V. DISCUSSION
bacterial population on the leaves was known to influence the disease development (Goto et al., 1965; Tagami and Mizukami, 1962; and Watanabe, 1975). This hypothesis was confirmed in an experiment on the leaf angle in relation to disease development in a cultivar at maximum tillering and flag leaf stages of the crop. The horizontal flag leaves of the cultivar showed comparatively less disease than the erect leaves of the same cultivar at the maximum tillering stage when the leaves were clip inoculated, but it was vice-versa when the leaves were spray inoculated. Thus, these results indicated that erect leaves which could not retain more amount of inoculum suffered less disease than the horizontal flag leaves which could hold much inoculum because of the leaf angle and this difference in the amount of inoculum retained on the leaf surface might have influenced the disease occurrence and development even under natural field conditions.

Moreover, the horizontal leaves not only tended to increase the humidity amongst the leaves but the wounds caused by frequent contact of the leaves also might have favoured the disease spread and dissemination of the pathogen in these leaves as surmised by Kiryu and Mizuta (1955a; b).

But no relationship was found between the length of the leaf and the severity of the disease in the present study. However, a strong correlation was found between the leaf texture with that of the disease occurrence. Cultivars
According to Premalatha Dath (1974), the hairy and glabrous cultivars did not show any clear-cut differences in the internal anatomy but showed a definite relation between the number and size of hairs on the lamina with that of the disease intensity. Glabrous leaves were completely devoid of any surface hairs. Though the marginal hairs were present, they were small in size, less in number and also widely placed. In contrast, the hairy leaves had large number of different kinds of surface hairs and also large number of big marginal hairs that were closely placed. In hairy leaves these hairs might have helped either in forming an effective "catching net" for the inoculum, or by providing more entry points to the pathogen when the hairs broke due to mechanical injury as the injury caused by wind during typhoons or storms enhance the disease development as reported by several workers (Bokura, 1911; Ishiyama, 1922; Hashioka, 1951; Goto et al., 1955; Mizukami, 1966; Devadath, 1969; Premalatha Dath, 1974).

These findings have some practical significance in bringing down the disease in the field. For instance, growing of cultivars with erect glabrous leaves in large areas would considerably reduce the disease severity, thereby arresting the inoculum build-up. However, this might not help in cyclone prone areas where the plants could be injured easily. Therefore, tolerance coupled with these advantageous leaf characters would be a suitable combination for reducing the inoculum build-up and spread of bacterial blight.
Micronutrients influenced the disease development remarkably. The lesion length was increased over no micronutrients added treatment (control) when the micronutrients were supplied in different combinations. When Mn was deleted from the culture solution, the lesion length was brought down. Surprisingly when Mn was doubled, the lesion length was also brought down. The lesion length was also brought down when Cu was doubled but when Cu was deleted, the lesion length was not brought down significantly over the treatment where all the micronutrients were supplied in normal level. When the leaf tissue of the plants grown in different combinations of micronutrients were analysed for different chemical constituents, no correlation was obtained between the total phenols, total sugars, reducing sugars, non-reducing sugars, amino and total nitrogen present in the leaf tissues and the lesion development. Therefore, the mechanism by which the lesion length was either brought down or increased in different treatments is not known.

According to the work carried out at the International Rice Research Institute (1966; 1974; Ou et al., 1971), the lesion length was not influenced by the amount of nitrogen present in the soil or culture solution. On the contrary, in the present study, the lesion length was increased as the nitrogen level increased in both tolerant and susceptible rice cultivars thus confirming the earlier reports of Devadath and Padmanabhan (1970), Matsuzaki et al.
(1972) and Uma Gupta (1975). However, the rate of increase in lesion length varied in different cultivars under varying levels of nitrogen thus corroborating the results of Kondo and Komura (1953) and Central Rice Research Institute (1970).

When the leaf tissues of both tolerant and susceptible rice cultivars grown under different nitrogen levels at different intervals after inoculation were analysed for different chemical constituents, no relationship was found between the totals of different chemical constituents detected in the leaf tissues and that of the level of nitrogen applied at all the intervals after inoculation. Further, no correlation was obtained between the totals of these chemical constituents with that of the lesion development in tolerant and susceptible rice cultivars, thus confirming the recent findings of Damodaram Naidu et al. (1977).

But, according to the studies carried out at the International Rice Research Institute (1967), the tolerant cultivars had a low ratio of reducing sugars:nitrogen while the susceptible ones had a high ratio. On the contrary, Moses et al. (1975) observed a reverse ratio for reducing sugars:total nitrogen. But according to these authors, there was a relation between the ratio of total sugars:total nitrogen with that of the tolerance or susceptibility of the rice cultivars.

Moreover, the levels of nitrogen in the leaf tissue did not seem to influence the disease development. It was
presumed that high levels of nitrogen applied to the semi-dwarf rice might not directly increase disease by making the tissue more susceptible but by increasing the number of leaves for the bacterium to infect and by changes in microclimate around the plants which favoured the disease build-up (International Rice Research Institute, 1974). Therefore, it may be surmised that the totals of the different chemical components of the leaf tissue may not throw any light in understanding the specific factor (S) responsible for the increase or decrease of the disease in tolerant or susceptible rice cultivars grown in different levels of nitrogen in the present study.

Phenolic compounds and their related oxidases were known to be associated with the defense mechanisms of plants because of their general accumulation near the wounded or infected tissues and that phenols and their oxidation products were highly toxic to the pathogens. A marked post-infectional change in the phenolic compounds and its oxidases have been observed in many host-parasite relationships (Uritani, 1961; Farkas and Kirlay, 1962; Cruickshank and Perrin, 1964; Rubin and Artsikhovskaya, 1964). However, according to Byrde et al. (1960), Mahadevan (1964) and Rubin and Artsikhovskaya (1963) high level of phenol does not always mean resistance. All these foregoing reports suggest that the analysis of leaf tissues for the totals not necessarily reflect on the mechanism of tolerance or susceptibility or the decrease or increase of the disease. Further, according to Ranga Reddy and Sridhar
(1975), at any given potassium level, the total phenols were more in healthy Taichung (Native) 1 than in healthy IR 8. Following infection, the total phenols reduced in Taichung (Native) 1 as compared to its corresponding healthy leaves while in IR 8, the total phenols increased after infection than the corresponding healthy leaves. If the total phenols were responsible for the disease inhibition, Taichung (Native) 1 which was associated with more phenols prior to infection should have effectively arrested/inhibited the growth of *X. oryzae* as compared to the less susceptible cultivar IR 8, where the phenols were less prior to infection. From their results one can clearly see that there was no relation between total phenols in the leaf tissues with that of the tolerance or susceptibility of a cultivar. Such conclusions could also be safely drawn in relation to total soluble amino acids, reducing sugars and non-reducing sugars.

Further, it was felt by the present author that if one tolerant and one susceptible rice cultivar was used in the study and then compared, some differences between these two cultivars in any one of the chemical constituents viz., total phenols, reducing, non-reducing sugars, amino and total nitrogen could be obtained; but if a group of tolerant and a group of susceptible cultivars were used and then compared such differences could not be obtained as in the present investigation. Hence, it was concluded that these factors were only the associated factors which might not play a real
causal role in determining the resistance or susceptibility of the rice cultivars or the intensity of the disease development.

Therefore, studies at the cellular level with sophisticated techniques would only throw much light on the mechanism of increase or decrease of the disease in different rice cultivars rather than the analysis of the leaf tissues for the totals of different chemical constituents.

In studies relating to the multiplication of *X. oryzae* in different rice cultivars, it was observed that the pathogen could grow in the leaf extracts of tolerant as well as susceptible rice cultivars and there was no relation between the degree of tolerance or susceptibility of the rice cultivars with that of the extent of growth of *X. oryzae* in their leaf extracts thus indicating that the compound(s) responsible for the tolerance was/were not constitutive but only formed during the host-pathogen interaction. The initial bacterial population at the point of inoculation was almost the same in different cultivars inoculated with both the colony types of *X. oryzae* but the population of these colony types showed marked difference in these cultivars when assayed 9 days after inoculation as it was observed earlier by Devadath (1969).

Multiplication of the pathogen was also found to be related to the virulence of the bacterial isolate. The virulent wild colony type multiplied more than the less virulent mutant type in both tolerant and susceptible rice cultivars, thus showing a relation between the degree of virulence of the
bacterial isolate and its multiplication in the host. These results are in conformity with Devadath (1969), Premalatha Dath (1974) and Mohiuddin and Kauffman (1975). But the multiplication of both the colony types was faster in susceptible cultivar than the tolerant cultivars thus corroborating the results of Sekiya and Watanabe (1957) and Watanabe et al. (1957), Devadath (1969), Reddy and Kauffman (1973), Nziguwe (1973) and Premalatha Dath (1974). The bacterial population was higher in the compatible host-isolate system than in the incompatible host-isolate system thus corroborating the reports of Devadath (1969), Premalatha Dath (1974) and Mohiuddin and Kauffman (1975).

Confirming the results of Devadath and Premalatha Dath (1970) and Kuo et al. (1970), the bacterial exudate isolated from both the colony types, irrespective of the degree of virulence induced wilting of the rice cuttings placed in different concentrations of the exudate. As reported by Devadath and Premalatha Dath (1970), the virulent isolate (wild colony type) produced more exudate than the less virulent isolate (mutant colony type) both in vitro and in vivo.

Fan and Kuo (1972) did not observe the presence of any enzyme action or the polysaccharide did not cause any damage to the cell permeability. Since wilting was caused by even low concentrations of the exudate it was thought to be caused by toxigenic action rather than by plugging of xylem vessels. On the contrary, the same authors reported that the exudate
blocked the acid fuschine transport in rice cuttings. Moreover, the same author reported that dextran with a molecular weight of 150,000 could also cause wilting thereby indicating that the wilting induced by the exudate was non-specific phenomenon. Evidence to show that the exudate played a major role in the mechanical occlusion of the xylem vessels (Devadath and Premalatha Dath, 1970) was also supported by the observation made by the author on the occurrence of wilt symptoms in the wax coated cuttings. The wilted cuttings recovered turgor when the wax coated basal portion of the shoot was severed and then placed in water. Similar recovery was also noted when the wilted shoots from different concentrations of the test solutions were severed and then placed in water. Moreover, with an increase in the dilution of the exudate, there was a corresponding delay in the wilt production. This result further strengthened the present hypothesis.

Rice roots played an important role in the activation of the causal bacterium. From the cotton soaked with the bacterial suspension and from the cotton soaked with the bacterial suspension + soil suspension, *X. oryzae* could be recovered only during the first two weeks of incubation. But when the roots of rice seedlings touching the inoculum in the cotton were plated on the medium, growth of *X. oryzae* could be observed around the roots, till the end of the experimental period of three months, thus indicating that the rice roots might have activated the *X. oryzae* cells that were
weakened or lost their reproductive ability as reported by Mizukami (1959; 1969). The prolonged survival of the pathogen might be due to the bacterial response to sugars, amino acids or oxygen secreted by the rice roots (Wakimoto, 1957; Mizukami, 1959; 1961) that restored the reproductive ability of the pathogen.

\textit{X. oryzae} is a strict aerobe (Dye, 1962; Devadath, 1969; Uma Gupta, 1975). When two agar layers were poured on the cotton soaked with the bacterial suspension, sufficient quantity of oxygen required for the multiplication of the pathogen might not have been available thereby weakening the cells of \textit{X. oryzae}. This might also be one of the reasons why \textit{X. oryzae} could not be recovered from both the treatments where rice roots did not touch the bacterial layer.

The soil microorganisms in the soil suspension did not seem to interfere with the activation of \textit{X. oryzae} by rice roots because there was no difference in the duration of the survival of the pathogen around the rice roots that came in contact with either bacterial suspension alone or the bacterial suspension + soil suspension. It might be presumed that \textit{X. oryzae} cells in the soilwater irrigation water in the double cropped areas when come in contact with the rice roots might get activated, multiply in the rhizosphere and incite infection when they enter the host plant.

In studies on the physiology of infected hosts, a positive correlation was observed between the ratio of
chlorophyll pigments a and b and the degree of susceptibility of the rice cultivars. In the susceptible cultivars, the ratio was much higher than the tolerant cultivars in the healthy leaves and also in the green region and water-soaked region of the infected leaves. However, in the straw-yellow region of the infected leaves, such clear-cut differences between the tolerant and susceptible cultivars were not observed.

There was also some positive correlation between the ratio of chlorophyll pigments in the leaf tissue with that of the photosynthetic efficiency. In the healthy leaves and green region of the infected leaves, the two susceptible cultivars showed more ratio of chlorophyll pigments and more photosynthetic efficiency than the tolerant cultivars. But in the water-soaked region, though both the susceptible cultivars had more ratio of chlorophyll pigments, its photosynthetic efficiency was less than the tolerant cultivars. But in the straw-yellow region no such relation was observed between the ratio of chlorophyll pigments and photosynthetic efficiency in the rice cultivars.

The reasons for such inconsistency in the chlorophyll content and photosynthetic efficiency of these infected regions in different cultivars is yet to be understood.

Differences in the amount of inorganic elements in the healthy and infected leaf tissues of tolerant and susceptible cultivars were found. Total nitrogen was more in
healthy leaves than in the infected leaves of both tolerant as well as susceptible cultivars thus indicating that the pathogen might have utilized the nitrogen sources in the leaf tissues thereby resulting in lesser amounts of total nitrogen in the infected leaf tissues. However, there was no relation between the total contents of different elements viz., nitrogen, phosphorus, calcium, iron and manganese either in healthy or in infected leaf tissues and the degree of tolerance or susceptibility of the rice cultivars. For example, healthy leaves of IR 1545-339-2-26 (tolerant) and IR 8 (susceptible) had the same amount of nitrogen but in infected leaves, IR 1545-339-2-26 had lesser amount of total nitrogen than IR 8 thus corroborating the results reported from the International Rice Research Institute (1974) wherein it was seen that the levels of nitrogen in the leaf tissues did not influence the lesion development. Recently, Ranga Reddy and Sridhar (1975) also reported that there was no relations between potassium content in the leaf tissues and disease development.

In the present study, total phosphorus and potassium (except in Taichung (Native) 1) was also more in healthy leaves than the infected leaves. But iron and manganese were more in the infected leaves than in the healthy leaves. On the contrary, Misawa and Miyazaki (1972) reported that the infected leaves had high contents of total phosphorus and insoluble phosphorus in the inoculated leaves.
In the present investigation, the wild colony type was more virulent than the mutant colony type. Such difference between these colony types in virulence was noted in *X. oryzae* by Goto and Okabe (1967) for the first time and subsequently confirmed by Goto (1972), International Rice Research Institute (1972), Premalatha Dath (1974), Reddy (1974) and Raoof (1975). However, Nwigwe (1973) did not observe any appreciable differences in the virulence of the colony types.

According to Goto (1972), the polysaccharide material (exudate) produced in abundance by the virulent wild type and absence of polysaccharide in mutant type played a critical role in the expression of phage susceptibility and virulence. Experiments conducted by Devadath and Premalatha Dath (1970), Kuo et al. (1970), Fan and Kuo (1972) and the author clearly indicated that the mechanism of "wilt" phase of bacterial blight was due to the exudate which directly affected the water economy of the plant system by plugging the xylem vessels. However, it is not possible to correlate the production of exudate with the virulence of the bacterium as far as blight phase was concerned.

The answer to the question of Goto and Okabe (1967) that "why the translucent mutants of this bacterium showed only the restricted growth in xylem tissues?" would probably help to understand the host-pathogen relationship. From the above question itself it might be surmised that the capacity
of the bacterium to multiply in the xylem vessels could be correlated with virulence. Therefore, the differences that were observed in the virulence of wild and mutant colony types might be attributed for their differential capacity of multiplication in the xylem vessels of the host as it was observed in the multiplication study of wild and mutant colony types in some rice cultivars by the author.

Many workers in Japan (Kuhara, 1956; Mizukami and Seki, 1956; Mizukami, 1961; Sekizawa, 1963) reported that the density of the bacterial suspension in the inoculation tests greatly influenced the percentage of plants infected, rapidity of infection and lesion enlargement. In the present investigation, only the higher inoculum levels (1.0 and 1.5 OD) showed significantly more lesion length than the lower inoculum levels (0.1-0.6 OD). Similar trends were also observed by Premalatha Dath (1974) though the differences were not statistically significant. According to Mizukami (1961), the minimum concentration required to incite infection was $10^4$ cells/ml. According to Reddy (1974) the reaction of susceptible or resistant cultivars was not influenced by the inoculum concentration. However, the tolerant cultivars at high inoculum concentrations ($10^9$ cells/ml) reacted as susceptible and at low concentrations ($10^6$ cells/ml), they reacted as moderately resistant—moderately susceptible at the seedling stage but the fluctuations in disease reactions were not so wide at the adult plant age.
The results obtained in the present investigation were not comparable with that of Kuhara (1956), Mizukami and Seki (1956), Mizukami (1961), Sekizawa (1963) Tagami et al. (1961; 1964), Yoshimura (1963), Yoshimura and Tagami (1967), Devadath (1969) and Kuo et al. (1971) because all these authors studied the effect of concentration of the inoculum by employing spray inoculation method, wherein the density of the pathogen was found to influence the disease development. The more the concentration of the inoculum, the more would be the chances for the bacterium to survive in the leaf surface and get into the host tissue. On the contrary, in the clip inoculation method the bacterium was introduced directly into the plant system to incite infection. This might be the reason why even the dilute concentrations could also initiate infection. However, why only 1.0 and 1.5 OD produced more lesion length than other inoculum concentrations in the present study as it was also observed by Premalatha Dath (1974) was not clearly understood.

Younger the plants (45 days) and more the concentration of the inoculum used (0.1 OD) more was the lesion length produced thus corroborating the results of Devadath (1969). On the contrary, Kuo et al. (1971) reported that younger plants (6 days old) required more concentration of the inoculum than the older plants (2 months old). In the present investigation when three lower concentrations of the inoculum were used they all differed with each other significantly in
the lesion development, thus showing the effect of inoculum concentration unlike the earlier experiment where 0.1-0.6 OD failed to differ significantly.

The lesion length produced on 45 days old plants was more than 60 and 75 days old plants which indicated that the tolerance increased with an increase in the age of the plant as reported earlier (Muko et al., 1963; Kuhara and Sekiya, 1967; Goto, 1965; International Rice Research Institute, 1968; 1972; Devadath, 1969; Devadath and Padmanabhan, 1969; All-India Coordinated Rice Improvement Project, 1970; Horino and Ezuka, 1973; Ezuka et al., 1974; Kauffman et al., 1974; Premalatha Dath, 1974; Reddy, 1974; Uma Gupta, 1975).

Even within a given cultivar, the tolerance increased with an increase in the age of the host from 60 to 75 days as it was seen in IR 20, IR 8 and Taichung (Native) 1 thus confirming the results of other workers (Reitsma and Schure, 1950; Sulaiman and Ahamed, 1965; Sulaiman et al., 1965; Devadath, 1969; All-India Coordinated Rice Improvement Project, 1971; Horino and Ezuka, 1973; Miah, 1973; Ezuka et al., 1974; Watanabe, 1975) but in other cultivars, the susceptibility increased as the plants increased in age from 60 to 75 days thus corroborating the results of Devadath (1969), Devadath and Padmanabhan (1969), Mahmood and Singh (1970), Kuo et al. (1971) and Kauffman et al. (1974).
On the contrary, according to International Rice Research Institute (1965; Ou, 1966; 1970; Ou et al., 1971), none of the resistant, moderately resistant and susceptible cultivars showed a striking difference in their reaction between seedling or flag leaf stage.

The lesion length produced by the three concentrations of the inoculum did not differ significantly on any one of the cultivars indicating that the lowest concentration of the inoculum used in the present experiment was sufficient enough to incite good infection in all the cultivars. However, the interaction between age levels of the cultivars and inoculum concentrations was significant as reported by Devadath (1969) and Reddy (1974).

Results of the study on the effect of inoculum concentration on disease development in tolerant and susceptible rice cultivars under different environmental conditions revealed that irrespective of the concentration of the inoculum, the lesion development in plantings 1, 10 and 11 was higher than in plantings 4, 5 and 6. The meteorological data collected during these favourable and unfavourable periods when compared indicated that neither the number of rainy days nor the amount of rainfall nor the relative humidity had any relation with the lesion development thus corroborating the results of Premalatha Dath (1974).

Though exhaustive literature is available correlating different weather factors like favourable temperature ranges,
more amount of rainfall, more number of rainy days and high relative humidity for the occurrence of the disease, all these factors were correlated basing on the natural incidence in the field ignoring the availability of the inoculum to the rice crop. So, the present results might not be comparable with the results obtained under natural field conditions since the inoculum was introduced straight inside the host system and then the disease development was correlated with different climatic factors as it was done earlier by Premalatha Dath (1974). According to her results, it was only the temperature which played a significant role in the lesion development and the other factors like the amount of rainfall, number of rainy days, and sunshine hours per day had no relation with the lesion development, though relative humidity played only a secondary role. However, in the present study, a positive correlation was obtained between temperature ranges as well as sunshine hours per day with that of the lesion development. But no correlation was obtained with the other factors.

This discrepancy in the results might be due to the fact that she had used sufficiently higher concentration of the inoculum (1.0 OD) which when inoculated into the host tissue could overcome the effect of sunlight because of the higher population of the causal bacterial cells in the inoculum. But in the present study, very low concentrations of the inoculum were used and the population of the pathogen
in the inoculum were very less so that they could easily be adversely affected at the site of inoculation due to prolonged sunshine hours. That was the reason why the favourable periods were associated with less sunshine hours and the unfavourable periods with more sunshine hours. Further, according to Chattopadhyay and Mukherjee (1973), less sunshine hours over the growth period of the crop not only favoured the multiplication of the pathogen but also weakened the plant.

Both tolerant and susceptible cultivars responded in the same way as reflected by similar trends in lesion length, to the weather factors, irrespective of their differential susceptibility thus agreeing with the results obtained by Premalatha Dath (1974).