Inst. Agr. Sci., Tokyo. Bull. 14 (Ser. B).

53. Janson, S. L., and Clark, F. E. 1952. Losses of N during decomposition of plant material in the presence of inorganic nitrogen. Soil Sci. Soc. Am. Proc. 16:334.

54. Jensen, H. L. 1930. Actinomyces in Danish soils. Soil Sci. 30:59-77.

55. Kopper, P. H. 1947. An atypical strain of Pseudomonas aeruginosa. J. Bact. 54:359-362.

56. Katznelson, H. 1946. The rhizosphere effect of mangels on certain groups of soil microorganisms. Soil Sci. 62:343-354.

57. Katznelson, H., Lochhead, A. G., and Timonin, M. I. 1948. Soil microorganisms and the rhizosphere. Botan. Rev. 14:9.

58. Katznelson, H., and Richardson, L. T. 1943. The microflora of the rhizosphere of tomato plants in relation to soil sterilization. Can. J. Res. 21(C):249-255.

59. Katznelson, H., Rouatt, J. W., and Payne, T. M. B. 1956. The liberation of amino acids and reducing compounds by plant roots. Plant and Soil 7:35-47.

60. Kefauver, M., and Allison, F. E. 1957. Nitrite reduction by Bacterium denitrificans in relation to oxidation-reduction potential and oxygen tension. J. Bacteriol. 73:8-14.

61. Kelley, W. P. 1912. The assimilation of nitrogen by rice. Hawaii Agr. Exp. Sta. Bull. 24.

62. Kluyver, H. J., and Verhoeven, W. 1954. Studies on true dissimilatory nitrate reduction. IV. An adaptation in Micrococcus denitrificans. Antonie Van Leeuwenhoek J. Microbiol. Serol. 20:337-358.

63. Kononova, M M. 1961. Soil organic matter. Pergamon Press. 450 p.

64. Krassilnikov, N. A. 1934. Influence of root secretion on the development of Azotobacter and other soil microbes. Microbiol. U. S. S. R. 3:343-359.

65. Krassilnikov, N. A., Kriss, A. E., and Litvinov, M. A. 1936. The effect of the root system on the soil microflora. Microbiol. U. S. S. R. 5:270-286.

66. Krassilnikov, N. A. 1958. Soil microorganisms and higher plants. Office of Technical Services, U. S. Dept. of Commerce, Washington, D.C.

67. Krassilnikov, N. A. 1960. The biological role of microbes: Antagonistic producers of antibiotic substances. Soil and Plant Food. Vol. 5, No. 4.

68. Knight, B. C., and Proom, H. 1950. A comparative survey of the nutrition and physiology of mesophilic species in the genus Bacillus. J. Gen. Microbiol. 4:508-538.

69. Langston, C. W., and Williams, P. P. 1962. Reduction of nitrate by Streptococci. J. Bacteriol. 84:603.

70. Lee, Y. C., and Lin, J. G. 1964. Rice [in Chinese]. In Crops and Fertilizer, Taiwan Fertilizer Corporation.

71. Lenhoff, H. 1963. An inverse relationship in the effects of oxygen and iron on the production of fluorescin and cytochrome by Pseudomonas fluorescens. Nature 199:601-602.

72. Linford, M. B. 1942. Methods of observing soil flora and fauna associated with roots. Soil Sci. 53:93-103.

73. Liu, P. V. 1964. Factors that influence toxigenicity of Pseudomonas aeruginosa. J. Bacteriol. 88:1421-1427.

74. Livingston, B. E. 1923. Physiological aspects of toxicity. J. Am. Soc. Agron. 15:313-323.

75. Livingston, B. E. 1907. Further studies on the properties of unproductive soils. U.S. Dept. of Agr., Bur. of Soils. Bull. 36.

76. Lochhead, A. G., Timonin, M. I., and West, P. M. 1939. The microflora of the rhizosphere in relation to resistance of plants to soil borne pathogens. Sci. Agr. 20:414-418.

77. Lochhead, A. G., and West, P. M. 1940. The nutritional requirements of soil bacteria--a basis for determining the bacterial equilibrium of soils. Soil Sci. 50:409-420.

78. Lochhead, A. G., and Chase, F. E. 1943. Qualitative studies of soil microorganisms. V. Nutritional requirements of the predominant bacterial flora. Soil Sci. 55:188-195.

79. Lochhead, A. G. 1946. Qualitative studies of soil microorganisms. III. Influence of plant growth on the character of the bacterial flora. Can. J. Research 18(C):42-53.

80. Lochhead, A.G., and Thexton, R. H. 1947. Qualitative studies of soil microorganisms. VII. The "rhizosphere effect" in relation to the amino acid nutrition of bacteria. Can. J. Res. 25(C):20-26.

81. Loehwing, W. F. 1937. Root interactions of plants. Botan. Rev. 3:195-239.

82. Lockett, J. L. 1937. Microbiological aspects of decomposition of clover and rye plants at different growth stages. Soil Sci. 44:425-439.

83. Loewenstein, H. 1957. Nitrogen loss in gaseous form from soils as influenced by fertilizer and management. Soil Sci. Soc. Am. Proc. 21:397-400.

84. Louw, H. A., and Webley, D. M. 1959. The bacteriology of the root region of the oat plant grown under controlled pot culture conditions. J. Appl. Bacteriol. 22:216-226.

85. Maloy, O.C., and Alexander, M. 1958. The most probable number method for estimating populations of plant pathogenic organisms in the soil. Phytopathol. 48:126-128.

86. Martin, J. P., and Ervin, J. O. 1958. Greenhouse studies on influence of other crops and of organic materials on growth of orange seedlings in old citrus soil. Soil Sci. 85:141.

87. McCalla, T., and Haskins, F. A. 1964. Phytotoxic substances from soil microorganisms and crop residues. Bacteriol. Rev. 28:181-207.

88. McElroy, W. D., and Glass, B. 1956. A symposium on inorganic nitrogen metabolism. John Hopkins Press, Baltimore. p. 233-259.

89. McFadden, B. A., and Howes, W. V. 1961. Pseudomonas indigofera. J. Bacteriol. 81:858-862.

90. McGarity, J. W., Gilmour, C. M., and Bollen, W. B. 1958. Use of an electrolytic respirometer to study denitrification in soil. Can J. Microbiol. 4:303-316.

91. Meiklejohn, J. 1940. Aerobic denitrification. Ann. Appl. Biol. 27:558-573.

92. Mitsui, S., Aso, S., Kumarawa, K., Ishizawa, T. 1954. The nutrient uptake of rice plants influenced by hydrogen sulfide and butyric acid abundantly evolving under water-logged soil conditions. Trans. Intern. Congr. Soil Sci., 5th., Leopoldville. 2:364-368.

93. Morihara, K. 1965. Production of proteinase on non-carbohydrate carbon sources by Pseudomonas aeruginosa. Appl. Microbiol. B(2):793-797.

94. Motomura, S. 1962. Effect of organic matter on the formation of ferrous iron in soils. Natl. Inst. Agr. Sci. Tokyo. Soil Science and Plant Nutrition Vol. 8, No. 1.

95. Myers, H. E., and Anderson, K. L. 1954. Bromegrass toxicity vs. nitrogen starvation. J. Am. Soc. Agron. 34:770.

96. Najjar, V. A., and Allen, M. B. 1954. Formation of nitrogen, nitrous oxide and nitric oxide by extracts of denitrifying bacteria. J. Biol. Chem. 206-214.

97. Nance, J., and Cunningham, L. W. 1951. Evolution of acetaldehyde by excised wheat roots in solutions of nitrate and nitrite salts. Am. J. Botany 38:604-609.

98. Nielsen, K. F., Cuddy, T. F., and Woods, W. B. 1960. The influence of the extract of some crops and soil residues on germination and growth. Can. J. Plant Sci. 40:188-197.

99. Nommik, H. 1956. Investigations on denitrification in soil. Acta. Agr. Scand. 6:195-228.

100. Okajima, H., and Takagi, S. 1955. Physiological behaviour of hydrogen sulphide in the rice plant. II. Effects of H_2S on the content of nutrients in the rice plant. Inst. Agr. Res. Tohoku Univ. Rep. No. 6:89-99.

101. Page, J., and Bodman, G. B. 1953. The effect of soil physical properties on availability of nutrients. In Mineral nutrition of plants. The Univ. of Wisc. Press.

102. Patrick, Z. A., and Koch, L. W. 1958. Inhibition of respiration, germination and growth by substances arising during the decomposition of certain plant residues in the soil. Can. J. Botany 36:621-647.

103. Peck, H. D. 1961. Symposium on metabolism of inorganic compounds. V. Comparative metabolism of inorganic sulfur compounds in microorganisms. Annual meeting of the Amer. Soc. for Microbiology in Chicago, on April 26, 1961.

104. Piper, C. S. 1950. Soil and Plant Analysis. Univ. of Adelaide, Adelaide, Australia.

105. Pearsall, W. A., and Marlimer, C. H. 1939. Oxidation reduction potentials in water-logged soils, natural water and muds. J. Ecol. 27:483-501.

106. Ponnamperuma, F. N., Bradfield, B., and Peech, M. 1955. Physiological disease of rice attributable to iron toxicity. Nature 175:265.

107. Rhodes, M., Best, A., and Payne, W. J. 1962. Electron donors and factors for denitrification by Pseudomonas perfectomarinus. Can. J. Microbiol. 9:799-807.

108. Robinson, W. O. 1930. Some chemical phases of the submerged soil condition. Soil Sci. 30:197.

109. Rouatt, J. W. and Atkinson, R. G. 1950. The effect of the incorporation of certain cover crops on the microbiological balance of potato scab infested soil. Can. J. Res. 28(C):140-152.

110. Rouatt, J. W., and Katznelson, H. 1961. A study of the bacteria on the root surface and in the rhizosphere soil of crop plants. J. Appl. Bacteriol. 24:164-171.

111. Rovira, A. D. 1953. Some quantitative and qualitative aspects of the rhizosphere. Proc. Soil Sci. Conf. Adelaide, June, 1953.

112. Rovira, A. D. 1956. Plant root excretions in relation to the rhizosphere effect. Plant and Soil 7:178-194.

113. Russell, E. J. and Russell, E. W. 1952. The soil atmosphere. In Soil conditions and plant growth. 8th Ed., Longmans, Green and Co., London, pp. 335-345.

114. Russell, E. W. 1961. Soil conditions and plant growth. 9th Ed. Longmans, Green and Co., London. 688 p.

115. Russell, M. B. 1957. Soil aeration and plant growth. In Soil physical conditions and plant growth. Agronomy Monograph 2, Academic Press, New York. pp. 253-301.

116. Sawada, Y., Nitta, K., and Igarashi, T. 1964. Injury of young plants caused by the decomposition of green manure. Soil Science and Plant Nutrition. Vol. 10, No. 4.

117. Schreiner, O., and Reed, H. S. 1908. The toxic action of certain organic plant constituents. Botan. Gaz. 45:73-102.

118. Schreiner, O., and Shorey, E. C. 1909. The isolation of harmful organic substances from soils. U.S. Dept. of Agr., Bur. of Soils Bull. 53.

119. Schreiner, O., and Shorey, E. C. 1911. Chemical nature of soil organic matter. U.S. Dept. of Agr. Bur. of Soils Bull. No. 74.

120. Schreiner, O., and Lathrop, E. C. 1911. Examination of soils for organic constituents, especially dehydroxystearic acid. U.S. Dept. of Agr. Bur. of Soils Bull. No. 80.

121. Skinner, F. A. 1960. The isolation of anaerobic cellulose decomposing bacteria from soil. J. Gen. Microbiol. 22:539-554.

122. Smith, N. R., and Dawson, V. T. 1944. The bacteriostatic action of rose bengal in media used for plate counts of soil fungi. Soil Sci. 58:467-471.

123. Somers, I. I., and Shive, J. W. 1942. The iron-manganese relation in plant metabolism. Plant Physiol. 17:582-607.

124. Stanier, R. Y., Doudoroff, M., and Adelberg, E. A. 1963. The microbial world. 2nd Ed. Prentice-Hall, Englewood Cliffs, N.J. 682 p.

125. Stansly, P. G., Shephard, R. G., and White H. J. 1947. Polymyxin, a new chemotherapeutic agent. Johns Hopkins Hosp. Bull. 81:43-55.

126. Starkey, R. L. 1929. Some influence of the development of higher plants upon the microorganisms in the soil. I. Historical and introductory. Soil Sci. 27:319-334.

127. Starkey, R. L. 1931. Plant growth and microorganisms. Soil Sci. 32:367-391.

128. Starkey, R. L. 1938. Some influences of the development of higher plants upon the microorganisms in the soil. VI. Microscopic examination of the rhizosphere. Soil Sci. 45:207-249.

129. Starkey, R. L. 1958. Interrelations between microorganisms and plant roots in the rhizosphere. Bacteriol. Rev. 22:154-172.

130. Suzuki, T., and Ishizawa, S. 1965. Soil microorganisms, their activities and soil fertility. Natl. Inst. of Agr. Sci., Tokyo. Ser. B (Soil and Fertilizers) No. 15:181-186.

131. Takahashi, J. 1960. Review of investigations into physiological disease of rice. Intern. Rice Commission Newsletter 9:1-6; 17-24.

132. Takahashi, J., and Chang, S. C. 1961. The physiological disease of rice in Taiwan [in Chinese]. Newsletter of Soils and Fertilizer, Taiwan, No. 125.

133. Takai, Y., and Asami, T. 1962. Formation of methyl mercaptan in paddy soil. Natl. Inst. Agr. Sci. Tkoyo. Soil Science and Plant Nutrition. Vol. 8, No. 3.

134. Takai, Y., Koyama, T., and Kamura, T. 1956. Microbial metabolism in reduction process of paddy soil. Part 1. Soil and Plant Food 2:63-66.

135. Takai, Y. 1960. Essays of the 3rd. Congress of Isotope Technology, Japan. p. 897.

136. Takijima, Y., Shiojima, M., and Konno, K. 1958. Studies on soils of peaty paddy fields. I. Geographical distribution, characteristics and classification of soils in Miyagi prefecture. Soil and Plant Food. Vol. 4, No. 2.

137. Takijima, Y., and Sakuma, H. 1961. Metabolism of organic acids in soil of peaty paddy field and their inhibitory effects on the growth of the rice plant. Part II. Production of organic acids in water-logged soil treated with green manure (Chinese milk vetch) and its relation to growth inhibition of rice seedlings. Natl. Inst. Agr. Sci. Tokyo. Ser. B (Soil and Fertilizers) No. 11:560-564.

138. Takijima, Y. 1963. Studies on behaviour of the growth inhibiting substances in paddy soil with special reference to the occurrence of root damage in the peaty paddy fields. Natl. Inst. of Agr. Sci., Tokyo. Ser. B (Soils and Fertilizers) No. 13: 118-245.

139. Takijima, Y. 1964. Studies on organic acids in paddy field soil with reference to their inhibitory effects on the growth of rice plants. Part I. Growth inhibiting action of organic acids and absorption and decomposition of them by soils. Soil Sci. and Plant Nutrition. Vol. 10, No. 5.

140. Takijima, Y. and Shiojima, M. 1958. Studies on soils of peaty paddy fields. II. Soil characteristics and metabolism under flooded conditions. Soil and Plant Food, Vol. 4, No. 3.

141. Takijima, Y. and Shiojima, M. 1951. The influence of plants on the mineralization of nitrogen and the maintenance of organic matter in the soil. J. Agr. Sci. 41:289-296.

142. Theron, J. J., and Haylett, D. G. 1953. The regeneration of soil humus under a grass ley. Emp. J. Exptl. Agr. 21:86-98.

143. Timonin, M. I. 1940. The interaction of higher plants and soil microorganisms. I. Microbial populations of rhizospheres of seedlings of certain cultivated plants. Can. J. Res. 18(C):307-317.

144. Timonin, M. I. 1940. The interaction of higher plants and soil microorganisms. II. Study of the microbial population of the rhizosphere in relation to resistances of plants to soil borne disease. Can. J. Res. 18(C):444-456.

145. Timonin, M. I. 1946. Microflora of the rhizosphere in relation to the manganese deficiency disease of oats. Soil Sci. Soc. Am. Proc. 11:284-292.

146. Totter, J. R., and Moseley, F. T. 1953. Influence of the concentration of iron on the production of fluorescin by Pseudomonas aeruginosa. J. Bacteriol. 65:45-47.

147. Tulecke, W. 1965. Isolation of an organism resembling Achromobacter liquefaciens. J. Bact. 89:905-906.

148. Twyman, E. S. 1963. The iron manganese balance and its effect on the growth and development of plants. New Phytol. 45:92.

149. Valera, C. L., and Alexander, M. 1961. Nutrition and physiology of denitrifying bacteria. Plant and Soil 15:268-280.

150. Vamos, R. 1956. The role of excess nitrogen in the "brusone" disease of rice. Acta. Biol. Szeged. 2:103-110.

151. Vamos, R. 1958. H_2S the cause of the brusone (akiochi) disease of rice. Soil and Plant Food. Vol. 4, No. 1: 37-40.

152. Vamos, R. 1959. "Brusone" disease of rice in Hungary. Plant and Soil 11:65-67.

153. Vandecaveye, S. C., and Katznelson, H. 1938. Microbial activities in soil. Soil Sci. 46:139-167.

154. Vlamis, J., and Davis, A. R. 1944. Effects of oxygen tension on certain physiological responses of rice, barley and tomato. Plant physiol. 19:33-51.

155. Voorhees, E. B. 1902. Studies in denitrification. J. Am. Chem. Soc. 24:785-823.

156. Wagner, G. H., and Smith, G. E. 1957. Nitrogen losses from soils fertilized with different nitrogen carriers. Soil Sci. 85:125-129.

157. Wahba, A. H. 1965. Pyrorubrin-producing Pseudomonas aeruginosa. Appl. Microbiol. 13:291-292.

158. Waksman, S. A. 1952. Soil Microbiology. Wiley, New York. pp. 183-189.

159. Wallace, T. H., and Lochhead, A. G. 1950. Qualitative studies of soil microorganisms. IX. Amino acid requirements of rhizosphere bacteria. Can. J. Res. 28(C):1-6.

160. Wang, S. C., and Chuang, T. T. 1966. Soil alcohols, their dynamics and their effects upon plant growth. Soil Sci. (unpublished).

161. Wang, S. C. 1966. Dynamics of soil organic acids (unpublished).

162. Wang, C. M., and Chang, S. C. 1964. Review of studies on the physiological disease of rice in Taiwan. Joint Commission on Rural Reconstruction. PID-C-281, February, 1964.

163. Wasserman, A. E. 1965. Absorption and fluorescence of water soluble pigments produced by four species of Pseudomonas. Appl. Microbiol. 13:175-180.

164. West, P. M. 1939. Excretion of thiamin and biotin by the roots of higher plants. Nature 144:1050.

165. West, P M., Lochhead, A. G. 1940. Qualitative studies of soil microorganisms. IV. The rhizosphere in relation to the nutritive requirements of soil bacteria. Can. J. Res. 18(C):129-135.

166. Wheeler, B. E. J. 1963. The conversion of amino acids in soils. II. Denitrification in percolated soil. Plant and Soil 19:219-232.

167. Wijler, J., and Delwiche, C. C. 1954. Investigations of the denitrifying process in soil. Plant and Soil 5:155-169.

168. Williamson, R. E. 1964. The effect of root aeration on growth. Soil Sci. Soc. Am. Proc. 28:86-90.

169. Woldendorp, J. W. 1962. The quantitative influence of the rhizosphere on denitrification. Plant and Soil 17:267-270.

170. Woldendorp, J. W. 1963. The influence of living plants on denitrification. Medeelingen van de Landbouwhogeschool te Wageningen, Nederland. 63(13):1-100.

171. Yamane, I. 1957. Nitrate reduction and denitrification in flooded soils. Soil and Plant Food Vol. 3:100-103.

172. Yamane, I. 1958. Metabolism in muck paddy soil. II. Determination of gases evolved from paddy field--estimation of decomposable organic matter. Soil and Plant Food, Vol. 4, No. 1.

173. Yanagiswawa, M., and Takahashi, J. 1964. Studies on the factors related to the productivity of paddy soil in Japan with special reference to the nutrition of the rice plant. Bull. of the Natl. Inst. of Agr. Sci. Japan. Ser. B (Soil and Fertilizers), No. 14.

174. Yoshida, T., and Sakai, H. 1963. Soil moisture condition and organic matter decomposition--microbiological studies on the soils of Hokkaido. Hokkaido Natl. Agr. Exp. Sta. Present address, Chugoku Natl. Agr. Exp. Sta. Bull. 6:197-202.

175. Yoshida, S. 1965. Chemical aspects of the role of silicon in physiology of the rice plant. Bull. Natl. Inst. Agr. Sci. Japan. Ser. B (Soil and Fertilizer) No. 15:1-56.

176. Young, G. 1947. Pigment production and antibiotic activity in cultures of Pseudonomas aeruginosa. J. Bact. 54:109-117.

APPENDIX

Table 22. Sample codes used in Tables 23 through 27.

Soil sample source:

C = Chungli (field location)

G = Lotung (greenhouse experiments)

L = Lotung (field operation)

P = Pingtung (field operation)

T = Taichung (field operation)

Subscript g = green manure added in greenhouse

Subscript st = straw added in greenhouse

Subscript s = seedling before or at time of transplanting

Plant condition:

H = healthy

D = disease

Sampling zone:

R = root

Rh = rhizosphere soil

S = edaphosphere soil

Examples:

TRs = Taichung soil, seedling root

DstR = Lotung soil, root count from diseased plant in straw-amended pot

HRh = Lotung soil, rhizosphere count from healthy plants

Table 23. Microbial numbers in the Lotung profile samples at various stages of growth of rice in the greenhouse. (First trial, fall crop rice, 1964.)

Time of sampling	Sample Code	Bacteria	Actino-myces	Fungi	Anae-robes	Cellulose Decomposers		Denitri-fiers	Ammoni-fiers
						Aerobic	Anaerobic		
		x 10^6	x 10^5	x 10^3	x 10^5	x 10^6	x 10^6	x 10^6	x 10^5
Aug. 8, 1964	TRs	332.5	25.0	9.6	67.9	217.1	121.2	5.4	0.8
	TSs	16.3	64.4	18.8	27.1	72.7	9.6	0.11	0.8
	LS	52.5	41.1	15.0	33.2	141.6	18.3	1.3	0.9
Seedling bed	PS	37.3	31.3	14.6	12.5	72.4	3.4	0.21	0.3
	CS	18.3	43.9	12.9	13.5	61.8	10.0	0.068	0.4
Aug. 12, 1964	HR	340.1	13.3	21.3	588.3	512.3	42.7	4.3	24.0
	DgR	1036.7	10.1	33.0	282.9	1090.0	65.0	16.0	7.0
	HS	150.2	43.1	18.2	32.2	133.4	18.1	5.4	1.3
Tillering	DgS	135.7	23.0	11.5	79.1	174.7	24.5	16.0	1.3
Sept. 3, 1964	HR	397.5	72.0	5.9	66.2	74.7	39.1	5.4	9.2
	DgR	650.1	60.2	45.6	113.0	66.7	24.0	11.0	13.0
Max.	DstR	1000.0	40.2	195.3	114.8	229.9	105.7	3.5	2.4
tiller	HS	74.6	58.6	3.2	26.3	12.6	5.3	2.8	54.0
no. stage	DgS	93.3	57.3	2.1	29.0	17.8	7.1	4.0	920.0
	DstS	129.9	38.4	5.2	18.5	20.0	8.8	1.1	77.2
Oct. 2, 1964	HR	207.8	6.4	58.7	5.9	11.2	2.0	9.5	13.0
	DgR	437.8	12.6	27.8	17.7	35.8	5.3	5.4	17.0
	DstR	276.1	11.3	9.4	6.6	15.6	2.6	9.5	7.9
Panicle	HS	173.7	3.7	30.1	22.2	11.4	1.8	0.49	7.9
develop-	DgS	274.6	11.9	30.7	13.5	18.0	2.7	4.6	10.0
ment	DstS	253.7	5.7	23.1	11.4	8.1	1.8	0.79	14.0

Table 23 (Continued).

Time of sampling	Sample Code	Bacteria	Actino-myces	Fungi	Anae-robes	Cellulose Decomposers		Denitri-fiers	Ammoni-fiers
						Aerobic	Anaerobic		
		x 10^6	x 10^5	x 10^3	x 10^5	x 10^6	x 10^6	x 10^6	x 10^5
Oct. 8, 1964	HR	174.9	17.1	49.9	39.5	17.5	10.1	16000.0	40.0
	DgR	217.5	5.2	41.2	131.0	30.3	4.7	16000.0	32.0
	DstR	318.3	16.1	25.8	39.5	23.6	6.3	690.0	19.2
Anthesis	HS	51.6	22.6	9.9	23.3	7.2	2.0	16000.0	4.7
	DgS	41.3	14.2	7.1	7.1	1.9	1.1	16000.0	3.4
	DstS	81.3	29.9	9.6	20.0	10.3	2.4	16000.0	4.0
Nov. 17, 1964	HR	158.6	16.3	38.6	36.4	16.5	9.0	16000.0	54.0
	DgR	189.7	11.2	32.5	89.7	25.4	4.6	16000.0	43.0
	DstR	268.1	17.1	25.8	78.6	21.3	5.9	16000.0	17.0
Maturation	HS	23.6	18.9	8.9	21.2	6.8	1.8	16000.0	13.0
	DgS	36.7	16.2	7.8	6.8	6.9	1.0	16000.0	3.4
	DstS	45.3	20.1	8.8	16.1	9.9	2.2	16000.0	4.0

*Note: For sample codes, see Table 6.
**Chromobacter violaceum isolated from this experiment.

Table 24. Microbial numbers in Lotung soil at various stages of growth of rice in the field. (First trial, fall crop rice, 1964.)

Time of sampling	Sample Code	Bacteria	Actino-myces	Fungi	Anae-robes	Cellulose Decomposers		Denitri-fiers	Ammoni-fiers
						Aerobic	Anaerobic		
		x 10^6	x 10^5	x 10^3	x 10^5	x 10^6	x 10^6	x 10^6	x 10^5
July 5,'64	Rs	596.7	140.4	70.2			34.2	0.02	0.2
Seedling	Rhs	92.3	32.2	15.0			13.7	0.17	1.7
bed	Ss	37.3	32.0	32.0			10.0	0.17	1.7
Aug. 2, '64	Rs	956.4	63.9	175.9	454.2	268.5	183.9	0.14	0.7
Tillering	Rhs	92.6	34.2	33.6	41.4	42.6	18.6	0.11	1.4
	Ss	114.2	40.7	31.0	21.2		10.2	0.11	0.9
	S	37.0	63.7	30.1	56.2	32.1	10.6	0.21	1.7
Aug. 15,'64	HR	308.6	5.5	3.9	25.3	220.0	10.8	9.2	13.0
	DR	434.8	25.9		131.3	316.4	25.4	9.2	20.8
Max. tiller	HRh	41.9	41.9	10.3	21.2	56.4	3.5	16.0	90.2
no. stage	DRh	96.2	29.3	12.1	23.6	107.7	3.5	5.4	10.3
	HS	46.3	56.3	7.3	23.7	58.2	4.6	3.5	160.0
	DS	30.0	34.2	4.2	13.7	40.5	4.1	0.5	13.0
Sept. 10,	HR	809.6	25.1	5.0	136.7	529.4	28.1	9.2	14.0
1964	DR	1722.5	26.5	11.8	537.9	884.7	33.5	16.0	4.0
Panicle	HRh	144.6	28.7	3.5	32.6	56.3	3.0	92.0	28.0
develop-	DRh	172.4	29.5	10.2	37.5	72.7	2.7	7.0	20.0
ment	HS	56.7	24.6	2.8	28.5	100.8	1.3	16.0	160.0
	DS	53.2	41.9	10.3	31.6	50.4	2.7	16.0	40.7

Table 24 (Continued.)

Time of sampling	Sample Code	Bacteria	Actino-myces	Fungi	Anae-robes	Cellulose Decomposers		Denitri-fiers	Ammoni-fiers
						Aerobic	Anaerobic		
		x 10^6	x 10^5	x 10^3	x 10^5	x 10^6	x 10^6	x 10^6	x 10^5
Sept. 26, 1964	HR	225.0	11.5	355.6	13.1	45.4	7.6	5.8	54.0
Anthesis	DR	236.8	18.3	84.0	7.2	8.8	6.9	28.0	14.0
	HRh	48.0	25.1	190.7	12.0	24.2	13.8	14.0	95.0
	DRh	66.7	12.7	92.1	13.2	10.7	6.9	160.0	140.0
	HS	82.5	30.0	138.7	17.2	12.5	4.7	0.7	35.0
	DS	42.7	22.5	29.8	13.5	12.9	3.8	1.1	540.0
Oct. 20, 1964	HR	99.3	14.8	4.9	13.4	9.9	2.6	11.0	5.4
	DR	230.8	7.8	5.8	20.4	15.9	2.8	22.0	17.0
	HRh	46.2	22.1	12.6	19.9	6.8	5.2	5.6	140.0
Maturation	DRh	62.8	27.2	3.0	9.0	6.2	4.5	3.3	22.0
	HS	34.4	17.8	17.2	16.6	9.4	2.6	11.0	17.0
	DS	66.5	37.6	14.8	14.8	12.8	10.4	11.0	210.0

Table 25. Microbial numbers in the Pingtung field soil sample at various stages of growth of rice (first trial, fall crop rice, 1964).

Time of sampling	Sample Code	Bacteria	Actino-myces	Fungi	Anae-robes	Cellulose Decomposers		Denitri-fiers	Ammoni-fiers
						Aerobic	Anaerobic		
		x 10^6	x 10^5	x 10^3	x 10^5	x 10^6	x 10^6	x 10^6	x 10^5
July 25, 1964 Seedling bed	Rs	98.1	146.0	301.6	1261.6	312.6	212.0	0.28	2.1
	Rhs	27.4	43.1	22.9	188.6	20.6	42.8	0.21	2.8
	Ss	23.4	63.8	17.0	23.9	17.6	10.2	0.28	1.7
	S	17.0	84.7	52.4	28.2	17.0	16.4	0.17	1.4
Aug. 5, 1964 Tillering	HR	554.0	53.1	17.7	17.7	28.0	22.4	0.24	1.1
	DR	124.9	137.8	37.4	8.7	12.9	97.7	0.28	1.4
	HS	12.4	60.7	25.0	20.8	16.7	24.0	0.17	0.9
	DS	11.4	47.4	21.7	24.9	28.1	12.1	0.14	1.1
Aug. 29, 1964 Max. tiller no. stage	HR	1087.6	27.8	85.0	29.4	110.9	47.3	7.0	35.0
	DR	1136.7	38.4	63.1	90.6	332.2	102.6	1.6	9.2
	HRh	20.0	46.5	23.8	8.6	15.2	9.2	0.9	16.0
	DRh	38.5	62.6	28.8	7.9	37.0	12.6	1.6	17.0
	HS	59.3	45.1	18.4	6.1	38.8	13.8	0.9	160.0
	DS	23.0	68.0	9.6	7.5	24.4	9.4	2.1	90.2

Table 25. (Continued.)

Time of sampling	Sample Code	Bacteria	Actino-myces	Fungi	Anae-robes	Cellulose Decomposers		Denitri-fiers	Ammoni-fiers
						Aerobic	Anaerobic		
		x 10^6	x 10^5	x 10^3	x 10^5	x 10^6	x 10^6	x 10^6	x 10^5
Sept. 19, 1964 Panicle develop-ment	HR	160.4	16.2	54.0	62.0	42.5	30.7	9.5	16.0
	DR	197.5	59.1	6.7	18.2	27.7	34.3	16.0	54.0
	HRh	43.1	51.2	15.1	20.0	14.2	11.9	2.6	33.0
	DRh	56.1	64.7	53.0	15.6	15.5	16.4	7.0	14.0
	HS	92.0	51.0	22.2	10.5	26.3	15.6	2.6	4.7
	DS	54.4	64.3	38.3	22.7	15.1	12.4	1.4	18.0
Sept. 26, 1964 Anthesis	HR	217.0	37.2	49.0	28.2	13.7	6.2	7.5	0.9
	DR	134.4	28.1	31.4	14.7	11.6	1.7	320.0	7.0
	HRh	46.3	8.1	16.7	5.8	5.3	1.0	2.1	26.0
	DRh	29.4	14.2	12.6	9.6	3.1	1.2	21.0	20.0
	HS	32.1	14.2	3.8	8.1	3.1	0.6	0.6	70.0
	DS	46.9	13.8	10.5	7.7	4.2	5.0	4.9	6.8
Oct. 28, 1964 Maturation	HR	189.2	29.3	32.0	25.0	12.7	5.9	2.1	14.0
	DR	120.2	21.2	28.7	13.2	9.9	2.7	2.8	13.0
	HRh	43.2	8.2	15.6	5.6	5.1	1.0	16.0	28.0
	DRh	40.8	13.2	14.2	8.7	3.6	1.1	17.0	22.0
	HS	38.1	12.7	4.7	7.1	3.6	0.6	3.4	54.0
	DS	42.3	11.7	9.6	8.2	4.2	3.9	4.7	35.0

Table 26. Microbial numbers in the Lotung profile sample at various stages of growth of rice in the greenhouse (second trial, spring crop rice, 1965).

Time of sampling	Sample Code	Bacteria	Acti-nomyces	Fungi	Anae-robes	Sulfate Reducers	Cellulose Decomposers		Denitri fiers	Ammoni-fiers
							Aerobic	Anaerobic		
		x 10^6	x 10^5	x 10^3	x 10^5	x 10^3	x 10^6	x 10^6	x 106	x 10^5
Mar. 10, 1965 Seedling bed	L_3	27.0	21.9	43.8	18.9	1.1	5.5	3.8	1.20	1.4
	L_{3g}	29.2	14.9	85.4	19.9	0.8	4.3	3.1	3.20	1.1
	L_{3s}	49.0	14.1	75.8	13.4	54.0	5.1	2.1	0.09	4.9
	Rs	80.9	9.7	107.1	45.8	0.4	4.9	5.9	0.48	2.3
	RsS	17.8	11.7	65.7	8.4		1.5	2.4	0.33	0.4
Mar. 27, 1965 Tillering	LHR	97.3	82.7	146.2	214.3		3.7	38.5	1.30	1.4
	DgR	808.6	251.5	537.0	233.4	1.7	82.6	60.2	1.40	2.6
	DstR	488.4	229.9	486.0	383.0	21.0	84.3	72.8	5.40	7.9
	HS	18.5	26.0	30.7	7.4	22.0	3.1	1.5	0.79	2.0
	DgS	31.9	19.0	20.4	14.5	13.0	4.0	1.6	0.49	14.0
	DstS	19.8	48.0	20.8	14.6	35.0	3.9	3.4	0.78	4.7
Apr. 26, 1965 Max. tiller no. stage	HR	210.4	60.1	358.9	46.7	7.9	5.3	3.8	8.0	11.0
	DgR	120.1	18.6	149.3	14.9	70.0	5.5	1.5	1600.0	28.0
	DstR	699.7	65.2	495.6	41.7	14.0	25.8	27.7	180.0	49.0
	HS	55.7	35.4	108.6	36.7	2.2	3.2	5.2	0.1	22.0
	DgS	61.7	63.8	94.2	48.1	13.0	6.2	3.3	0.2	21.0
	DstS	279.7	67.7	78.7	22.1	4.6	6.0	3.0	0.1	3.9

Table 26. (Continued.)

Time of sampling	Sample Code	Bacteria	Actinomyces	Fungi	Anaerobes	Sulfate Reducers	Cellulose Decomposers		Denitrifiers	Ammonifiers
							Aerobic	Anaerobic		
		x 10^6	x 10^5	x 10^3	x 10^5	x 10^3	x 10^6	x 10^6	x 10^6	x 10^5
May 18, 1964 Panicle development	HR	21.8	6.5	9.6	4.3	7.0	2.4	5.8	11.1	18.0
	DgR	13.5	7.6	5.8	5.1	22.0	3.0	1.5	16.0	35.0
	DsR	47.9	6.8	4.3	8.2	22.0	4.3	4.8	3.7	22.0
	HS	4.1	9.4	3.6	3.1	3.3	1.8	1.4	0.2	2.4
	DgS	5.3	8.4	3.0	8.1	3.8	0.7	0.6	0.3	22.0
	DsS	7.0	11.3	7.1	3.7	0.6	1.1	0.6	0.3	18.0
June 1, 1965 Anthesis	HR	20.1	6.0	9.5	4.7	6.8	2.6	5.7	9.5	11.0
	DgR	13.5	7.2	6.7	5.6	17.0	2.9	1.5	46.0	27.0
	DstR	47.8	7.8	5.3	8.9	16.0	4.3	3.7	31.0	22.0
	HS	4.0	10.5	4.7	3.6	2.8	1.1	1.5	0.2	7.0
	DgS	5.8	9.7	5.0	8.7	42.9	0.8	0.8	0.3	1.4
	DstS	6.7	12.6	7.4	4.8	2.9	1.0	0.6	0.3	9.0
July 3, 1965 Maturation	HR	21.2	5.6	7.6	4.0	6.0	2.2	5.3	3.7	1.7
	DgR	12.7	7.6	6.2	4.6	17.0	2.9	1.1	16.0	16.0
	DstR	46.7	6.5	4.7	5.6	17.0	4.2	3.8	5.6	17.0
	HS	6.1	11.2	5.8	2.1	2.2	1.6	1.3	0.2	1.2
	DgS	7.6	10.3	6.3	7.6	2.6	0.8	0.7	0.3	1.5
	DstS	6.0	10.6	8.9	3.6	2.6	1.0	0.6	0.2	1.3

Table 27. Microbial numbers in Lotung soil at various stages of growth of rice in the field (second trial, spring crop rice, 1965).

Time of sampling	Sample Code	Bacteria	Actinomyces	Fungi	Anaerobes	Sulfate reducers	Cellulose Decomposers		Denitrifiers	Ammonifiers
							Aerobic	Anaerobic		
		x 10^6	x 10^5	x 10^3	x 10^5	x 10^3	x 10^6	x 10^6	x 10^6	x 10^5
Mar. 3, 1965 Seedling bed	S	13.9	4.8	24.0	3.4	4.8	1.3	0.9		1.1
	Rs	180.7	14.2	263.8	40.5	54.0	9.1	1.2	0.49	11.0
	RsS	90.3	13.3	42.4	9.5	92.0	2.1	1.6	0.26	92.0
Mar. 24, 1965 Tillering	HR	29.2	97.3	114.5	23.3	3.3	36.6	17.2	0.48	1.7
	DR	23.0	42.8	89.9	74.4	14.0	54.8	17.4	3.50	1.7
	HS	52.8	28.2	54.0	11.4	7.0	5.3	3.7	0.14	4.7
	DS	39.6	25.8	13.2	11.6	11.0	4.8	1.4	0.17	1.7
April 12, 1965 Max. tiller no. stage	HR	472.3	21.2	141.7	19.8	4.6	7.5	1.3	1.70	2.5
	DR	148.9	33.8	75.1	8.7	4.8	5.6	0.9	2.10	9.5
	HS	218.3	24.1	129.0	22.9	7.9	1.5	1.3	0.04	1.4
	DS	59.1	38.4	53.2	31.3	8.4	1.6	0.8	0.06	3.9
May 7, 1965 Panicle development	HR	66.0	8.5	7.1	8.5	7.0	3.7	4.5		2.4
	DR	141.4	15.0	13.7	26.0	24.0	21.8	10.6		3.3
	HS	18.6	13.7	1.7	6.0	2.7	3.7	1.3		2.0
	DS	19.6	14.8	3.5	8.6	11.0	3.9	2.3		14.0

Table 27. (Continued.)

Time of sampling	Sample Code	Bacteria	Actinomyces	Fungi	Anaerobes	Sulfate Reducers	Cellulose Decomposers		Denitrifiers	Ammonifiers
							Aerobic	Anaerobic		
		x 10^6	x 10^5	x 10^3	x 10^5	x 10^3	x 10^6	x 10^6	x 10^6	x 10^5
May 30, 1965 Anthesis	HR	53.2	7.6	7.0	7.6	6.8	3.6	4.4	16.0	1.7
	DR	136.4	15.3	12.7	23.8	23.0	20.8	10.0	46.0	3.8
	HS	19.7	12.7	1.6	5.8	2.1	3.6	1.1	0.1	2.5
	DS	28.2	13.8	2.9	8.7	9.5	3.9	3.1	0.5	11.0
June 24, 1965 Maturation	HR	47.8	6.8	6.9	6.9	6.2	3.5	4.2	9.5	1.7
	DR	125.1	14.2	10.3	22.6	17.0	19.9	7.9	12.0	2.5
	HS	17.4	11.2	1.2	4.8	2.1	3.4	1.0	0.1	1.4
	DS	18.2	12.7	2.5	7.6	10.0	3.8	2.0	0.2	2.1