INTERACTION OF RICE WITH ISOLATES OF COMPATIBLE AND INCOMPATIBLE *XANTHOMONAS CAMPESTRIS* PV. *ORYZAE* AND RACES

I. EXAMINATION OF RICE WATER PORES IN COMPATIBLE AND INCOMPATIBLE INTERACTIONS WITH SCANNING ELECTRON MICROSCOPY

II. CHARACTERIZATION OF BACTERIAL MULTIPLICATION DYNAMICS

by

AILAN GUO

B.S., Shandong Agricultural University

A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Plant Pathology

KANSAS STATE UNIVERSITY
MANHATTAN, KANSAS

1988

Approved by:

Jan E Leach

Major Professor

ACKNOWLEDGMENTS

I wish to express my sincerest thanks to my major professor, Dr. J. E. Leach, for her encouragement, outstanding professional guidance, valuable suggestions and friendship. I also wish to thank my committee members, Dr. William F. Pfender, Dr. Peter P. Wong and Dr. James A. Guikema, for their advice and help. I would like to thank Timothy C. Todd and Charles A. S. Pearson, for their help in statistical analyses.

I wish to express my special thanks to the People's Republic of China, for the financial support and encouragement.

TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

Interaction of rice with isolates of compatible and incompatible *Xanthomonas campestris* pv. *oryzae* races

I. Examination of rice water pores in compatible and incompatible interactions with scanning electron microscopy

II. Characterization of bacterial multiplication dynamics

INTRODUCTION

Bacterial blight of rice (*Oryza sativa* L.), caused by *Xanthomonas campestris* pv. *oryzae*, is one of the most destructive diseases in Asia (19, 29). Recently, the disease has been observed not only in all major rice-producing countries of Asia, but also in the Sahelian countries of Africa (1), in South America (18) and in the United States (J. E. Leach, personal communication). Ou (29) reported that in Japan 300,000 to 400,000 hectares have been affected annually by the disease in recent years. Yield losses in severely infected fields range from 20 to 30%, and may on rare occasions be up to 50%. The damage is usually more severe in the tropics, such as in the Philippines and Indonesia.

Bacterial leaf blight symptoms. Bacterial leaf blight symptoms may appear as kresek or leaf blight (19, 29). Kresek usually occurs on seedlings. Infected seedlings first show green water-soaked spots. As the spots enlarge, the leaves turn grayish-green, then roll and wither. Finally, the entire young plant dies

(29). In leaf blight, lesions may start at one or both edges of the leaves. While still green, infected blades wilt and roll, then, as the disease advances, white lesions cover the entire blade. In severely diseased fields, grains may also be infected. Symptoms on the glumes are discolored spots surrounded by a watersoaked margin. At maturity, these spots are gray or yellowish white (29).

Pathogenic specialization of X. c. pv. oryzae to rice. Resistant cultivars are traditionally used to control the disease. Inheritance of resistance to bacterial blight in rice has been investigated by several workers (4, 26, 34, 28, 31, 38, 39, 40, 46). The genetics of resistance in rice to X. c. pv. oryzae was investigated in Japan; four dominant genes in rice [Xa-1, Xa-2, Xa-3 (Xa-w), and Xa-kg] were identified (4, 26, 34). Five additional genes (Xa-4, xa-5, Xa-7, Xa-10, and Xa-11) have been identified at the International Rice Research Institute (IRRI, Los Banos, Philippines) (28, 31, 38, 39, 40, 46).

For many years it was unclear whether there was pathogenic specialization of X. c. pv. oryzae to rice (19). When the previously resistant rice cultivar Asakaze was unexpectedly and severely attacked by bacterial blight in Japan, scientists realized that the bacterial pathogen was variable. Attempts have been made to study pathogenic specialization in the last twenty years. Five bacterial groups were identified based on differential reactions to a set of rice cultivars in Japan (16, 35, 45, 9). Bacterial isolates collected in the Philippines in the 1960s and 1970s were

evaluated on three selected cultivars. Four bacterial groups were recognized based on a set of rice cultivars with different genes for resistance (21). Through repeated tests and various experimental designs and analyses (22), the lesion length was found to be significantly different between compatible and incompatible combinations. Thus, the interactions between rice cultivars with specific resistance genes and X. c. pv. oryzae are race-specific. Several rice differential systems for X. c. pv. oryzae have been established. For example, there are five bacterial groups in Japan, six races in the Philippines, and nine bacterial groups in Indonesia (10, 23, 24). Since the differential cultivars and bacterial groups used in each system were not the same, these bacterial groups or races are not directly comparable.

The differential system established at IRRI was used in our research. In this system, six races of the pathogen have been identified based on their specific interactions with differential rice cultivars. Four races (race 1, race 2, race 4, and race 6), and four rice cultivars [IR20 (Xa-4), Cas 209 (Xa-10), IR1545-339 (xa-5) and DV85 (Xa-7, xa-5)], were used in our studies (Table 1). IR20 is resistant only to race 1; Cas 209 is resistant only to race 2; IR1545-339 is resistant to both race 1 and 2; and DV85 is resistant to race 1, 2, and 4.

Gene for gene system. In race-specific interactions it has been suggested that the specificity of compatibility and incompatibility is regulated by gene-for-gene interactions (3).

According to the gene-for-gene hypothesis, incompatibilility (resistance) results from the interaction of the product of a specific dominant avirulence gene in the pathogen with the product of a dominant resistance gene in the host. That is, the expression of disease resistance in the plant host is dependent on the occurence of dominant alleles in the host for resistance and in the pathogen for avirulence. Thus, in gene-for-gene relationships, avirulence is a positive function, rather than the loss of a function. Compatibility is allowed by a lack of recognition for incompatibility.

Because X. c. pv. oryzae has been demonstrated to exhibit pathogenic specialization to rice, it has been suggested that the specificity of compatibility and incompatibility is regulated by the gene-for-gene interactions described above (3). Therefore, we can predict that for each resistance gene described in rice, there should be a corresponding gene in X. c. pv. oryzae governing avirulence. For example, X. c. pv. oryzae race 2 would have two or three avirulence genes which correspond with host resistance genes Xa-10, xa-5, and possibly Xa-7 (Table 1). Recently, Kelemu and Leach cloned an avirulence gene from race 2 which conditioned incompatibility on Cas 209 (J. E. Leach, personal communication). When the cloned race 2 avirulence gene was introduced into a race 6 isolate (compatible on Cas 209), the resulting interaction phenotype was changed from compatible to incompatible on only cultivar Cas 209. Therefore, the cloned avirulence gene behaved as a specific

Table 1. Interactions of rice cultivars with Xanthomonas campestris pv. oryzae isolates[a]

Rice cultivar (R gene)	X. c. pv. oryzae isolate (race)[b]				
	PXO61(1)	PXO86(2)	IRN793(2)	PXO71(4)	PXO99(6)
CAS 209 (Xa-10)	C	I	I	C	C
IR20 (Xa-4)	I	C	C	C	C
IR1545-339 (xa-5)	I	I	I	C	C
DV85 (xa-5, Xa-7)	I	I	I	I	C

[a]Adapted from Mew, 1987 (7).

[b]C=compatible ; I=incompatible.

dominant character in the interaction of rice and X. c. pv. oryzae.

Terminology. In phytobacteriology, the term avirulent is often used to describe a pathogen that has lost pathogenicity to its host or the ability to colonize the host, that is, it has lost a basic compatibility factor (6). This is clearly distinct from the use of avirulence in the context of the gene-for-gene hypothesis reviewed by Ellingboe (3). To avoid confusion, in this thesis, I will generally avoid the use of the term avirulence unless it is in the context of others' work. Here, I will describe bacterial isolates in terms of their interactions (compatible or incompatible) with specific host cultivars.

Ultrastructure of the X. c. pv. oryzae/rice interaction. X. c. pv. oryzae, like other phytopathogenic bacteria, lacks active mechanisms for penetrating the protective barriers of rice (42). The bacteria enter the rice host through hydathodes and wounds. Horino (12, 13) compared the water pore morphology between two hosts of X. c. pv. oryzae, Oryza sativa and Leersia japonica (a weed host), and found that the outer ledges of water pores on the upper side of O. sativa were distinctly shorter compared with those of L. japonica. Thus, it was suggested that L. japonica water pores were less available to penetration by the bacteria and, therefore, more resistant. This type of resistance is preformed and passive, and not likely to be involved in race-specific resistance.

To study active and inducible host resistant mechanisms,

ultrastructural changes of hydathodes and tissues of rice plants were examined in compatible and incompatible interactions with X. c. pv. oryzae isolates (7, 8, 11, 44). For example, transmission electron microscopy (TEM) studies of incompatible combinations revealed that by 3 days after inoculation bacterial cells were irregular in shape and were enveloped by abundant fibrillar material (FM) (8, 10). On the contrary, in the compatible combination, FM was not observed until 20 days after inoculation and bacteria appeared to be normal in shape and to multiply actively. Horino (8) concluded that FM originated from the host cell wall and cytoplasm because 1) no FM was observed in cultured bacteria, 2) the bacterial capsule was different from FM in ultrastructure, and 3) no FM was observed around cultured bacterial cells even if the cells were treated with a bacteriocidal chemical (7). Thus, he suggested FM might participate in the defense of host tissue against incompatible pathogens.

Mew et al. (20) suggested that water pores of the hydathode were not only portals of entry for X. c. pv. oryzae, but were also involved in the specificity of rice cultivar/X. c. pv. oryzae interaction. When rice cultivar Cas 209 was spray-inoculated with isolate PXO86 (an incompatible combination), the water pores were sealed off with an exudate by 24-48 h after inoculation. Bacterial cells were embedded in the exudate. On the other hand, no exudate was observed in a compatible combination with isolate PXO61. PXO61 cell numbers increased significantly and formed masses around or inside the opening of the water pores. Bacterial cells in the

incompatible combinations did not increase. In this study, bacterial numbers were determined at 24 and 48 h after inoculation; the number of water pores with exudate were not quantified in compatible or incompatible interactions. Though the study indicated that there is site specificity for phytopathogenic bacterial penetration through natural openings, it did not reveal whether or not the water pore exudate was involved in race-specific resistance of rice to X. c. pv. oryzae. Thus, a quantitative measure of water pore exudate formation in compatible and incompatible combinations of rice and X. c. pv. oryzae is needed to determine if water pore plugging is a general response in race-specific resistance induction.

Growth dynamics of X. c. pv. oryzae in compatible and incompatible interactions with rice. Another approach to study resistance mechanisms is to monitor bacterial growth dynamics in compatible and incompatible interactions. In most dicotyledonous plants race-specific resistance to bacteria appears in the form of a hypersensitive response (HR) (15, 36, 37). In general, bacterial multiplication in an incompatible combination is inhibited and host tissues rapidly collapse and desiccate. In the rice / X. c. pv. oryzae interaction, a typical HR has not been detected (30). Mew (19) indicated that resistance in the interaction is not always clear-cut, that is, resistance and susceptibility are both relative terms and are determined by measuring and comparing lesion length or lesion area. The effects of resistance on multiplication and spread

of X. c. pv. oryzae have been investigated by several laboratories (25, 30, 32, Barton-Willis et al., appendix A), but the results are controversial. It is clear from all studies that X. c. pv. oryzae cells multiply in rice leaves in both compatible and incompatible interactions. Parry and Callow (30) found bacterial numbers per 10-cm leaf section in the compatible interaction were only fiv-to ten-fold of that in the incompatible interaction. Thus, bacteria multiplied to the almost same extent in both interactions, and yet lesions were much longer in compatible than in incompatible interaction (30). They also assessed spread of bacteria in infected leaves by scoring for presence of bacterial ooze around the ends of 1 cm leaf sections and observed little difference in the spread of bacteria. They concluded that the major difference between compatible and incompatible interactions was in the response (i.e., symptom expression) of the rice plant, not the multiplication and spread of bacteria. They sampled plants and examined bacterial numbers within 210 h (8.75 days) after inoculation. Later work (Barton-Willis et al., appendix A) indicated that differences in bacterial growth trends of compatible and incompatible races may not be apparent until after this time (8-12 days).

In incompatible interactions, it has been demonstrated that bacterial numbers were 100-1000 fold less than those in compatible interactions (25, 32, Barton-Willis et at., appendix A). Mohiuddin and Kauffman (25) found no bacterial population differences in compatible or incompatible combinations if leaf samples were taken only at the inoculation point. However, at 1-3 cm basal to the

inoculation point, bacterial numbers in the compatible interaction were much higher than those in the incompatible combination and 100-fold (cfu/5 mm disc) differences in bacterial numbers were observed. Using whole leaf samples differences of growth more than 1000 fold (cfu/leaf) were often observed (Barton-Willis et al., appendix A).

Not only were multiplication rates higher in compatible interactions with rice, but the spread of X. c. pv. oryzae in compatible interactions was much faster than in incompatible interactions (32, Barton-Willis, et al. appendix A). Barton-Willis et al. (appendix A) monitored spread of bacteria in rice leaves by assessing bacterial numbers in 2 cm sections taken up and down the leaf from the inoculation site. They found that bacteria within compatible interactions spread more rapidly than those in incompatible interactions. Further, bacterial numbers per 2-cm section in compatible interactions were higher than those in incompatible interactions in each section 4-12 days after inoculation. The results suggested that resistance in rice to X. c. pv. oryzae was reflected in reduced bacterial multiplication and spread. Lesion lengths, in general, were positively correlated with bacterial numbers (Barton-Willis et al., appendix A). To summarize, in contrast to the conclusions that the major difference between compatible and incompatible interactions was in the response of the rice plant not the multiplication and spread of bacteria (30), more detailed studies indicated that resistance in the X. c. pv. oryzae rice interactions is characterized by reduced lesion lengths and restricted bacterial multiplication and spread (32, 33, Barton-

Willis et al., appendix A).

Effects of mixed inoculation on bacterial growth and symptom expression in rice. In gene-for-gene systems it is hypothesized that incompatibility is the specifically determined trait and that compatibility results from the passive failure of host cells to detect the bacteria and initiate a hypersensitive defense response (14). If this is true, then the incompatible trait should be physiologically predominant to compatibility. This has been observed in other interactions. For example, in the Pseudomonas syringae pv. glycinea /soybean interaction, when bacteria from compatible and incompatible races are infiltrated into soybean leaves at 1:1 ratios, a hypersensitive response and restriction of bacterial populations (similar to that induced in leaves inoculated only with the incompatible race) are observed (17). This has been further confirmed by the cloning of an avirulence (incompatibility) gene from a race 6 P. s. pv. glycinea isolate which conferred race 6 incompatibilities when transferred to other P. s. pv. glycinea races (41). Thus, avirulence (incompatibility) is a positive function, and when bacteria encoding this function are introduced into hosts with corresponding resistance genes, a resistant response should be observed.

Firm conclusions as to whether resistance is physiologically predominant to susceptibility in mixed inoculations of rice with X. c. pv. oryzae isolates can not be drawn. A protective effect (reduction in lesion length and restriction of virulent bacterial

populations) was observed when rice leaves were inoculated with mixtures of an avirulent (not pathogenic to any cultivar) mutant and its virulent parent (5). Also, when rice plants were inoculated with mixed inocula of a virulent isolate and a less virulent isolate, lesion lengths were significantly reduced in direct proportion to the ratio of virulent and less virulent cells in the inoculum (2). However, these were not race-specific interactions because the less virulent isolate were less aggressive to all cultivars, regardless of genotype.

Introduction of 1:1 mixtures containing bacteria of compatible and incompatible races into rice leaves (race-specific interactions) did not reduce lesion lengths compared with the singly inoculated compatible control (30, 33). Bacterial numbers were not measured (30) or were not different from single control inoculations when 0.5 cm discs were sampled at the inoculation point (33). Similar results were obtained in compatible and incompatible combinations with two rice cultivars (Cas 209 and IR1545-339) (Barton-Willis et al., appendix A). In both hosts, inoculations with 1:1 ratios resulted in lesions similar to the compatible control. Multiplication of isolates representing the compatible interactions was the same in mixed and individual inoculations. However, multiplication of bacteria of incompatible races was inhibited in mixed inoculations more than when the isolates (incompatible) were inoculated alone. To determine if the inhibition of incompatible isolates was the result of competition between compatible and incompatible isolates, a host (IR8) which was susceptible to

isolates of both races (1 and 2) was inoculated with mixtures and individual isolates. Populations in mixed inoculations followed a pattern similar to that observed in interactions of race 1 and 2 with Cas 209. The race 1 (compatible to IR8 and Cas 209) isolate in mixed inoculations increased at a rate equal to that observed when the isolate was inoculated alone, while populations of the race 2 (compatible to IR8) isolate in mixed inoculations were reduced. The authors concluded that competition between the isolates in plants was occurring and that this confounded any interpretations of physiological dominance of resistance.

In the rice/X. c. pv. oryzae interaction, the hypersensitive response (HR) is absent (30). The HR is a rapid response, that is, it is completed within 18-24 hr and bacterial growth is restricted within that time (36). In incompatible interactions with rice, it was demonstrated by infiltrating leaves with different bacterial numbers that bacteria multiply until a threshold level of 10^7-10^8 cfu/leaf is reached (a threshold level), then multiplication is restricted (Barton-Willis et al., appendix A). It is possible that in mixed inoculations, nutritional competition between isolates prevents host recognition because the isolate from the incompatible race can not reach the level necessary for resistance induction.

To avoid competition between isolates such that the physiological predominance of resistance could be addressed, three approaches were considered: (1) mixed inoculation of rice with higher ratios (greater than 1:1) of incompatible : compatible races, (2) inoculation of plants with isolates of incompatible races prior

to challenge by bacteria of compatible races, (3) using host isolines (which differ only in those resistance genes of interest) in combinations with isogenic bacteria (which differ from the wild type only in their resistance inducing capabilities) to compare bacterial growth in mixed inoculations. Although approach (3) would give more definitive answers, isogenic bacteria are not yet available and near-isogenic lines have just recently been developed at IRRI (27). Therefore, in this study, I used the first and second approaches.

Two groups provided preliminary evidence that approaches (1) and (2) might give bacteria of the incompatible race a competitive advantage such that the threshold level of bacteria needed for resistance induction might be reached before competition inhibited growth. Horino (8) observed that if rice leaves were first inoculated with an isolate of an incompatible race followed by a challenge with an isolate of a compatible race, symptom development in rice leaves was reduced. He did not study the effects of pre-inoculation with incompatible or compatible races on bacterial populations. Reddy and Kauffman (33) observed a 65% reduction in lesion lengths if leaves were inoculated 20:1 (incompatible to compatible) ratio of bacteria. The bacterial population of the compatible race in 5 mm leaf discs was reduced by 10-fold in comparison with a singly inoculated control (compatible alone). Populations of bacteria of the incompatible race were not affected. In these studies, resistance (reduction in symptoms) was the observed phenotype when bacteria of incompatible races were

introduced into rice leaves either at a higher ratio then compatible races (33) or prior to inoculation with compatible races (8). The effects of these treatments on bacterial growth rates and final populations, however, were not thoroughly examined.

Objectives. This study includes two parts: I. an examination of rice water pores in compatible and incompatible host/pathogen interactions with scanning electron microscopy, and II. a characterization of bacterial growth dynamics in the rice/X. c. pv. oryzae race interaction. In Part I, my objectives were: (1) to determine if water pore exudate formation is a resistance mechanism, and, if so, (2) to determine if it is a general response in race-specific resistance induction. In the second part, my objective was to study the effect of rice resistance on the growth of X. c. pv. oryzae and on lesion development in the host in mixed and time-interval inoculations.

LITERATURE CITED

1. Awoderu, V. A., and John, V. T. 1984. Occurrence of bacterial leaf blight on rice in four Sahelian countries: Senegal, Mali, Niger and Upper Volta. WARDA Tech. Newsl. 5(1):36-39.

2. Devadath, S. 1970. Effect of mixture of inoculations of virulent and less virulent isolates of Xanthomonas oryzae. Current Sci. 39,420.

3. Ellingboe, A. H. 1976. Genetics of host-parasite interactions. In: R. Heitefuss, P. Williams, eds, Physiological Plant Pathology. Springer-Verlag, Berlin, pp 761-778.

4. Ezuka, A., Horino, O., Toriyama, K., Shinoda, H., and Morinaka, T. 1975. Inheritance of resistance of rice variety Wase Aikoku 3 to Xanthomonas oryzae. Bull. Tokai-Kinki Nat. Agric. Exp. Stn. 28:124-130.

5. Fugii, H. 1976. Variation in virulence of Xanthomonas oryzae (3) Interaction between avirulent mutants and virulent wild-type isolates. Ann. Phytopath. Soc. Jpn. 42:526-532.

6. Gabriel, D. W. 1986. Specificity and gene function in plant-pathogen interactions. Features. 52:19-25.

7. Horino, O. 1973. Ultrastructure of Xanthomonas oryzae and its morphological changes by chemical treatment. Ann. Phytopath. Soc. Jpn. 39:14-26.

8. Horino, O. 1976. Induction of bacterial leaf blight resistance by incompatible strains of Xanthomonas oryzae in rice. Pages 43-55 in: Biochemistry and Cytology of Plant Parasite Interactions. K. Tomiyama, J. M. Daly, I. Uritani, H. Oku, and S. Ouchi, eds. Kodansha, Ltd., Tokyo. 256 pp.

9. Horino, O. 1979. Distribution of pathogenic strains of Xanthomonas oryzae (Uyeda et Ishiyama) Dowson in Japan in 1973 and 1975. Ann. Phytopathol. Soc. Jpn. 44:297-304.

10. Horino, O. 1981. A survey of geographical distribution of pathogenic groups of Xanthomonas campestris pv. oryzae. Ann. Phytopathol. Soc. Jpn. 47(1):50-57.

11. Horino, O. 1981. Ultrastructural histopathology of rice leaves infected with Xanthomonas campestris pv. oryzae on Kogyok group rice varieties with different levels of resistance at the seedling stage.

12. Horino, O. 1983. Ultrastructural observation on the water pores in Oryza sativa L. and Leersia japonica Makino in relation to the hydathodal invasion of Xanthomonas campestris pv. oryzae.

Proceedings of the Association for Plant Protection of Hokuriku 31:37-43.

13. Horino, O. 1984. Ultrastructure of water pores in Leersia japonica Makino and Oryza sativa L.: its correlation with the resistance to hydathodal invasion of Xanthomonas campestris pv. oryzae. Ann. Phytopath. Soc. Jpn. 50:72-76.

14. Keen, N. T., and Holliday, M. J. 1982. Recognition of bacterial pathogens by plants. Pp. 179-217 in Phytopathogenic Prokaryotes, Vol. 2. M. S. Mount and G. H. Lacy, eds. Academic Press, New York. 506 pp.

15. Klement, Z. 1982. Hypersensitivity. In : Phytopathogenic prokaryotes. M. S. Mount and G. H. Lacy (eds.). Academic Press, New York, pp. 149-177.

16. Kozaka, T. 1969. Control of rice diseases with resistant varieties. Agric. Hortic. 44:208-212.

17. Long, M., Barton-Willis, P., Staskawicz, B. J., Dahlbeck, D., and Keen, N. T. 1985. Further studies on the relationship between glyceollin accumulation and the resistance of soybean leaves to Pseudomonas syringae pv. glycinea. Phytopathology 75:235-239.

18. Lozano, J. C. 1977. Identification of bacteial leaf blight in rice caused by Xanthomonas oryzae in America. Plant Dis. Rep. 61:644-648.

19. Mew, T. W., 1987. Current status and future prospects of research on bacterial blight of rice. Annu. Rev. Phytopathol. 25:359-382.

20. Mew, T. W., Mew, I-pin C., and Huang, J. D. 1984. Scanning electron microscopy of virulent and avirulent strains of Xanthomonas campestris pv. oryzae on rice leaves. Phytopathology 74:635-641.

21. Mew, T. W., and Vera Cruz, C. M. 1979. Variability of Xanthomonas oryzae: specificity in infection of rice differentials. Phytopathology 69:152-155.

22. Mew, T. W., Vera Cruz, C. M., and Raina, G. L. 1982. Response of rice with different gene resistance to Xanthomonas campestris pv. oryzae. Proc. 5th Int. Conf. Plant Pathogenic Bact., Cali, Colombia, pp. 323-331.

23. Mew, T. W., Vera Cruz, C. M., and Reyes, R. C. 1982. Interaction of Xanthomonas campestris pv. oryzae and a resistant rice cultivar. Phytopathology 72:786-789.

24. Mew, T. W., Wu, S. Z., and Horino, O. 1982. Pathotypes of Xanthomonas campestris pv. oryzae in Asia. IRRI Res. Pap. Ser. 75. 7pp.

25. Mohiuddin, M. S., and Kauffman, H. E. 1975. Multiplication studies of Xanthomonas oryzae isolates on differential rice varieties. Current Sci. 44:637-638.

26. Ogawa, T., Morinaka, T., Fujii, K., and Kimura, T. 1978. Inheritance of resistance of rice varieties Kogyoku and Java 14 to bacterial group V of Xanthomonas oryzae. Ann. Phytopathol. Soc. Jpn. 44(2):137-141.

27. Ogawa, T., and Yamamoto, T. 1987. Selection of recurrent parents to develop near-isogenic lines resistant to bacterial leaf blight of rice. Jpn. Agric. Res. Q. 21:65-69.

28. Olufowote, J. O., Khush, G. S., and Kauffman, H. E. 1977. Inheritance of bacterial blight resistance in rice. Phytopathology 67:772-775.

29. Ou, S. H., 1985. Rice Diseases. Kew, England: Commonwealth Mycological Institute. 380 pp.

30. Parry, R. W. H., and Callow, J. A. 1986. The dynamics of homologous and heterologous interactions between rice and

strains of Xanthomonas campestris. Plant Pathol. 35:380-389.

31. Petpisit, V., Khush, G. S., and Kauffman, H. E. 1976. Inheritance of resistance to bacterial blight in rice. Crop Sci. 17:551-554.

32. Reddy, A. P. K. and Kauffman, H. E. 1973. Multiplication and movement of Xanthomonas oryzae in susceptible and resistant hosts. Plant Dis. Rep. 57:784-787.

33. Reddy, A. P. K. and Kauffman, H. E. 1974. Population studies of mixed inoculum of Xanthomonas oryzae in susceptible and resistant varieties of rice. Ann. Phytopathol. Soc. Jpn. 40:93-97.

34. Saksguchi, S. 1967. Linkage studies in the resistance to bacterial blight resistance gene in some rice cultivars. Phytopathology 68:461-463.

35. Sato, T., Choi, Y. S., Iwasaki, M., and Watanabe, B. 1976. Distribution of races of Xanthomonas oryzae in Kyushu. Ann. Phytopathol. Soc. Jpn. 17:42. (Abstr.)

36. Sequeira, L. 1976. Induction and suppression of the hypersensitive reaction caused by phytopathogenic bacteria in specific and non-specific components. In: Specificity in Plant

Diseases (Ed. by R. K. S. Wood & A. Graniti), pp. 289-309. Plenum Press, New York.

37. Sequeira, L. 1984. Plant-bacterial interactions. In: Encyclopedia of Plant Physiology Vol. 17 (Ed. by H. F. Linskens & J. Heslop-Harrison), pp. 187-211. Springer-Verlag, Berlin.

38. Sidhu, G. S., and Khush, G. S. 1978. Dominance reversal of a bacterial blight resistance gene in some rice cultivars. Phytopathology 68:461-463.

39. Sidhu, G. S., Khush, G. S., and Mew, T. W. 1978. Genetic analysis of bacterial blight resistance in seventy-four cultivars of rice Oryza sativa L. Theor. Appl. Genet. 53:105-111.

40. Singh, R. J., Khush, G. S., and Mew, T. W. 1983. A new gene for resistance to bacterial blight of rice. Crop Sci. 23.

41. Staskawicz, B. J., Dahlbeck, D., and Keen, N. T. 1984. Cloned avirulence gene of Pseudomonas syringae pv. glycinea determines race-specific incompatibility on Glycine max (L.) Merr. Proc. Natl. Acad. Sci. USA 81:6024-6028.

42. Tabei, H. 1977. Anatomical studies of rice plant affected

with bacterial leaf blight Xanthomonas oryzae (Uyeda et Ishiyama) Dowson. Bull. Kyushu Agric. Exp. Stn. 19:193-257.

43. Tsuchiya, K., Mew, T. W., and Wakimoto, S. 1982. Bacteriological and pathological characteristics of wild type and induced mutants of Xanthomonas campestris pv. oryzae. Phytopathology 72:43-46.

44. Tsuno, K., and Wakimoto, S. 1983. Ultrastructural investigations on the host-parasite interactions in the rice leaf parenchymatous tissue infiltrated with bacterial leaf blight and leaf streak pathogens. Ann. Phytopath. Soc. Jpn. 49:659-669.

45. Yamamoto, T., Machmud, M., Hifni, H. R. 1977. Variation in pathogenicity of Xanthomonas oryzae and classification of rice varieties based on their reaction to the pathogen. Contr. Gen. Agric. Res. Stn., Bogor, Indonesia 8:33-45.

46. Yoshimura, A., Mew, T. W., Khush, G. S., and Omura, T. 1983. Inheritance of resistance to bacterial blight in rice cultivar CAS 209. Phytopathology 73:1409-1412.

I. Examination of Rice Water Pores in compatible and incomaptible interactions with scanning electron microscopy

INTRODUCTION

The rice bacterial blight pathogen, Xanthomonas campestris pv. oryzae (Ishiyama) Dye, is primarily a vascular pathogen (10,11). The pathogen does not actively or directly penetrate host barriers, but enters the plant through hydathode water pores or wounds (10,11). Hydathodes (1-5 mm in length, 10-15 per leaf) are located near the edge of each leaf. Each hydathode contains 10 to 20 water pores, which are similar in appearance to stomata (Fig. 1A), but are about two to four times larger (7).

Once bacteria have entered a water pore, they multiply in the epithem and invade the vessels through the vascular pass (11). Horino (3,4) used transmission electron microscopy (TEM) to observe compatible and incompatible interactions between X. c. pv. oryzae isolates and rice leaf vessels. Three days after inoculation, bacterial cells within vessels in incompatible interactions were irregular in shape and enveloped by abundant host-produced fibrillar material (FM). In contrast, bacterial cells in compatible combinations appeared normal and were not surrounded by FM until 20 days after inoculation. These and another (13) TEM study suggested that production of FM in vessels might be involved in host resistance to X. c. pv. oryzae.

Mew and coworkers (7) used scanning electron microscopy (SEM)

to investigate the interactions between X. c. pv. oryzae isolates and hydathodes of resistant and susceptible rice cultivars. In incompatible combinations, bacteria were embedded in an exudate which appeared to emanate from the rice hydathode water pores by 24-48 hr after inoculation. An example of a water pore with exudate is shown in Fig. 1B. The composition of the exudate, which often sealed the pore opening, and its relation to FM are not known. Mew et al (7) did not observe exudates in compatible combinations. They found that bacterial numbers did not increase on the water pore surfaces during incompatible combinations, whereas within compatible interactions, bacteria multiplied significantly. The authors concluded that immobilization and inhibition of multiplication at the infection court were important events in resistance and that these were the result of the host water pore excretions. Because the excretions were observed only in incompatible interactions, it seemed possible that induction of the excretions might be a race-specific event. However, these studies included a limited number of rice cultivar/X. c. pv. oryzae race combinations. Therefore, our objective was to determine whether water pore exudate formation is a general response in race-specific resistance induction. To do so, we quantified water pore plugging in interactions between four rice cultivars carrying different bacterial blight resistance genes and isolates of three X. c. pv. oryzae races.

MATERIALS AND METHODS

Bacterial cultures. X. c. pv. oryzae isolates (PX061, race 1; PX086, race 2; and PX071, race 4) were obtained from T. W. Mew at the International Rice Research Institute (IRRI), Los Banos, Philippines. Bacteria were maintained on peptone sucrose agar (PSA, 12) at 28 C for routine use and stored at -80 C in 15% glycerol (6) or lyophilized for long-term storage. For experiments, bacteria from 2-day-old PSA cultures were inoculated into peptone sucrose broth and incubated overnight at 28 C with shaking. The cells were washed twice with sterile distilled water by centrifuging at 6750 g for 10 min and then adjusted to 10^8 cfu/ml in sterile distilled water.

Rice cultivars. Four rice cultivars with known genes for bacterial blight resistance [IR20 (Xa-4), Cas 209 (Xa-10), IR1545-339 (xa-5), and DV85 (xa-5, Xa-7)] were obtained from T. W. Mew at IRRI. IR20 is resistant to isolates of race 1, Cas 209 is resistant to isolates of race 2, IR1545-339 is resistant to isolates of races 1 and 2, and DV85 is resistant to races 1, 2, and 4 (8). Two seeds of each cultivar were planted in 8.9 cm square pots containing Bacto potting soil (Michigan Peat Co., Houston, TX) supplemented with fertilizer (Peter's 20-20-20, W. R. Grace, Cambridge, Mass.). The plants were grown in a greenhouse with 28-32 C/22-26 C day/night temperatures. At 2 wk past sowing, the pots containing rice seedlings were placed in trays with 2" of water.

Inoculation procedure. Rice leaves were inoculated as described by Mew (7). Prior to inoculation, 55-day-old rice plants

were placed in large plastic bags and incubated in the dark overnight in a growth chamber (28 C). Leaves were sprayed with bacterial suspensions using an atomizer (No. 152, The DeVilbiss Co., Somerset, PA) until fine droplets uniformly covered the leaf blades (7). The atomizer was held 50 to 60 cm away from leaves during spraying to avoid damage to leaf surface structures. Plants sprayed with distilled water or untreated plants served as controls. All plants were returned to bags and incubated for 1 hr at 28 C with light (175 - 185 lux from a combination of cool white fluorescent tubes and tungsten incandescent bulbs) in the growth chamber. The plants were then removed from the bags and placed in a greenhouse (28-32 C/22-26 C day/night temperatures) until sampling.

Sampling and preparation for SEM. Two leaves of plants from different pots were randomly sampled at 24 and 48 hr after inoculation for each treatment. Six sections (1 x 8 mm) containing hydathodes were cut from the leaf margin of each leaf with a razor blade. The 12 sections from each treatment were combined, fixed in 0.025 M potassium phosphate buffer (PB, pH 7.0) containing 3% glutaraldehyde for 24 hr at 4 C, washed three times with PB, and then dehydrated at room temperature in a graded ethanol series of 30%, 50%, 75%, 80%, 85%, 90%, and 95%. The sections were critical-point dried with liquid carbon dioxide, coated with platinum, and then examined with SEM (ETEC Autoscan, Perkin-Elmer Electron Beam Technology, Hayward, CA). Individual water pores (between 50-400) of several hydathodes were counted and scored for presence or absence of plugs. Since sections were pooled, treatments within an

experiment could not be compared statistically. In order to compare treatments, experiments (replicated over time) were analyzed using an augmented randomized complete block design (1,2). In this design, experiments (blocks) were augmented with additional treatments. There were two kinds of treatments, those that occurred once in every block (standard treatments) and those that occurred in only a portion of the blocks (new treatments). Cas 209 plants treated with X. c. pv. oryzae PX086 (incompatible) or water served as standard treatments for all analyses. First, an analysis of variance (9) of the data (percentage of water pores with exudate) from the 48 hr samplings of Cas 209 plants was calculated to determine if treatments on that cultivar were different (Experiments 1, 2, and the Cas 209 portion of 3). Then, the 48 hr data from all experiments were analyzed to determine if there were any cultivar/treatment interactions.

RESULTS AND DISCUSSION

Exudate was observed in some water pores in all treatments, including compatible and incompatible interactions or water inoculation (Table 1). The overall average percent of water pores plugged in the 48 hr data from Cas 209 was not greater for treatments with bacteria from incompatible races (20% plugged) than that from treatments with compatible races (16%) or water (23%). Further, the exudate was not induced by the inoculation procedure, because water pore plugs were also present on healthy, untreated Cas 209 leaves (Table 1, Expt. 2). Analysis of the 48 hr data

(percentage of water pores with exudate) from Cas 209 treatments indicated no significant difference ($P = 0.05$) on that cultivar among any treatments. In addition, analysis of the 48 hr data from all cultivars indicated no significant difference ($P = 0.05$) among any combinations of cultivars and treatments. Thus, although counts varied from treatment to treatment and experiment to experiment, the numbers of water pore plugs were not correlated with any interactions between rice cultivar and incompatible or compatible bacteria or water. Similar results were observed when cultivar IR20 was treated with PXO86 (compatible, 23%), PXO61 (incompatible, 16%), and water (11%).

The number of pores with exudate did not differ among treatments with bacteria whether sampled at 24 or 48 hr. Further, the number of plugs present in any treatment at either time did not exceed that of the water or untreated controls (Table 1). To determine if differential plugging was dependent on experimental conditions other than those described above, variables such as plant age (4-5 wk vs. 7-8 wk), bacterial concentrations (10^6 vs. 10^9 cfu/ml), humidity levels (70% vs. 100% RH), and tissue dehydration procedures [critical point drying vs. freeze drying (5)] were tested (data not shown). Although the percentage of water pores with exudate varied from treatment to treatment, again no difference that would indicate specific induction by any treatment was observed. For example, when Cas 209 leaves were treated with PXO86 were incubated at 100% or 70% RH for 24 hr, 13% or 11% plugged water pores were observed, respectively.

The SEM study by Mew et al (7) indicated that incompatible interactions between X. c. pv. oryzae isolates [races 0 (avirulent), 1, and 2] and rice cultivars (Cas 209 resistant to race 2; and TN-1 susceptible to races 1 and 2) were characterized by induction of a host exudate which emanated from and sealed off water pores. Water pore plugs were not observed in compatible interactions. These studies suggested that exudate formation was 1) involved in resistance and 2) was specifically induced in an incompatible combination. Our results indicate that induction of water pore exudate induction was not specific to incompatible X. c. pv. oryzae/rice interactions as had previously been suggested. Further, because the percentages of water pores with exudate were similar in treated versus untreated tissues, water pore plugging does not appear to be a general resistance mechanism in rice to X. c. pv. oryzae.

LITERATURE CITED

1. Federer, W. T. 1961. Augmented designs with one-way elimination of heterogeneity. Biometrics 17:447-473.

2. Federer, W. T., and Raghavarao, D. 1975. On augmented designs. Biometrics 31:29-35.

3. Horino, O. 1976. Induction of bacterial leaf blight resistance by incompatible strains of Xanthomonas oryzae in rice. Pages 43-55 in: Biochemistry and Cytology of Plant Parasite Interactions. K. Tomiyama, J. M. Daly, I. Uritani, H. Oku, and S. Ouchi, eds. Kodansha, Ltd., Tokyo. 256 pp.

4. Horino, O. 1981. Ultrastructural histopathology of rice leaves infected with Xanthomonas campestris pv. oryzae on Kogyok group rice varieties with different levels of resistance at the seedling stage. Ann. Phytopathol. Soc. Jpn. 47:501-509.

5. Humphreys, W. J. 1975. Drying soft biological tissue for scanning electron microscopy. Scanning Electron Microsc. 2:707-714.

6. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor

Laboratory. Cold Spring Harbor, NY. 545 pp.

7. Mew, T. W., Mew, I-pin C., and Huang, J. D. 1984. Scanning electron microscopy of virulent and avirulent strains of Xanthomonas campestris pv. oryzae on rice leaves. Phytopathology 74:635-641.

8. Mew, T. W. 1987. Current status and future prospects of research on bacterial blight of rice. Annu. Rev. Phytopathol. 25:359-382.

9. Snedecor, G. W., and Cochran, W. G. 1980. Statistical Methods. 7th ed. Iowa State University Press, Ames, IA. 593 pp.

10. Tabei, H. 1967. Anatomical studies of rice plant affected with bacterial leaf blight. Ann. Phytopathol. Soc. Jpn. 33:12-16.

11. Tabei, H. 1977. Anatomical studies of rice plant affected with bacterial leaf blight Xanthomonas oryzae (Uyeda et Ishiyama) Dowson. Bull. Kyushu Agric. Exp. Stn. 19:193-257.

12. Tsuchiya, K., Mew, T. W., and Wakimoto, S. 1982. Bacteriological and pathological characteristics of wild types and induced mutants of Xanthomonas campestris pv. oryzae.

Phytopathology 72:43-46.

13. Tsuno, K., and Wakimoto, S. 1983. Ultrastructural investigations on the host-parasite interactions in the rice leaf parenchymatous tissue infiltrated with bacterial leaf blight and leaf streak pathogens. Ann. Phytopathol. Soc. Jpn. 49:659-669.

TABLE 1. Percentage water pores with exudate after treatment of rice cultivars Cas 209, IR1545-339 or DV85 with *Xanthomonas campestris* pv. *oryzae* isolates (PXO86, PXO61, or PXO71) or water.

Rice cultivar	Bacterial isolate	Interaction phenotype[b]	% water pores with exudate at postinoculation time[a] 24 hr	48 hr
Experiment 1				
Cas 209	PXO86	I	21 (13/62)	35 (44/127)
	PXO61	C	14 (35/249)	31 (68/219)
	Water	-	16 (19/117)	44 (53/120)
Experiment 2				
Cas 209	PXO86	I	6 (6/97)	10 (23/240)
	PXO61	C	6 (7/119)	14 (23/165)
	Water	-	11 (9/80)	13 45/358)
	No treatment	-	14 (15/107)	14 (28/202)
Experiment 3				
Cas 209	PXO86	I	ND[c]	14 (28/199)
	PXO71	C	ND	3 (5/152)
	Water	-	ND	11 (23/203)
Pr>F for all treatments on Cas 209				0.57[d]
IR1545-339	PXO86	I	2 (1/60)	17 (19/112)
	PXO71	C	8 (4/51)	9 (6/65)
	Water	-	ND	2 (1/50)
DV85	PXO86	I	15 (19/129)	10 (16/157)
	PXO71	I	11 (9/83)	5 (8/148)
	Water	-	ND	11 (6/55)
Pr>F for all treatments on all cultivars				0.62[e]

[a]Individual water pores with and without exudate were counted. Data in parentheses = number of water pores with exudate/total water pores counted.
[b]I = incompatible; C = compatible.
[c]ND - not determined.
[d]Cas 209 - treatment combinations (percentage water pores with exudates at 48 hr samplings) were not significantly different at P = 0.05.
[e]No cultivar-treatment combinations at 48 hr samplings were significantly different at P = 0.05.

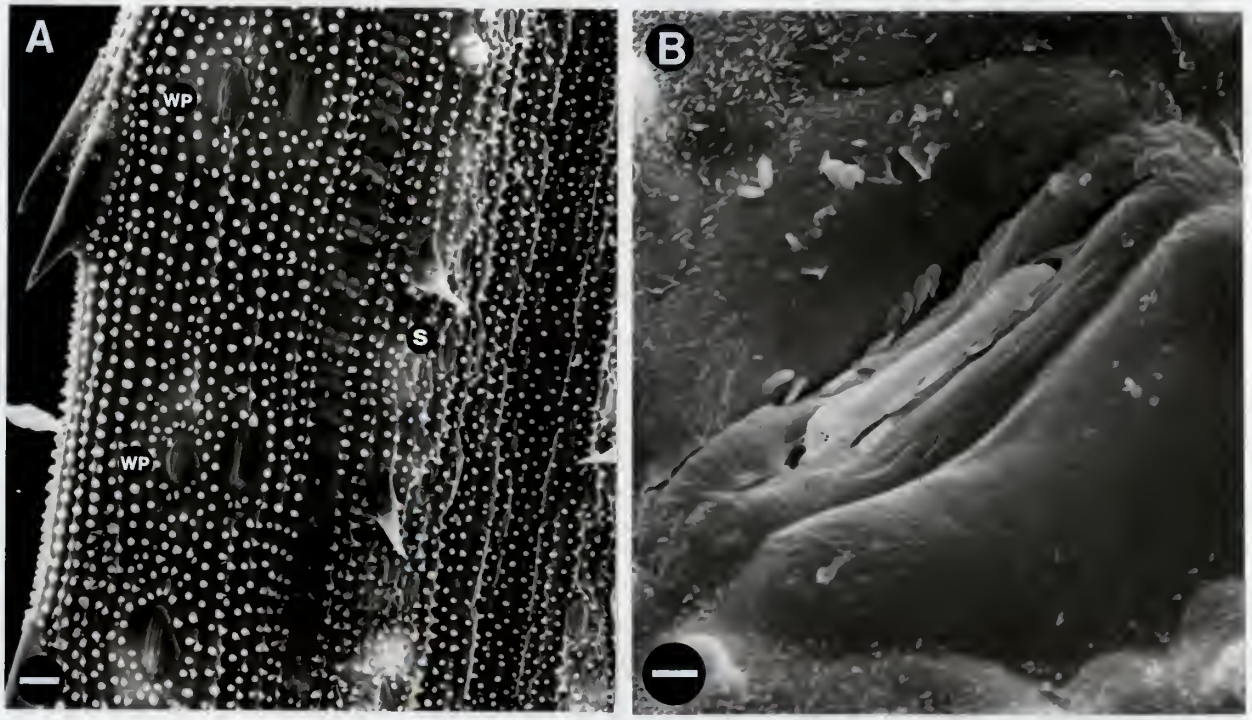


Fig. 1. Scanning electron micrographs of Cas 209 rice leaf surfaces. A, Leaf section showing stomata (s) and a portion of a hydathode with several water pores (wp). Bar = 20 m. B, Leaf section 24 hr after inoculation with Xanthomonas campestris pv. oryzae isolate PX086. Bacteria can be seen on the surface of the water pore. An exudate plugs the water pore opening. Bar = 2 m.

II. Characterization of bacterial multiplication dynamics in the rice/*Xanthomonas campestris* pv. *oryzae* race interaction

Introduction

Pathogenic specialization of *Xanthomonas campestris* pv. *oryzae*, the bacterial blight pathogen, has been demonstrated on rice cultivars with specific resistance genes (for review, see 9). Expression of resistance in interactions of *X. c.* pv. *oryzae* races with rice is not qualitative, as is the typical hypersensitive response (HR) observed in many dicot/bacterial race-specific interactions (5). Instead, resistance is quantitative and is measured by a reduction in lesion lengths (9, 13) and as a restriction in bacterial multiplication and spread (10, 16, Barton-Willis *et al.*, appendix A).

It is not clear whether resistance in rice to incompatible *X. c.* pv. *oryzae* races is physiologically predominant to disease expression in mixed-race inoculations. In race-specific interactions between dicotyledonous hosts and bacteria where resistance is expressed as an HR, if mixtures (1:1) of bacteria from incompatible and compatible races are used as inocula, the resistant (incompatible) phenotype is physiologically predominant (6). The response is rapid and is characterized by tissue collapse, restricted multiplication of bacteria from both races and, often, accumulation of compounds associated with resistance (eg. phytoalexins) (1, 6, 7). When rice leaves were inoculated with 1:1 mixtures (compatible to incompatible) of bacterial races, the

resulting lesion lengths were not significantly different from those observed in compatible interactions (16, 14, Barton-Willis et al., apendix A). Barton-Willis and coworkers (appendix A) found that multiplication of isolates of incompatible races was restricted after inoculation with 1:1 mixtures much earlier and more dramatically than observed in incompatible controls (incompatible alone). Growth of isolates from compatible races was the same whether in 1:1 mixtures or when inoculated alone. Similar growth patterns were observed when a host cultivar susceptible to both races was inoculated with isolates of each race individually and with 1:1 mixtures. That is, in mixtures, whereas one isolate grew at a rate equal to that observed when it was inoculated alone, the other isolate's growth was severely restricted by comparison with control individual inoculations. These results indicated that competition existed between X. c. pv. oryzae isolates in rice leaves and that this competition confounded any conclusions about the physiological predominance of resistance.

When a higher ratio (20:1, incompatible:compatible) was used as mixed inocula, 16 observed a 65% reduction in lesion lengths on rce. Similarly, Horino (2) demonstrated that if rice leaves were first inoculated with isolates of incompatible races and then, after 0-4 days, were challenged with isolates of compatible races, symptom development was reduced when compared to that observed when isolates of compatible races were inoculated alone. The results of Horino (2) and Reddy and Kauffman (17) could be interpreted in two ways: 1) if isolates of incompatible races reached a threshold level

resistance was induced, and, once induced, was dominant; or 2) isolates of the incompatible race were given a competitive advantage by introducing higher numbers or by growth after pre-inoculation. Because the authors did not measure bacterial populations in whole leaves, whether the effects on lesion lengths are from competition or resistance is not clear.

In an attempt to clarify whether competition or expression of resistance was responsible for the reduction in lesion lengths, we compared bacterial multiplication and final bacterial populations and lesion development in whole rice leaves after 1) inoculation with 10:1 and 100:1 bacterial ratios (incompatible to compatible) and 2) preinoculation with bacteria of an incompatible race prior to challenge with bacteria of a compatible race. In addition, we tested different cultivar/isolate combinations than were used by Barton-Willis *et al*. (appendix A).

MATERIALS AND METHODS

Bacterial strains. X. c. pv. oryzae isolates IRN793^{Rif} (resistant to rifampicin, race 2), hereafter designated I2, and PX099^{Sm} (resistant to streptomycin, race 6), hereafter designated C6, were obtained from H. Leung [the International Rice Research Institute, Los Banos, the Philippines (IRRI)]. Bacterial cultures were maintained in 15% glycerol (8) at -70 C. The cultures were recovered on peptone sucrose agar (18) and transferred to casein glucose peptone agar (CPG, 4) medium containing rifampicin or streptomycin, respectively.

Rice plants. Rice seed was obtained from T. Mew at IRRI. The rice cultivars used, IR1545-339 (xa-5) and Cas 209 (Xa-10), were resistant to isolate I2, but susceptible to C6. Two seeds were planted in each square 8.9 cm pot containing Bacto potting soil (Michigan Peat Co., Houston, Tx) supplemented with complete fertilizer (Peter's 20-20-20, W. R. Grace, Cambridge, MA.). Plants were greenhouse grown (28-32 C days and 22-26 C nights). After 2 weeks, pots containing seedlings were placed in flats and flooded continually with 2" water.

Experimental Design. Experiments were set up in a completely randomized design with four replications per treatment. In addition, each experiment was repeated twice. The initial bacterial growth rates (slopes) were calculated from the linear portions of

the growth curves (days 2 through 6 for Cas 209 and days 0 through 6 for IR1545-339, Fig. 1-7) and compared by orthogonal contrasts (t test). Data presented are from one representative experiment.

Inoculation and sampling of rice leaves. Bacteria grown at 28 C for 2 days on CPG containing streptomycin or rifampicin were inoculated into peptone sucrose broth (18) and incubated overnight at 28 C with shaking (120 rpm). The cells were harvested by centrifugation at 6750 g for 10 min and then washed twice by centrifugation in sterile distilled water. Finally, the cells were resuspended in sterile distilled water and adjusted to 1 X 10^{10} cfu/ml (100 Klett units, Klett-Summerson photoelectric colorimeter, Klett Mfg. Co., Inc., N.Y.). For inoculation with a single bacterial isolate, an equal volume of sterile distilled water was added to the bacterial suspension to yield 5 X 10^{9} cfu/ml. Mixed inocula were prepared by combining each individual bacterial suspension (1 X 10^{10} cfu/ml) in 1:1, 10:1, or 100:1 ratios (incompatible to compatible). In the mixtures, bacterial numbers of the incompatible race were constant (5 X 10^{9} cfu/ml), and numbers of the compatible race varied.

Fully expanded leaves (second from the top) of 50-day-old rice plants were inoculated by using a double needle technique of Muko and Yoshida (11) as following: sterile double sewing machine needles (Schmetz, 2.0/80, Herzogenrath, Germany) were dipped into the bacterial suspension, then punched gently into a leaf blade beside the midrib 15 cm (for cultivar IR1545-339, a semidwarf variety) or

25 cm (for cultivar Cas 209, a full size variety) from the tip. Two plants were grown in one pot and only one leaf from each plant was inoculated. Plants were then placed in a growth chamber with 22 C day/32 C night temperatures, and a 12 h light period (175-185 lux from tungsten incandescent bulbs and cool-white fluorescent tubes) until sampled.

For experiments where inocula were applied at different times, leaves were first inoculated at the same position as described for a single inoculation with isolates C6 (compatible) or I2 (incompatible). After four days incubation in a growth chamber (as above), the leaves were challenged with bacteria from the alternate race at a site 1 cm above the original inoculation site. Plants were then returned to the growth chamber until sampling.

Estimation of bacterial multiplication and lesion measurement. Four inoculated leaves per treatment were randomly sampled from different plants at 0, 2, 4, 6, 8, 10, 12, and 14 days after inoculation. Lesions were measured and the leaves were ground individually for estimation of bacterial numbers. To assess bacterial numbers at time zero, 5-cm-long sections of rice leaves, 2.5 cm on either side of the inoculation point, were sampled immediately after inoculation. On subsequent days, whole leaves were detached and ground in a sterile mortar containing 10 ml sterile distilled water. To enhance tissue maceration, sterile sand was added. Ten-fold serial dilutions of the samples were plated onto CPG containing cycloheximide (750 ppm) using a modified micro-

plating technique (3). Grids that sectioned the plate into nine approximately equal zones were drawn on the bottom of petri dishes. Twenty ul samples of each dilution were pipetted onto a section on each of four agar plates. The plates were incubated at 28 C for 3-5 days or until bacterial colonies could be counted using a 10 X stereo microscope.

RESULTS

Bacterial multiplication and symptom development in control inoculations (I2 and C6 alone). In compatible and incompatible interactions with both cultivars IR1545-339 and Cas 209, bacterial numbers increased steadily and at the same rate in rice leaves for 6 days post inoculation (I2 vs C6 not different at P=0.05; Table 1, 2; Fig. 1). After I2 (incompatible) had reached 10^6 - 10^8 cfu/leaf, the net increase in bacteria was reduced. In rice cultivar IR1545-339, isolate C6 (compatible) multiplied at a steady rate in leaves for about 10 days after inoculation, whereas the multiplication of isolate I2 (incompatible) leveled off by day 6 (Fig. 1A). In Cas 209, growth of isolate I2 (incompatible) paralleled that of C6 (compatible) until 8 days after inoculation (Fig. 1B). After this time, increase of I2 (incompatible) ceased.

Generally, within 8 days after inoculation typical lesions developed on leaves of both cultivars inoculated with C6 (compatible). Lesions contained watersoaked tissue in early phases then turned gray-green in color. The lesions extended both directions from the inoculation point. Leaves containing lesions were curled and wilted. Lesion lengths varied between experiments, however those in compatible interactions were clearly distinguishable in size from those of incompatible interactions. In IR1545-339, symptoms appeared earlier in the compatible interaction than in the incompatible interaction (6-8 vs 8-10 days). Lesion

Figure 1. Multiplication of Xanthomonas campestris pv. oryzae isolates C6 (■, compatible) and I2 (●, incompatible) in leaves of cultivars (A) IR1545-339 and (B) Cas 209. Data are means and standard errors (vertical bars) of four replications from each treatment. Slopes (± se) are calculated from linear portions of the curves (Cas 209, days 2-6; IR1545-339, days 0-6).

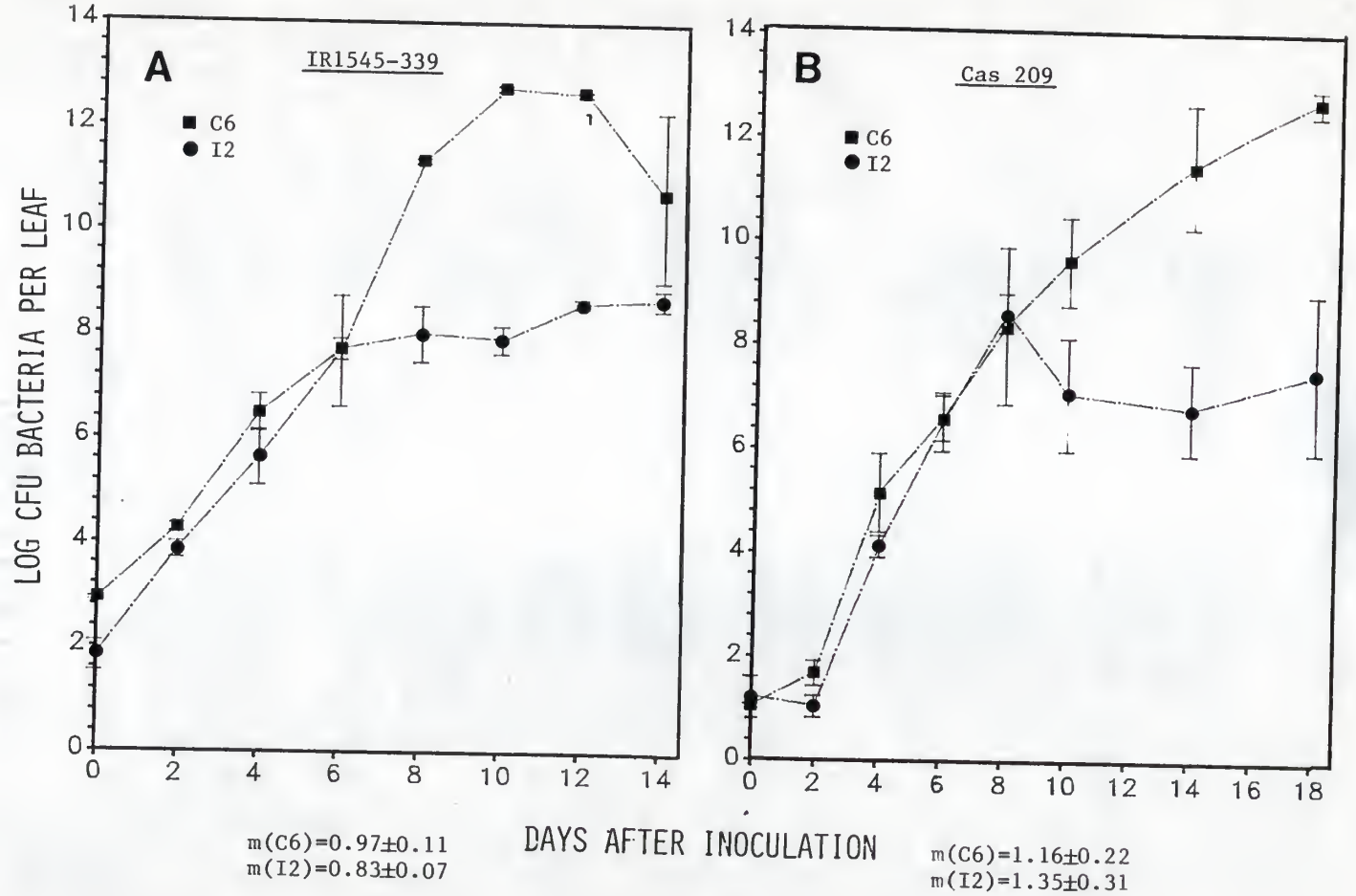

A
IR1545-339
C6
I2
B
Cas 209
C6
I2
LOG CFU BACTERIA PER LEAF
DAYS AFTER INOCULATION
m(C6)=0.97±0.11
m(I2)=0.83±0.07
m(C6)=1.16±0.22
m(I2)=1.35±0.31

Table 1. Comparison of initial growth rates (slopes) in interactions of Xanthomonas campestris pv. oryzae with rice cultivar IR1545-339.

Comparison[a]	
I2 vs C6	I2(1:1) vs C6***
I2(1:1)[b] vs I2***	I2(1:1) vs C6(1:1)***
I2(1:1) vs I2(10:1)***	I2(1:1) vs C6(10:1)***
I2(1:1) vs I2(100:1)***	I2(1:1) vs C6(100:1)*
C6 vs I2	C6(10:1) vs I2(10:1)
C6 vs C6(1:1)	C6(100:1) vs I2(100:1)
C6 vs C6(10:1)*	
C6 vs C6(100:1)	

aSlopes were calculated from linear portions the growth curves and compared using orthogonal contrasts. Comparisons are significantly different at: *, P<0.05; **, P<0.005; ***, P<0.001.

b1:1, 10:1 and 100:1 = incompatible : compatible ratios.

Table 2. Comparison of initial growth rates (slopes) in interactions of Xanthomonas campestris pv. oryzae in rice cultivars IR1545-339 and Cas 209.

Comparison[a]	IR1545-339	Cas 209
I2 vs C6	NS	NS
I2 vs I2(1:1)[b]	**	*
I2 vs I2(preI2)[c]	NS	*
I2 vs I2(preC6)[d]	NS	NS
I2(1:1) vs I2(preI2)	**	**
I2(1:1) vs C6(preI2)	***	*
C6 vs C6(1:1)	NS	NS
C6 vs C6(preI2)	NS	NS
C6 vs C6(preC6)	NS	NS
C6(1:1) vs C6(preC6)	NS	NS

[a]Slopes were calculated from linear portions of the growth curves (Cas 209, days 2-6; IR1545-339, days 0-6) and compared with orthogonal contrasts. Comparisons are significantly different at: *, $P<0.05$; **, $P<0.005$; ***, $P<0.001$. NS = not significant.

[b]1:1=I2:C6 (incompatible:compatible) ratio.

[c]PreI=Preinoculation with I2, than challenge with C6.

[d]PreC=Preinoculation with C6, than challenge with I2.

lengths on IR1545-339 were much longer in compatible combinations compared to those in incompatible combinations at the same sampling days (Fig. 2). For example, lesions were 13.4 cm vs 0.3 cm and 23.3 cm vs 5 cm in IR1545-339 cultivar 10 days and 12 days postinoculation, respectively (Fig. 2A). Symptom development was slower in cultivar Cas 209 (10-14 days) than in IR1545-339 (6-8 days) and lesions were not observed in incompatible combinations of Cas 209 and I2 (Fig. 2B). Lesion lengths were correlated with numbers of isolate C6 (compatible). The higher bacterial numbers of C6 in rice leaves, the longer lesions were observed.

Mixed inoculations. In rice cultivar IR1545-339 inoculated with a 1:1 mixture of isolate I2 (incompatible) to isolate C6 (compatible), the multiplication rate of C6 (compatible) was similar to that observed when IR1545-339 was inoculated with C6 alone (Fig. 3A). Numbers of C6 cells increased logarithmically until 8-10 days after inoculation, then reached a plateau (Fig. 3A). However, the growth rate of I2 (incompatible) in the 1:1 (incompatible to compatible) mixed inoculations was much slower than the growth rate in control inoculations (I2 alone or in 10:1 and 100:1 mixtures (incompatible to compatible) (Figs. 3A, 4; Table 2). The growth curve of I2 in 1:1 mixtures was flat and bacterial populations did not exceed 10^5 cfu/leaf. Similar results were also observed when Cas 209 was inoculated with 1:1 mixtures (Fig. 3B).

When IR1545-339 was inoculated with mixtures containing 10:1 and 100:1 ratios of I2 (incompatible) to C6 (compatible) bacteria,

Figure 2. Lesion lengths on rice cultivars (A) IR1545-339 and (B) Cas 209 after inoculation with isolates C6 (compatible) or I2 (incompatible) individually, or with 1:1, 10:1 and 100:1 mixtures of the isolates (I2:C6). Data are means of four replications from each treatment.

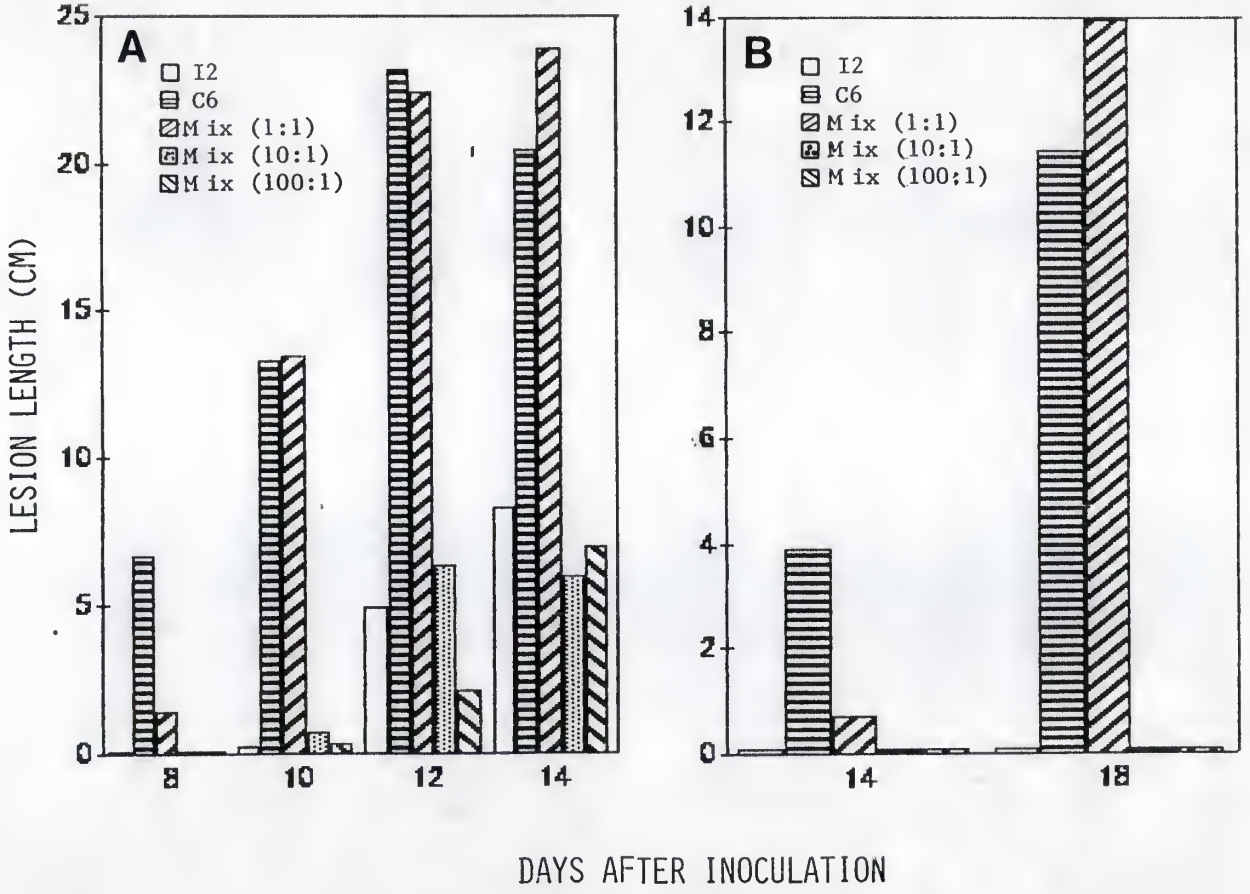
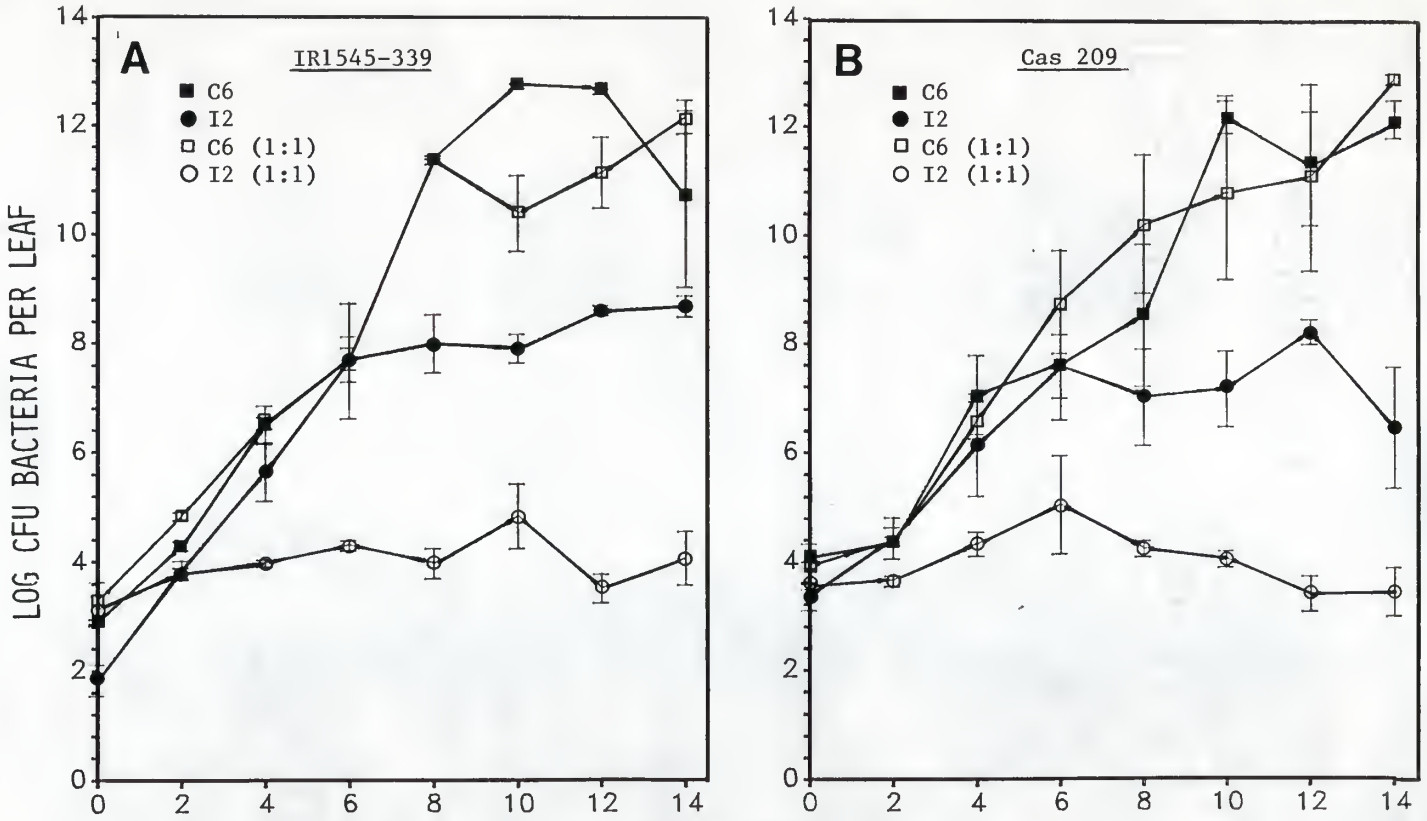

A
I2
C6
Mix (1:1)
Mix (10:1)
Mix (100:1)
LESION LENGTH (CM)
25
20
15
10
5
0
8
10
12
14
B
I2
C6
Mix (1:1)
Mix (10:1)
Mix (100;1)
14
12
10
8
6
4
2
0
14
18
DAYS AFTER INOCULATION

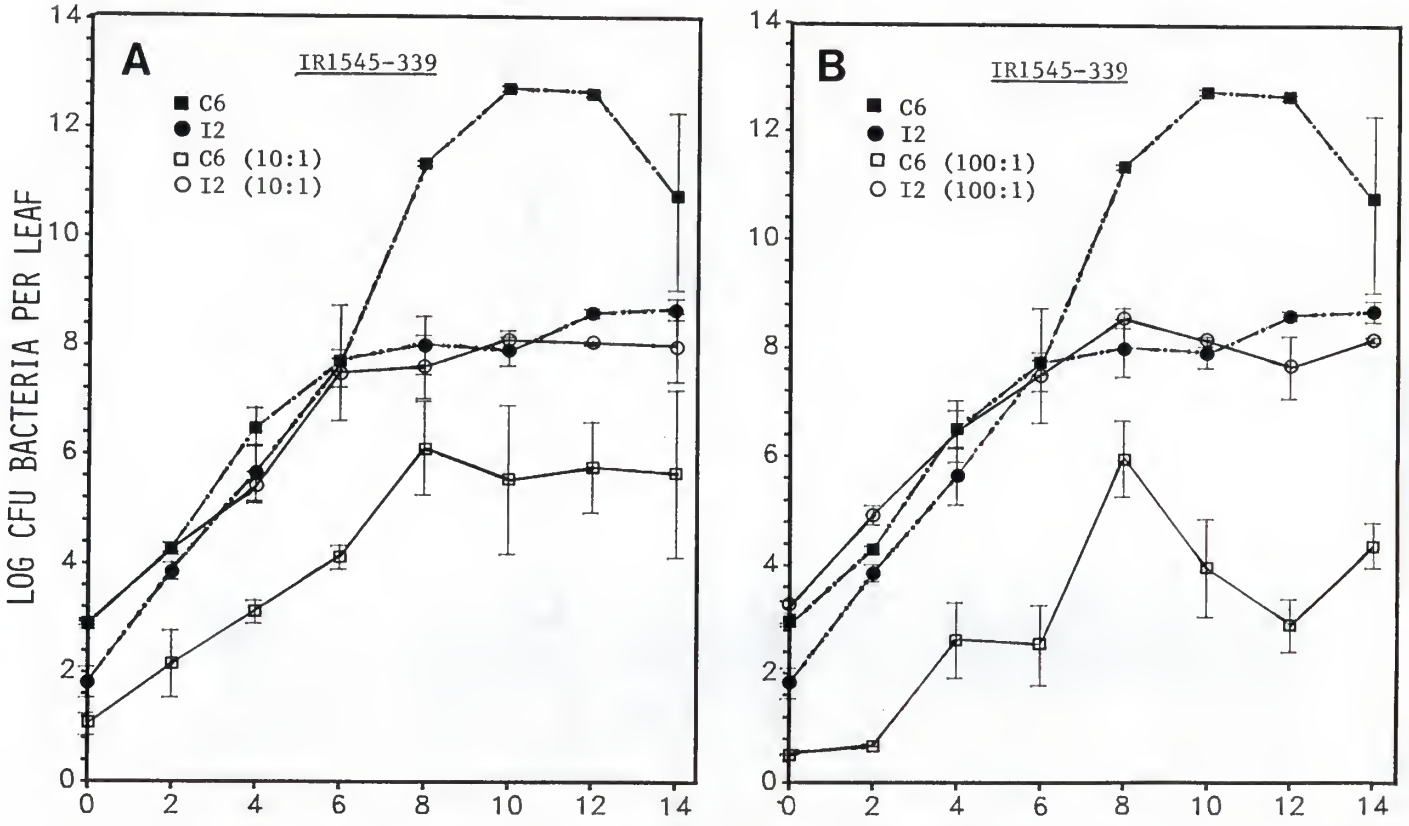
Figure 3. Multiplication of Xanthomonas campestris pv. oryzae in leaves of rice cultivars (A) IR1545-339 and (B) Cas 209 inoculated individually with C6 (■, compatible) or I2 (●, incompatible), or with a 1:1 mixture of C6 (□) and I2 (○). Data are means and standard errors (vertical bars) of four replications from each treatment. Slopes (±se) are calculated from the linear portions of the curves (IR1545-339, days 0-6; Cas 209, days 2-6).



m(C6)=0.97±0.11
m(I2)=0.83±0.09
m(C6,1:1)=0.81±0.10
m(I2,1:1)=0.22±0.18

m(C6)=0.82±0.30
m(I2)=0.79±0.20
m(C6,1:1)=1.10±0.20
m(I2,1:1)=0.11±0.22

Figure 4. Multiplication of Xanthomonas campestris pv. oryzae in leaves of rice cultivar IR1545-339 inoculated individually with C6 (■, compatible) or I2 (●, incompatible), or with a 10:1 (A) or 100:1 (B) mixture of C6 (□) and I2 (○). Data are means and standard errors (vertical bars) of four replications from each treatment. Slopes (±se) are calculated from the linear portions of the curves (0-6 days).



m(C6)=0.97±0.11
m(I2)=0.83±0.07
m(C6,10:1)=0.61±0.10
m(I2,10:1)=0.75±0.06

m(C6)=0.97±0.11
m(I2)=0.83±0.07
m(C6,100:1)=0.83±0.18
m(I2,100:1)=0.72±0.07

the growth rates of C6 (compatible) were reduced by comparison with C6 alone and C6 in 1:1 mixtures (Fig. 4A, B). However, growth rates of C6 in the 10:1 and 100:1 mixtures were not as dramatically affected as that of I2 (incompatible) in the 1:1 mixtures (Fig. 3A, Table 1). The growth rate of I2 in 10:1 and 100:1 mixtures (incompatible to compatible) in IR1545-339 was not significantly different than in control inoculations, but was greater than that of I2 in 1:1 mixtures (Fig. 4, Table 1). In all but the 1:1 mixture, populations of I2 (incompatible) reached 10^7 cfu/leaf by 6-8 days after inoculation, then levelled off. In the 10:1 and 100:1 mixtures, C6 populations did not exceed 10^6 cfu/leaf at 8 days after inoculation (Figs. 4A, B).

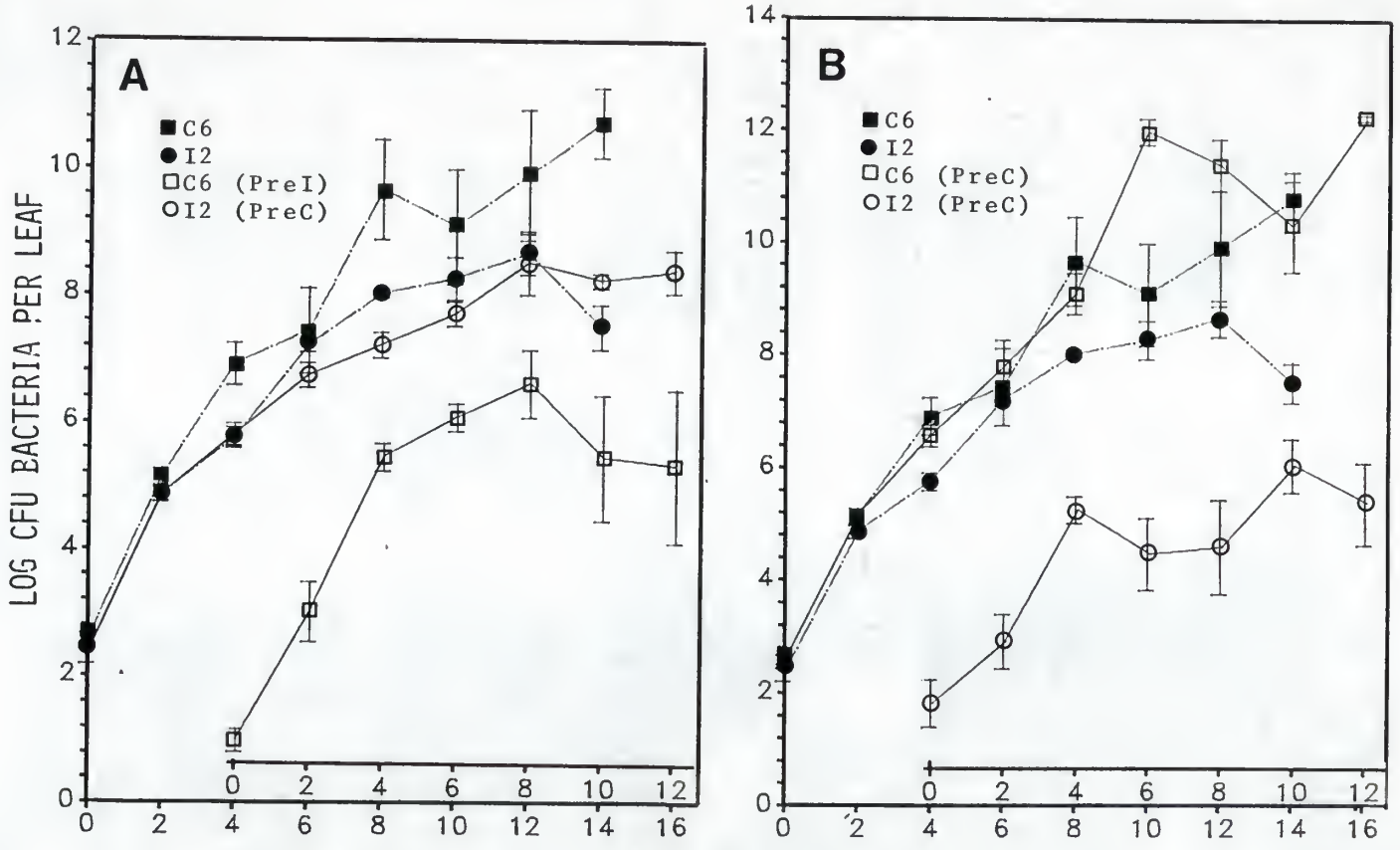
In rice cultivar Cas 209, growth patterns observed with I2 in 1:1 (Fig. 3B), 10:1, and 100:1 (data not shown) mixtures were similar to those in IR1545-339. When a 10:1 ratio of I2 (incompatible) to C6 (compatible) bacteria was used, the multiplication rate of C6 was slower than that observed in the compatible control (C6 alone) or C6 in the 1:1 mixture (Table 2). In a 100:1 mixture, C6 (compatible) bacteria were not detectable after day 2 in Cas 209 leaves.

Lesions were measured in plants inoculated with bacterial mixtures and compared to those induced by inoculation with C6 (compatible) or I2 (incompatible) alone. In both cultivars IR1545-339 and Cas 209, lesion lengths inoculated with the C6 (compatible) alone and the 1:1 mixture were similar. However, a very distinct effect was observed when the mixture ratios were changed to 10:1 and 100:1. For example, lesion lengths in IR1545-339 were reduced at day

14 by 73% in the 10:1 and by 70% in the 100:1 mixed inoculum compared to the 1:1 inoculum (Fig. 2A). Average lesion lengths from IR1545-339 leaves inoculated with C6 (compatible) alone and with a 1:1 mixture were 20.5±3.57 cm and 24±1.08 cm, respectively, at 14 days after inoculation, whereas average lesion lengths from rice leaves inoculated with I2 (incompatible) alone or 10:1 or 100:1 mixtures of I2 (incompatible) to C6 (compatible) were 8.4 cm, 6 cm and 7 cm respectively (Fig. 2A). In Cas 209, lesions were observed only in leaves inoculated with C6 (compatible) alone or with a 1:1 mixture (Fig. 2B). Intensity of symptom development correlated with numbers of compatible bacteria, that is, longer lesions were observed when higher numbers of bacteria from the compatible race were present.

Time interval inoculations. Rice leaves were first inoculated with isolate I2 (incompatible) then subsequently challenged with isolate C6 (compatible), or vice versa to determine if establishment of I2 would result in the resistance phenotype. When cultivar IR1545-339 was first inoculated with I2, then challenged with C6, the growth rate of I2 was the same as in the incompatible control (Fig. 5A). Growth of the challenge organism (C6) was at the same rate as the singly inoculated C6 control until 10^5 - 10^6 cfu/leaf was reached. Then, the growth of C6 (challenge, compatible) leveled off and populations did not reach the levels observed when C6 was inoculated alone (Fig. 5A). Similar results were observed if I2 (incompatible) was pre-inoculated into rice cultivar Cas 209

Figure 5. Multiplication of Xanthomonas campestris pv. oryzae in leaves of rice cultivar IR1545-339 inoculated with C6 (■, compatible) or I2 (●, incompatible); or (A) with isolate I2 (○, incompatible) four days before C6 (□, compatible); or (B) with isolate C6 (□) four days before I2 (○). Data are means and standard errors (vertical bars) of four replications from each treatment. Slopes (±se) are calculated from the linear portions of the curves (0-6 days).


A
B
C6
I2
C6 (PreI)
C6 (PreC)
I2 (PreC)
LOG CFU BACTERIA PER LEAF
DAYS AFTER INOCULATION
m(C6)=1.09±0.10
m(I2)=0.85±0.18
m(C6,PreI)=1.12±0.10
m(I2,PreC)=0.86±0.18
m(C6)=1.09±0.10
m(I2)=0.85±0.18
m(C6,PreC)=1.00±0.10
m(I2,PreC)=0.44±0.18

(Fig. 6). When C6 (compatible) was inoculated into IR1545-339 before I2 (incompatible), the rate of multiplication of I2 (incompatible) was slower, but by 14 days, the I2 population reached the same level as if it were inoculated alone (Fig. 5B).

Lesion length was positively correlated with bacterial numbers of the compatible race. In IR1545-339, if the rice plants were inoculated with C6 (compatible) bacteria 4 days before I2 (incompatible), the lesion lengths were similar to those caused by inoculated with C6 (compatible) alone or a 1:1 mixture (Fig. 7A). However, if rice leaves were inoculated with I2 (incompatible) prior to C6 (compatible), the lesion lengths were markedly reduced (Fig. 7A). Similar results were observed in rice cultivar Cas 209 (Fig. 7B).

Figure 6. Multiplication of Xanthomonas campestris pv. oryzae in leaves of rice cultivar Cas 209 inoculated C6 (■, compatible) or I2 (●, incompatible); or (A) with isolate I2 (○, incompatible) four days prior to challenge with C6 (□, compatible); or (B) with C6 (□) four days prior to challenge with I2 (○). Data are means and standard errors (vertical bars) of four replications from each treatment. Slopes (±se) are calculated from the linear portions of the curves (2-6 days).

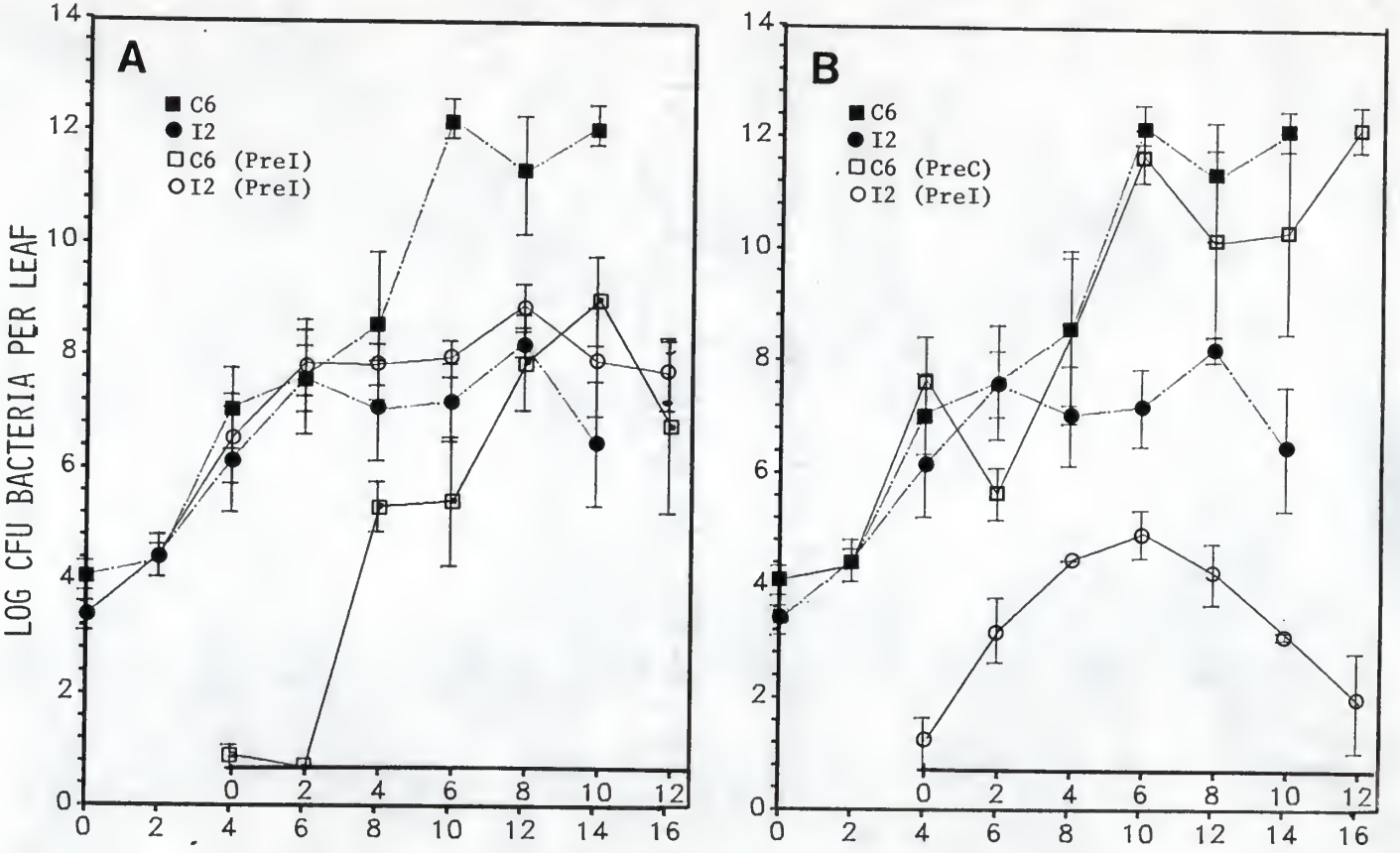
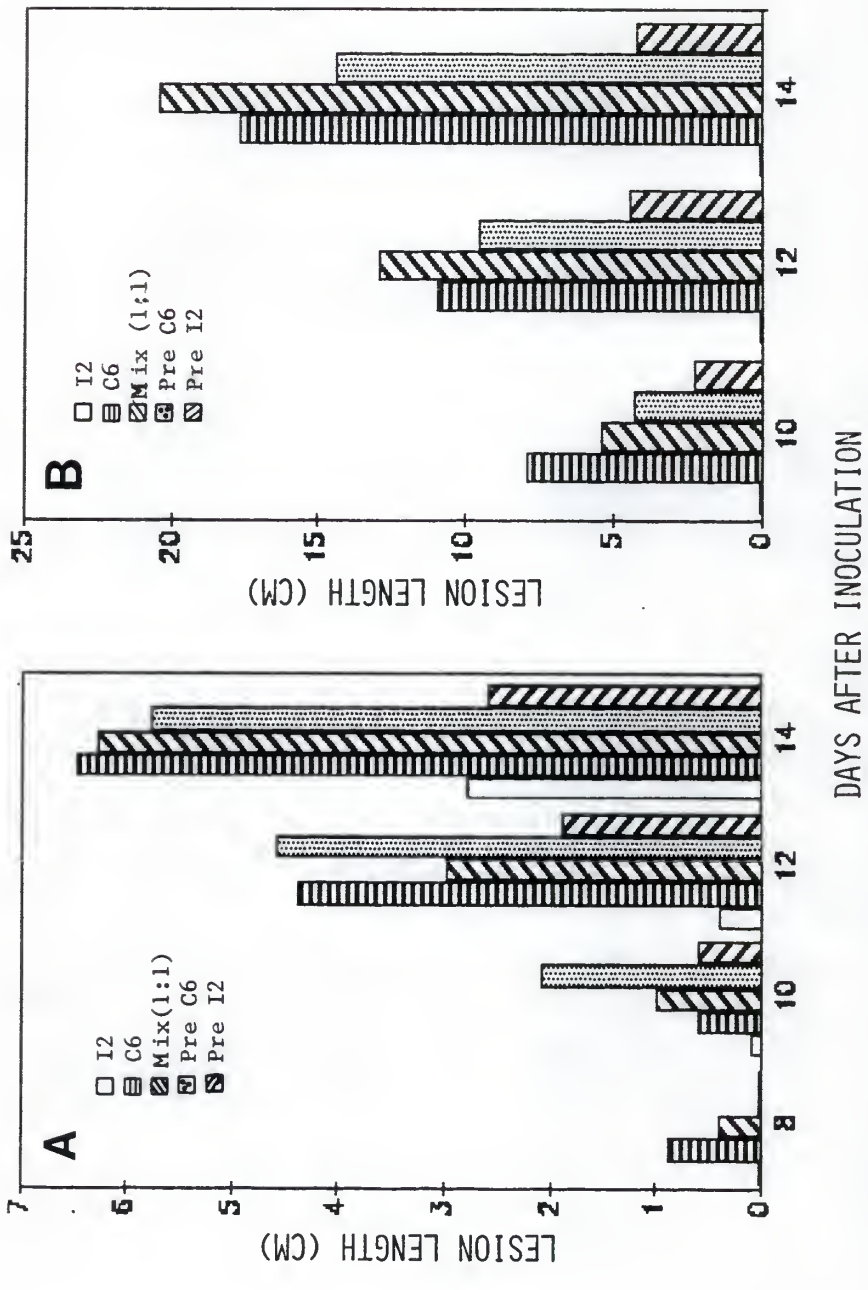

A
C6
I2
C6 (PreI)
I2 (PreI)
B
C6
I2
C6 (PreC)
I2 (PreI)
LOG CFU BACTERIA PER LEAF
DAYS AFTER INOCULATION
m(C6)=0.82±0.30
m(I2)=0.79±0.20
m(C6,PreI)=1.19±0.34
m(I2,PreC)=0.98±0.20
m(C6)=0.82±0.30
m(I2)=0.79±0.20
m(C6,PreC)=0.56±0.20
m(I2,PreC)=0.56±0.22

Figure 7. Lesion lengths on rice cultivars (A) IR1545-339 and (B) Cas 209 after inoculation with isolates C6 (compatible) or I2 (incompatible) individually, with 1:1 mixtures of the isolates or with isolate I2 four days prior to challenge with C6 (preI2), or with isolate C6 four days prior to challenge with I2 (preC6). Data are means of four replications from each treatment.


A
B
I2
C6
Mix(1:1)
Pre C6
Pre I2
LESION LENGTH (CM)
DAYS AFTER INOCULATION

Discussion

Our results confirm that in rice, resistance to the bacterial blight pathogen, X. c. pv. oryzae, is characterized by reduced lesion lengths and restricted bacterial multiplication. In both cultivars IR1545-339 and Cas 209, bacterial numbers in compatible and incompatible combinations increased initially at the same rate (Fig. 1, Tables 1, 2). However, once 10^6-10^8 cfu/leaf were reached, distinguishable trends in multiplication were observed. In incompatible combinations, bacterial numbers were maintained at 10^6-10^8 cfu/leaf for the remainder of the experiment. In compatible combinations, bacterial numbers increased to 10^{12}-10^{13} cfu/leaf. The differences in bacterial numbers between compatible and incompatible interactions were 10^4-10^6 cfu/leaf. Others (10, 14, 16) reported only 10^1-10^2 fold differences. Our values are most likely higher because we sampled whole leaves (25-30 cm on IR1545-339 and 40-45 cm on Cas 209), whereas others sampled only sections of leaves (0.5-10 cm) (10, 14, 16).

Lesion lengths were clearly distinguishable between compatible and incompatible combinations in both cultivars IR1545-339 and Cas 209. In general, symptoms appeared earlier and lesion lengths were longer in compatible combinations than in incompatible combinations. Lesion development correlated positively with numbers of compatible bacteria.

The effects of mixed inoculations of rice with compatible an incompatible X. c. pv. oryzae races has been previously studied (14,

17, Barton-Willis et al., appendix A). In general, in race-specific interactions between X. c. pv. oryzae and rice, lesion lengths were not reduced when leaves were inoculated with 1:1 ratios (compatible to incompatible races). Our work using different isolates confirms this. That is, in both rice cultivars IR1545-339 and Cas 209, lesion lengths were not reduced in the 1:1 mixture compared to those when isolates of compatible races were inoculated alone (Fig. 2). Multiplication and final bacterial numbers of C6 (compatible) in 1:1 mixed inoculations paralleled that of the compatible control (C6 alone, Fig. 3; Tables 1, 2). Lesion lengths after 1:1 mixed inoculation were similar to those of the compatible control (C6 alone) (Fig. 2,7).

The growth rate of I2 (incompatible) in 1:1 mixed inoculations was suppressed much more than that in the incompatible control (I2 alone) or in the 10:1 and 100:1 (incompatible to compatible) mixtures. Barton-Willis and coworkers (appendix A) presented evidence that competition exists between isolates of compatible and incompatible races in 1:1 mixed inoculations. We used different isolates (C6, PXO99^{Sm}, compatible; I2, IRN793^{Rif}, incompatible) than were used in their experiments (PXO61^{Sm} compatible; PXO86^{Rif}, incompatible). The growth curves of I2 in 1:1 mixtures in rice leaves indicate competition may also exist between isolates C6 and I2 (Fig. 3A). We compared growth trends of isolates C6 and I2 individually or in 1:1 mixtures in liquid culture media and found that C6 multiplied more rapidly then did I2 (data not shown). However, there was no apparent inhibition of the growth of one

bacteria by the other because when the two were mixed (1:1 or 10:1 incompatible to compatible) in rich culture medium the growth rates were similar to those of the individual inoculations. This does not preclude that competition may occur in leaves where some nutrients may be limited or lacking.

In incompatible interactions with Cas 209, a threshold level of 10^6-10^7 cfu/leaf is reached before the effects of resistance are observed (Barton-Willis et al., appendix A). Thus, C6 (compatible) may simply outgrow I2 (incompatible) in rice leaves and prevent the I2 population from reaching the threshold level necessary for resistance induction. We reasoned that if isolates of incompatible races could reach the threshold level, resistance would ensue and would be the observed phenotype in mixed inoculations. To test this hypothesis, leaves of cultivars IR1545-339 and Cas 209 were inoculated with different ratios [10:1 and 100:1, I2 (incompatible) to C6 (compatible)], or preinoculated with isolate I2 four days prior to inoculation with C6. In these experiments, lesion enlargement (Figs. 2A, 7A), multiplication rates (Tables 1, 2), and final populations (Figs. 4, 5) of isolate C6 (compatible) were inhibited in comparison with compatible controls or 1:1 mixtures in cultivar IR1545-339. I2 (incompatible) growth rates and final population levels were similar to those in incompatible controls, but were significantly greater than those of I2 observed in inoculations with 1:1 mixtures. Similar results were observed in Cas 209, except that inhibition of C6 multiplication in 10:1 and 100:1 mixtures was more dramatic than in IR1545-339. In fact, no C6

cells could be isolated after 2 days in 100:1 mixture. This host difference is also reflected in symptom expression; lesions in a Cas 209 resistant response are negligible by comparison with a compatible response, whereas in IR1545-339 lesions in incompatible responses can reach 40% the length of those in compatible responses.

In summary, when rice leaves were inoculated with incompatible and compatible X. c. pv. oryzae races at ratios of 10:1 (incompatible to compatible) or greater, or when leaves were first inoculated with an isolate of an incompatible race and challenged after four days with an isolate of a compatible race, then the resulting interaction phenotype was resistant. Thus, in the rice/X. c. pv. oryzae interaction, resistance is apparently physiologically predominant to susceptibility. In our experiments, bacteria representing the incompatible race were introduced at or allowed to grow to higher levels than those of the compatible race. Therefore, we cannot rule out that the effects observed were merely a reversal of the competitive advantage. However, in IR1545-339 the slope of the linear portion of the I2 (incompatible) growth curve in 1:1 mixtures was significantly lower than those of C6 in 10:1 or 100:1 mixtures or in the delayed challenge experiments (Table 1, 2), that is, the initial growth rate of I2 in 1:1 mixtures was much slower and the populations did not reach as high a level. In 10:1 and 100:1 mixtures (incompatible to compatible) growth of C6 paralleled that of I2 initially, but shortly after the growth of I2 leveled off, that of C6 also leveled off. Thus, the growth of C6 was

apparently not inhibited until resistance expression was observed (i.e., when the growth of I2 was also inhibited) indicating no competition from isolate I2 for at least the first 2-8 days of growth. We suggest that in these experiments the effects of resistance rather than competition were measured and the results would, therefore, support the hypothesis that resistance is physiologically predominant over susceptibility.

In order to critically evaluate the effects of resistance on isolates of compatible races, host isolines which differ in only those resistance genes of interest and pathogen mutants or clones which differ in only their resistance inducing capabilities are needed. The host isolines have just recently been developed (12). Recently a genomic fragment was cloned from a race 2 X. c. pv. oryzae isolate (incompatible to Cas 209), when introduced into a race 6 isolate (compatible to Cas 209), conferred incompatibility to rice cultivar Cas 209 (J. E. Leach, personal communication). The fact that such a clone could be identified indicates that resistance is physiologically predominant to susceptibility in the rice/X. c. pv. oryzae interaction. Mutants of race 2 isolates with a transposable element inserted in the avirulence gene are being constructed to characterize the effects race-specific resistance expression on bacterial multiplication and symptom development.

LITERATURE CITED

1. Holliday, M. J., and Keen, N. T. 1982. The role of phytoalexins in the resistance of soybean leaves to bacteria: Effects of glyphosate on glyceollin accumulation. Phytopathology 72:1470-1474.

2. Horino, O. 1976. Induction of bacterial leaf blight resistance by incompatible strains of Xanthomonas oryzae in rice. Pages 43-55 in: Biochemistry and Cytology of Plant Parasite Interactions. K. Tomiyama, J. M. Daly, I. Uritani, H. Oku, and S. Ouchi, eds. Kodansha, Ltd., Tokyo. 256 pp.

3. Keen, N. T., Ersek, T., Long, M., Bruegger, B., and Holliday, M. 1981. Inhibition of the hypersensitive reaction of soybean leaves to incompatible Pseudomonas spp. by blasticidin S, streptomycin or elevated temperature. Physiol. Plant Pathol. 18:325-337.

4. Kelman, A. 1954. The relationship of pathogenicity in Pseudomonas solanacearum to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.

5. Klement, Z. 1982. Hypersensitivity. In : Phytopathogenic prokaryotes. M. S. Mount and G. H. Lacy (eds.). Academic Press, New York, pp. 149-177.

6. Long, M., Barton-Willis, P., Staskawicz, B. J., Dahlbeck, D., and Keen, N. T. 1985. Further studies on the relationship between glyceollin accumulation and the resistance of soybean leaves to Pseudomonas syringae pv. glycinea. Phytopathology 75:235-239.

7. Lyon, F. M., and Wood, R. K. S. 1975. Production of phaseollin, coumestrol and related compounds in bean leaves inoculated with Pseudomonas spp. Physiol. Plant Pathol. 6:117-124.

8. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory. Cold Spring Harbor, NY. 545 pp.

9. Mew, T. W., 1987. Current status and future prospects of research on bacterial blight of rice. Annu. Rev. Phytopathol. 25:359-382.

10. Mohiuddin, M. S., and Kauffman, H. E. 1975. Multiplication studies of Xanthomonas oryzae isolates on differential rice varieties. Current Sci. 44:637-638.

11. Muko, M., and Yoshida, K. 1951. A needle inoculation method for bacterial leaf blight disease of rice. Ann. Phytopathol. Soc. Jpn. 15:179.

12. Ogawa, T., and Yamamoto, T. 1987. Selection of recurrent parents to develop near-isogenic lines resistant to bacterial leaf blight of rice. Jpn. Agric. Res. Q. 21:65-69.

13. Ou, S. H., 1985. Rice Diseases. Kew, England: Commonwealth Mycological Institute. 380 pp.

14. Parry, R. W. H., and Callow, J. A. 1986. The dynamics of homologous and heterologous interactions between rice and strains of Xanthomonas campestris. Plant Pathol. 35:380-389.

15. Petpisit, V., Khush, G. S., and Kauffman, H. E. 1976. Inheritance of resistance to bacterial blight in rice. Crop Sci. 17:551-554.

16. Reddy, A. P. K. and Kauffman, H. E. 1973. Multiplication and movement of Xanthomonas oryzae in susceptible and resistant hosts. Plant Dis. Rep. 57:784-787.

17. Reddy, A. P. K. and Kauffman, H. E. 1974. Population studies of mixed inoculum of Xanthomonas oryzae in susceptible and resistant varieties of rice. Ann. Phytopathol. Soc. Jpn. 40:93- 97.

18. Tsuchiya, K., Mew, T. W., and Wakimoto, S. 1982. Bacterio- Bacteriological and pathological characteristics of wild type and induced mutants of Xanthomonas campestris pv. oryzae.

Phytopathology 72:43-46.

APPENDIX A: Manuscript in Preparation

Growth Dynamics of Xanthomonas campestris pv. oryzae in Leaves of Rice Differential Cultivars

P. A. Barton-Willis, P. D. Roberts, A. Guo, and J. E. Leach

Department of Plant Pathology, Throckmorton Hall, Kansas State University, Manhattan, KS 66506.

ABSTRACT

Bacterial multiplication and spread were monitored in race-specific interactions of Xanthomonas campestris pv. oryzae and rice. Bacterial numbers in compatible and incompatible interactions increased equally until levels of 10^7-10^8 cfu/leaf were reached. Thereafter, the bacterial growth rate in incompatible combinations slowed in comparison to those in compatible combinations. Maximum bacterial numbers per leaf were dependent on host cultivar. In both compatible and incompatible interactions, bacteria advanced out from the inoculation point, however bacteria in compatible interactions spread more rapidly. Symptoms were not observed in advance of bacteria. Lesion lengths, in general, were positively correlated with bacterial numbers. In leaves inoculated with bacterial mixtures (1:1 compatible to incompatible races) the growth rates of isolates of the compatible race ws the same as in compatible controls (compatible alone). Growth rates of isolates from incompatible races, however, were severely restricted. The same effects were observed in a host in which both races are compatible,

thus analysis of the effect mixed inoculations on bacterial growth and lesion development was confounded by competition between bacterial isolates in rice leaves.

INTRODUCTION

Pathogenic specialization of Xanthomonas campestris pv. oryzae, the causal agent of bacterial blight, has been demonstrated on rice cultivars that have specific genes for resistance to the disease (for review see Mew, 1987). In the rice/X. c. pv. oryzae interaction, the classic hypersensitive response (Klement, 1982) is absent (Parry and Callow, 1986) and relative lesion lengths are the main criteria with which to assign host reaction type or resistance (Ou, 1985). Lesion length data are most useful for critical analysis of compatibility vs. incompatibility if compared with bacterial multiplication data.

When multiplication of an isolate of one race is compared in resistant and susceptible dicotyledonous hosts, incompatibility is generally reflected in lower pathogen numbers (Borkar and Verma, 1985; Keen et al., 1981; Stall and Cook, 1966). Few studies of bacterial multiplication dynamics in race-specific interactions with monocotyledonous plants are available. Where it exists in the rice/X. c. pv. oryzae interaction, the information is contradictory. Mohiuddin and Kauffman (1975) monitored X. c. pv. oryzae multiplication in 20-mm discs of rice leaves taken from the inoculation site or from 1 and 3 cm below the inoculation site. At 1 and 3 cm below the inoculation site, bacterial numbers in incompatible interactions were 100-fold less than in the compatible

interactions at 12-14 days after inoculation. At the inoculation site, no difference in bacterial numbers was detected. Thus, they concluded that incompatible interactions were characterized by lower bacterial numbers and reduced pathogen spread. In contrast, Parry and Callow (1986) found little difference in multiplication or spread of the pathogen in 10-cm leaf sections during either compatible or incompatible interactions although lesions lengths were substantially different. They concluded that resistance expression in rice is not characterized by lower bacterial numbers, but is the result of reduced symptom expression by the host.

Results from mixed inoculation experiments using isolates of two different X. c. pv. oryzae races are also not clear-cut. In a race-specific interaction, physiological dominance of resistance induced in incompatible interactions is expected (Ellingboe, 1976). For example, when soybean leaves were inoculated with a 1:1 mixture of isolates representing compatible and incompatible Pseudomonas syringae pv. glycinea races, multiplication of bacteria from the compatible race is restricted, presumably because the expression of hypersensitivity inhibits growth of both isolates (Long et al., 1985). In rice leaves inoculated with isolates of compatible and incompatible X. c. pv. oryzae races (1:1 ratio), lesion lengths were equal to the singly inoculated compatible control (Reddy and Kauffman, 1973 and Parry and Callow, 1986) or, in some cultivars, intermediate in length to those of the compatible and incompatible controls (Parry and Callow, 1986). Bacterial numbers were either not measured (Parry and Callow) or, when determined from leaf

samples 1 cm immediately around the inoculation site, were not different from single control inoculations (Reddy and Kauffman, 1973). Thus, in these race-specific interactions, physiological dominance of resistance was not apparent. On the other hand, if low virulence and high virulence X. c. pv. oryzae isolates were introduced into rice at ratios of 20:1 or greater, lesion development in the host and growth of the high virulence isolate were substantially reduced (K & R, 1974, Devadeth, 1970). Although the isolates were not grouped into races, their interactions with different rice cultivars suggested race-specificity. Overall, however, a clear idea of the effects of mixed inoculations on host reaction has not emerged.

To more critically evaluate race-specific interactions between X. c. pv. oryzae and rice, we studied the effects of the host's response on bacterial multiplication in compatible and incompatible combinations and in mixed inoculations. We report here bacterial multiplication in whole rice leaf samples, movement of bacterial populations out from the inoculation point throughout the leaf, and the effect of different inoculum levels on the time and onset of resistance. Finally, we present the effects of mixed inocula (compatible and incompatible X. c. pv. oryzae isolates) on bacterial multiplication and lesion development in different rice cultivars.

MATERIALS AND METHODS

Bacterial isolates. Philippine X. c. pv. oryzae isolates PXO61 (race 1), PXO86 (race 2), and PXO99 (race 6) were obtained from Dr.

T. W. Mew at the International Rice Research Institute (IRRI, Manila, Philippines). Bacterial cultures were grown on peptone sucrose agar (PSA; Tsuchiya et al., 1982) for immediate use. For long-term storage, bacteria were suspended in 15% glycerol (Maniatis) and frozen at -80 C or suspended in sterile skim milk (Difco, Detroit, MI) and lyophilized. X. c. pv. oryzae lose virulence to rice upon repeated transfer of cultures (Dr. T. W. Mew, personal communication). Therefore, cultures for experiments were grown fresh from glycerol stocks (one to two transfers) or lyophilization.

Bacterial suspensions for inoculations were prepared by growing bacteria in nutrient broth (NB, Difco) overnight on a rotary shaker (200 RPM) at 28 C. Cells were collected by centrifugation at 17,000 g for 10 min at 22 C. The bacterial pellet was washed twice, resuspended in distilled water, and adjusted to desired concentrations using a Klett-Summerson meter with a red #66 filter (Klett Mfg. Co., NY).

To select streptomycin (Sm) resistant PXO61 or PXO99 strains ($PXO61^{Sm}$ and $PXO99^{Sm}$), 1 ml of 17 h PXO61 and PXO99 cultures were inoculated into 15 ml PSB containing 50 g/ml streptomycin. After incubation overnight with shaking, streptomycin-resistant colonies were selected on PSA containing 100 g/ml streptomycin. A rifampicin (Rif) resistant PXO86 strain ($PXO86^{rif}$) was obtained using a gradient plate technique (Szybalski, 1952).

Rice cultivars. Seed of rice (Oryza sativa) cultivars IR8 (Xa-11 gene for resistance to X. c. pv. oryzae, Mew, 1987), IR20 (Xa-4,

Petpist, 1977), Cas 209 (Xa-10, Yoshimura, 1983), and IR1545-339 (xa-5, Olufowote, 1977) were supplied by Dr. T. Mew (IRRI). The interactions of these cultivars with isolates of X. c. pv. oryzae races 1, 2, and 6, are shown in Table 1 (for review, see Mew, 1987).

Inoculation and sampling of rice plants. Rice seedlings were greenhouse grown (28-32 C days and 22-26 C nights) in 4" pots sitting in 2" of water. A double sewing machine needle (Schmeth, 2.0/80, Herzogenrath, Germany) dipped into inoculum was used to stab fully expanded rice leaves (30-40 days past sowing) once between the margin and midrib, 10 cm from the leaf tip. The double-needle inoculation technique is adapted from that of Muko and Yoshida (1951). In all inoculations, only the second youngest leaf per plant was used. After inoculation, plants were incubated in a growth chamber (32 C day, and 22 C night; 12 hr photoperiod; 70% RH). Lesions were measured at each sampling day.

For experiments where varied bacterial concentrations were used in inocula, a Hagborg device (Hagborg, 1970) was used to infiltrate a circular area 1.5 cm in diameter in the center of the leaf blade, 10 cm from the leaf tip of 8-wk-old plants.

To assess the reproducibility of double-needle inoculation technique, 18 plants of each of cultivars Cas 209 and IR20 were inoculated with PXO61^{Sm} (5 X 10^{10} cfu/ml). Leaves were ground immediately after inoculation and diluted samples plated as described to determine the number of bacteria deposited per leaf. Reproducibility of the Hagborg inoculation technique was assessed by inoculating leaves with each of two PXO61^{Sm} concentrations (5 X 10^{10}

cfu/ml and 5 X 10^8 cfu/ml). Leaves were sampled as described.

Estimation of bacterial growth in single and mixed inoculations. Unless otherwise noted, four whole rice leaves per treatment were sampled immediately after inoculation and then at days 1-6, 8, 10, and 12. The leaves were triturated individually in 2-10 ml water using mortars and pestles under aseptic conditions. Ten-fold serial dilutions of the leaf suspensions were made using a microplating technique (Keen et al, 1981) and dilutions were plated onto casein-peptone glucose agar (CPG; Kelman, 1954) containing cycloheximide (75 g/ml) and either streptomycin (100 g/ml) or rifampicin (20 g/ml). Plates were incubated at 28 C and colonies counted within 3-5 days. All experiments were repeated at least twice.

To assess bacterial growth in rice leaves, plants were double needle inoculated with bacterial suspensions adjusted to 5 X 10^9 cfu/ml. For mixed inocula, 5 X 10^{10} cfu/ml suspensions of PX061^{Sm} and PX086^{Rif} or PX099^{Sm} and PX086^{Rif} were combined in equal amounts. A second inoculum was obtained by combining the bacterial mixture (1:1) with an equal portion of water.

Assessment of spread of bacteria in inoculated leaves. Suspensions of PX061^{Sm} and PX086^{Rif} (5 X 10^9 cfu/ml) were used to double needle inoculate leaves of rice cultivar Cas 209 as described above. Leaves were sampled immediately after inoculation and every two days through day 12. The top 1 cm of leaf tissue was discarded and the adjacent 12 cm of the leaf were cut into six 2-cm sections such that the inoculation point was in the center of the 5th section (section E, Table 2). Each section was ground individually; dilutions and

plating were as described. Lesions were measured on sampling days.

Effect of inoculum density on expression of resistance. A Hagborg device was used to infiltrate leaves of cultivar Cas 209 in separate inoculations with PXO61^{Sm} and PXO86^{Rif} at high (5 X 10^{10} cfu/ml) and low (5 X 10^{8} cfu/ml) inoculum densities. Plants were infiltrated just prior to the beginning of the daily photoperiod. Leaf samples were processed as described.

RESULTS

Antibiotic resistant strains and sampling procedures. Antibiotic resistant X. c. pv. oryzae strains were selected to expediate specific detection and allow differentiation of isolates in mixed inoculations. Antibiotic resistant strains had growth rates identical to the wild types both in planta and in culture and produced similar lesion lengths on rice leaves (data not shown). Grinding leaves in phosphate buffer instead of water did not increase the number of bacteria recovered (data not shown). Double-needle inoculations with 5 X 10^{9} cfu/ml delivered 2.5 ($\pm$ 0.4) X 10^{4} cfu/leaf. The number is mean $\pm$ the standard error. Hagborg infiltration of Cas 209 resulted 1.6 ($\pm$ 0.1) X 10^{7} cfu/leaf when a 5 X 10^{10} cfu/ml suspension was used and 2.7 ($\pm$ 0.6) X 10^{4} when a 5 X 10^{8} cfu/ml suspension was used.

Bacterial multiplication in rice leaves. In compatible and incompatible combinations with Cas 209 (Fig. 1) and IR1545-339 (Fig. 2), bacteria multiplied steadily and equally in rice leaves for 4-6 days after inoculation to 10^{7}-10^{8} cfu/leaf. Thereafter, bacterial

growth in incompatible combinations with these hosts slowed by comparison with those in compatible combinations. Bacterial numbers in compatible interactions with Cas 209 (Fig. 1), IR1545-339 (Fig. 2), and IR8 (Fig. 3) reached 10^{11}–10^{13} cfu/leaf within 8-10 days after inoculation. Inoculating Cas 209 with PXO99^{Sm} (compatible) and PXO86^{Rif} (incompatible) or with PXO99^{Sm} (compatible) and IRN793^{Rif} (incompatible) resulted in the same multiplication pattern (data not shown) as those shown in Fig. 1 . In IR20 the differences in multiplication were less definitive in that bacterial numbers did not exceed 10^9 cfu/leaf in compatible combinations and they were only about tenfold higher than in incompatible combinations.

Mixed inoculations. In mixtures of PXO61^{Sm} (compatible) and PXO86^{Rif} (incompatible) in Cas 209, the PXO61^{Sm} population increased at the same rate as when in the compatible control (PXO61^{Sm} inoculated alone) (Fig. 1). However, the PXO86^{Rif} growth rate was significantly reduced compared to the incompatible control (PXO86^{Rif} alone) and the final population did not exceed 10^5 cfu/leaf. Similar patterns were observed in mixed inoculations of IR1545-339 with PXO86^{Rif} (incompatible) and PXO99^{Sm} (compatible) (Fig. 2). In cultivar IR20, both bacterial populations (PXO86^{Rif}, compatible and PXO61^{Sm}, incompatible) were reduced about tenfold in comparison with that of PXO86^{Rif} alone, and were similar to the levels reached in incompatible interactions (PXO61^{Sm}) on that host (Fig. 4). In IR8 where both race 1 and 2 isolates result in compatible interactions, populations followed a pattern similar to that observed in interactions of race 1 and 2 with Cas 209 and IR1545-

339; that is, the race 1 isolate (PXO61^{Sm}) in mixed inoculations increased at a rate equal to that when the isolate was inoculated alone. Populations of the race 2 isolate (PXO86^{Rif}) on IR8 however, did not increase above 10^4 cfu/leaf.

Symptom development. Lesions on all hosts began to appear 6-8 days after inoculation (Fig. 5). Average lesion lengths at day 12 differed among cultivars (Fig. 5). Lesions on Cas 209 and IRI545-338 resulting from mixed inoculations were not substantially different from those inoculated only with the compatible race. On IR20, mixed inoculations resulted in shorter lesion lengths by comparison with the compatible control. Lesions produced on IR8 where interactions with both race 1 and 2 are compatible were similar in length.

Spread of bacteria. Bacteria in both compatible (PXO61^{Sm}) and incompatible (PXO86^{Rif}) combinations with Cas 209 multiplied and moved outward from the inoculation point (Table 2). In the incompatible combination, however, bacteria multiplied less rapidly and moved to adjacent sections at a much slower rate. Symptoms were never observed in advance of the bacteria; rather, bacterial numbers in a given section approached 10^8 cfu prior to symptom expression. The same trend was observed in a second experiment.

Effect of inoculum density on expression of resistance. The initial water soaking from Hagborg infiltration of Cas 209 leaves disappeared within 4 hr. At both high and low inoculum levels, PXO61^{Sm} (compatible) multiplied steadily (after a lag of 1-2 days) through day 8 (Fig. 6). After day 10, multiplication reached a

plateau. Numbers of PXO86^{Rif} (incompatible) at high inoculum did not significantly increase above the initial 10^7 cfu/leaf. At low inoculum, the bacteria multiplied to 10^7 cfu/leaf by day 6 and did not increase beyond that level. After Hagborg inoculation with a high inoculum density, water soaking was observed in the compatible combination at 48-72 hr. In incompatible combinations, either no symptoms appeared by day 4 or the leaf tissue which was infiltrated became dry and gray in color. The same results were observed in a second experiment.

DISCUSSION

Our investigations indicate that incompatibility in the interaction between X. c. pv. oryzae isolates and cultivars Cas 209, IR1545-339, and IR20 was reflected in lower bacterial numbers and reduced lesion lengths, although these parameters were much less pronounced in IR20. In general, lesion lengths corresponded with bacterial numbers in the interactions; that is, the higher the bacterial numbers, the longer the lesions.

In cultivar IR20, bacterial numbers in compatible interactions do not reach the high levels observed in cultivars Cas 209, IR1545-339, and IR8, suggesting that some level of resistance even in the compatible exists. In comparison with the other cultivars, compatible interactions in IR20 result in reduced lesion development (Horino et al., 1982; this work). Thus, IR20 must contain some means by which to inhibit bacterial growth and restrict lesion development; whether or not this resistance is a feature of the Xa-4 gene for bacterial blight resistance carried by IR20 is not clear.

Resistance to X. c. pv. oryzae conferred by IR20 is not complete (for review, see Mew, 1987; Ou, 1985). Horino et al., (1982) reported that gene Xa-4 is influenced by temperature. At high temperatures (33 C day, 25 C night), lesions in incompatible interactions developed up to five times longer than lesions at low temperatures (29 C day, 21 C night). The effect of temperature on bacterial multiplication was not measured. Our conditions (32 C day, 22 C night) approached those which would favor longer lesions in incompatible interactions and, thus, may have minimized the expected differences in compatible and incompatible interactions.

To assess whether the activation of host defense mechanisms was dependent on time or a threshold number of bacteria, varying inoculum levels were used and bacterial multiplication was monitored. As shown in Fig. 1 (initial inoculum about 10^3 cfu/leaf) and in the inoculum density experiment (Fig. 6 ; initial inoculum about 10^5 and 10^7 cfu/leaf), bacteria in incompatible combinations multiplied to 10^6-10^8 cfu/leaf and then leveled off regardless of the time necessary to reach that number. Thus, bacterial concentration was critical in the activation of rice defense mechanisms. Although the reduction of the growth rate was observed when bacterial numbers had reached 10^7-10^8 cfu/leaf, activation of host defenses probably took place earlier.

Parry and Callow (1986) measured bacterial movement down rice leaves by plating sections and scoring for presence or absence of bacterial growth from the ends of those sections. Bacteria were found in the same number of leaf sections in both compatible (PXO71)

and incompatible (PXO86) combinations with Cas 209. They concluded that since in both interactions bacteria were present throughout the leaf, the difference between compatible and incompatible interactions was in the expression of symptoms by the host; that is, longer lesions did not necessarily result from more bacterial growth and spread. However, they did not quantify bacterial numbers in the leaf sections. In our experiments, isolates of both compatible and incompatible X. c. pv. oryzae races colonized leaf tissues. However, in the incompatible combination, bacteria did not invade the leaf as aggressively as did those in the compatible combination (Table 2). In addition, when bacteria were present in a section in the incompatible combination, numbers were always one to two orders of magnitude lower than those in the compatible interaction. Previous reports indicated differences in bacterial numbers between compatible and incompatible interactions were only one hundred-fold or less (Parry, 1986; Reddy, 1973; Mohiuddin, 1975; Watanabe, 1975; Nakanishi, 1977). However, in those studies leaf sections of 15 cm or less were sampled. Our experiments suggest that because 1) bacteria in incompatible interactions do grow and move in leaves to a limited extent, 2) bacterial numbers in both combinations were similar at the inoculation site (E), and 3) compatible bacteria accumulate to fairly high concentrations in the lower portions of the leaf prior to symptom expression (Ou, 1985; our data, not shown), whole leaf samples give a more reliable estimate of the true differences in compatible and incompatible interactions. No symptoms were seen in sections with less than 10^7 cfu/leaf,

indicating a minimum bacterial number necessary for symptom expression.

In mixed inoculation experiments of Cas 209 or IR1545-339, if the mixture contained equal numbers of bacteria from both races, bacterial multiplication of isolates representing the compatible combination was not restricted by comparison with growth in leaves inoculated with only the compatible isolate (Figs.1,2). However, multiplication of bacteria representing incompatible races was inhibited, and bacterial numbers dropped over time. We observed the same phenomenon in IR8 (Fig. 3) which carries no known genes for resistance to these isolates, suggesting that inhibition of incompatible bacterial growth was likely due to competition between the isolates. No inhibition of one isolate by another was observed in *in vitro* mixed cultures, but the race 2 isolate ($PXO86^{Rif}$) multiplied at a slightly slower rate than did the race 1 isolate ($PXO61^{Sm}$, data not shown). Other isolates ($IRN793^{Rif}$, race 2 and $PXO99^{Sm}$, race 6) in Cas 209 produce similar growth curves in single and mixed inoculations. It is possible that in the host plant, isolates such as $PXO61^{Sm}$ or $PXO99^{Sm}$ can out-compete other isolates for available nutrients. In IR20, growth of the isolate $PXO86^{Rif}$ (compatible) was not reduced to the same extent in mixed inoculations with $PXO61^{Sm}$ (incompatible), as was apparent in cultivars Cas 209 and IR8. Multiplication patterns of isolate $PXO61^{Sm}$ (incompatible) were similar in mixed and single inoculations. Thus, in IR20 competition is not obvious and resistance is apparently physiologically dominant to

susceptibility.

As did others (Parry, Reddy), we observed that in some cultivars (Cas 209 and IR1545-339) inoculated with 1:1 mixtures of compatible and incompatible races the lesion lengths were not significantly different from those of leaves inoculated with only compatible bacteria. In IR20, lesions lengths were reduced by comparison with the compatible control, but in our conditions, lesions between compatible and incompatible interactions were not significantly different. Collectively, the population and lesion length data suggest that incompatibility is not physiologically dominant to compatibility in the rice/X. c. pv. oryzae interaction (with the exception of perhaps IR20). The apparent competition between isolates in mixed inoculations, however, confounds this conclusion. In an attempt to avoid or mask the effects of competition, several approaches are underway. Preliminary evidence indicates that if higher ratios (incompatible to compatible) of bacteria are inoculated to rice leaves of cultivars Cas 209 and IR1545-339 or incompatible races are inoculated prior to inoculation with compatible races, then lesion lengths and multiplication of bacteria from both races are reduced. In addition, we are developing isogenic mutants of X. c. pv. oryzae differing only in the gene responsible for incompatibility to a particular host. These will be used in concert with newly released host isolines which differ only in those resistance genes of interest (Ogawa, 1987) to more critically evaluate race-specific induction of resistance.

LITERATURE CITED

1. Borkar, S. G. and Verma, J. P. 1985. Population dynamics of Xanthomonas campestris pv. malvacearum (Xcm) in compatible and incompatible reaction of cotton cultivars. Acta Phytopathol. Acad. Sci. Hungaricae 20:31-34.
2. Devadath, S. 1970. Effect of mixture of inoculations of virulent and less virulent isolates of Xanthomonas oryzae. Curr. Sci. 39:420-421.
3. Ellingboe, A. H. 1976. Genetics of host-parasite interactions. Pages 761-778 in: Physiological Plant Pathology. R. Heitefuss and P. H. Williams, eds. Springer-Verlag, Berlin.
4. Fugii, H. 1976. Variation in the virulence of Xanthomonas oryzae (3) Interaction between avirulent mutants and virulent wild type isolates. Ann. Phytopathol. Soc. Jpn. 42:526-532.
5. Hagborg, W. A. F. 1970. A device for injecting solutions and suspensions into thin leaves of plants. Can. J. Bot. 48:1135-1136.
6. Horino, O., Mew, T. W., and Yamada, T. 1982. The effect of temperature on the development of bacterial leaf blight on rice. Ann. Phytopathol. Soc. Jpn. 48:72-75.
7. Keen, N. T., Ersek, T., Long, M., Bruegger, B., and Holliday, M. 1981. Inhibition of the hypersensitive reaction of soybean leaves to incompatible Pseudomonas spp. by blasticidin S, streptomycin or elevated temperature. Physiol. Plant Pathol. 18:325-337.
8. Kelman, A. 1954. The relationship of pathogenicity in Pseudomonas solanacearum to colony appearance on a tetrazolium medium. Phytopathology 44:693-695.

9. Klement, Z., Farkas, G. L. and Lovrekovich, L. 1982. Hypersensitivity. Pages 149-177 in: Phytopathogenic Prokaryotes. M. S. Mount and G. H. Lacy, eds. Academic Press, New York.

10. Long, M., Barton-Willis, P., Staskawicz, B. J., Dahlbeck, D., and Keen, N. T. 1985. Further studies on the relationship between glyceollin accumulation and the resistance of soybean leaves to Pseudomonas syringae pv. glycinea. Phytopathology 75:235-239.

11. Maniatis, T., Fritsch, E. F., and Sambrook, J. 1982. Molecular-cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 545 pp.

12. Mew, T. W. 1987. Current status and future prospects of research on bacterial blight of rice. Annu. Rev. Phytopathol. 25:359-382.

13. Mohiuddin, M. S., and Kauffman, H. E. 1975. Multiplication studies of Xanthomonas oryzae isolates on differential rice varieties. Curr. Sci. 44:637-638.

14. Muko, H., and Yoshida, K. 1951. A needle inoculation method for bacterial leaf blight disease of rice. Ann. Phytopathol. Soc. Jpn. 15:179.

15. Nakanishi, K., and Watanabe, M. 1977a. Studies on the mechanism of resistance of rice plants against Xanthomonas oryzae. III. Relationship between the rate of production of antibacterial substances and of multiplication of pathogenic bacteria in infected leaves of resistant and susceptible varieties. Ann. Phytopathol. Soc. Jpn. 4:265-269.

16. Nakanishi, K., and Watanabe, M. 1977b. Studies on the mechanism of resistance of rice plants against Xanthomonas oryzae. IV.

Extraction and partial purification of antibacterial substances from infected leaves. Ann. Phytopathol. Soc. Jpn. 4:449-454.

17. Ogawa, T. and Yamamoto, T. 1987. Selection of recurrent parents to develop near-isogenic lines resistant to bacterial leaf blight of rice. Jpn. Agric. Res. Q. 21:65-69.

18. Olufowote, J. O., Khush, G. S., and Kauffman, H. E. 1977. Inheritance of bacterial blight resistance in rice. Phytopathology 67:772-775.

19. Ou, S. H. 1985. Rice Diseases. Commonwealth Mycological Institute, U.K.

20. Parry, R. W. H. and Callow, J. A. 1986. The dynamics of homologous and heterologous interactions between rice and strains of Xanthomonas campestris. Plant Pathol. 35:380-389.

21. Petpisit, V., Khush, G. S., and Kauffman, H. E. 1977. Inheritance of resistance to bacterial blight in rice. Crop Sci. 17:551-554.

22. Reddy, A. P. K., and Kauffman, H. E. 1973. Multiplication and movement of Xanthomonas oryzae in susceptible and resistant hosts. Plant Dis. Rep. 57:784-787.

23. Reddy, A. P. K., and Kauffman, H. E. 1974. Population studies of mixed inoculum of Xanthomonas oryzae in susceptible and resistant varieties of rice. Ann. Phytopathol. Soc. Jpn. 40:93-97.

24. Stall, R. E., and Cook, A. A. 1966. Multiplication of Xanthomonas vesicatoria and lesion development in resistant and susceptible pepper. Phytopathology 56:1152-1154.

25. Szybalski, W. 1952. Microbial Selection. I. Gradient plate technique for study of bacterial resistance. Science 116:46-48.

26. Tsuchiya, K., Mew, T. W., and Wakimoto, S. 1982. Bacteriological and pathological characteristics of wild types and induced mutants of Xanthomonas campestris pv. oryzae. Phytopathology 72:43-46.

27. Watanabe, M., and Asaumi, T. 1975. Studies on the physiological changes in the rice plants infected with Xanthomonas campestris pv. oryzae. I. Relationship between the increase in respiratory rate and multiplication of the bacteria in infected leaves. Ann. Phytopathol. Soc. Jpn. 41:364-372.

28. Yoshimura, A., Mew, T. W., Khush, G. S., and Omura, T. 1983. Inheritance of resistance to bacterial blight in rice cultivar Cas 209. Phytopathology 73:1409-1412.

Figure Legends

Fig. 1. Growth of Xanthomonas campestris pv. oryzae isolates PX061^{Sm} (race 1, compatible) and PX086^{Rif} (race 2, incompatible) in leaves of rice cultivar Cas 209. Rice leaves were inoculated individually with each isolate (PX061^{Sm} or PX086^{Rif}), or with a mixture of the two bacteria at a 1:1 ratio. Data are means and standard errors of four replications from each treatment. The experiment was repeated three times with similar results.

Fig. 2. Growth of Xanthomonas campestris pv. oryzae isolates PX099^{Sm} (race 6, compatible) and PX086^{Rif} (race 2, incompatible) in leaves of rice cultivar IR1545-339. Rice leaves were inoculated individually with each isolate (PX099^{Sm} or PX086^{Rif}), or with a mixture of the two bacteria at a 1:1 ratio. The experiment was repeated twice with similar results.

Fig. 3. Growth of Xanthomonas campestris pv. oryzae isolates PX086^{Rif} (race 2, compatible) and PX061^{Sm} (race 1, incompatible) in leaves of rice cultivar IR20. Rice leaves were inoculated individually with each isolate (PX086^{Rif} or PX061^{Sm}), or with a mixture of the two bacteria at a 1:1 ratio. The experiment was repeated twice with similar results.

Fig. 4. Growth of Xanthomonas campestris pv. oryzae isolates PX061^{Sm} (race 1, compatible) and PX086^{Rif} (race 2, compatible) in leaves of rice cultivar IR8. Rice leaves were inoculated individually with each isolate (PX061^{Sm} or PX086^{Rif}), or with a mixture of the two bacteria at a 1:1 ratio. The experiment was repeated twice with similar results.

Fig. 5. Lesion lengths on four rice cultivars 12 days after inoculation

with single ($PXO86^{Rif}$; $PXO61^{Sm}$ or $PXO99^{Sm}$) or mixed ($PXO86^{Rif}/PXO61^{Sm}$ or $PXO86^{Rif}/PXO99^{Sm}$) isolates of Xanthomonas campestris pv. oryzae. Bars represent standard errors of the means.

Fig. 6. Effect of inoculum density on growth of Xanthomonas campestris pv. oryzae isolates $PXO61^{Sm}$ (race 1, compatible) and $PXO86^{Rif}$ (race 2, incompatible) per leaf of rice cultivar Cas 209. Rice leaves were infiltrated using a Hagborg apparatus with starting inocula of 5×10^{10} ($PXO61^{Sm}$, $PXO86^{Rif}$) and 5×10^{8} ($PXO61^{Sm}$, $PXO86^{Rif}$) cfu/leaf.

TABLE 1. Interactions of X. campestris pv. oryzae Philippine races with rice cultivars IR8, IR20, Cas 209 and IR1545-339 (Adapted from Mew, 1987)

Differential Cultivar (R gene)	1	2	6
IR8 (Xa-11)	C[a]	C	C
IR20 (Xa-4)	I[b]	C	C
Cas 209 (Xa-10)	C	I	C
IR1545-339 (xa-5)	I	I	C

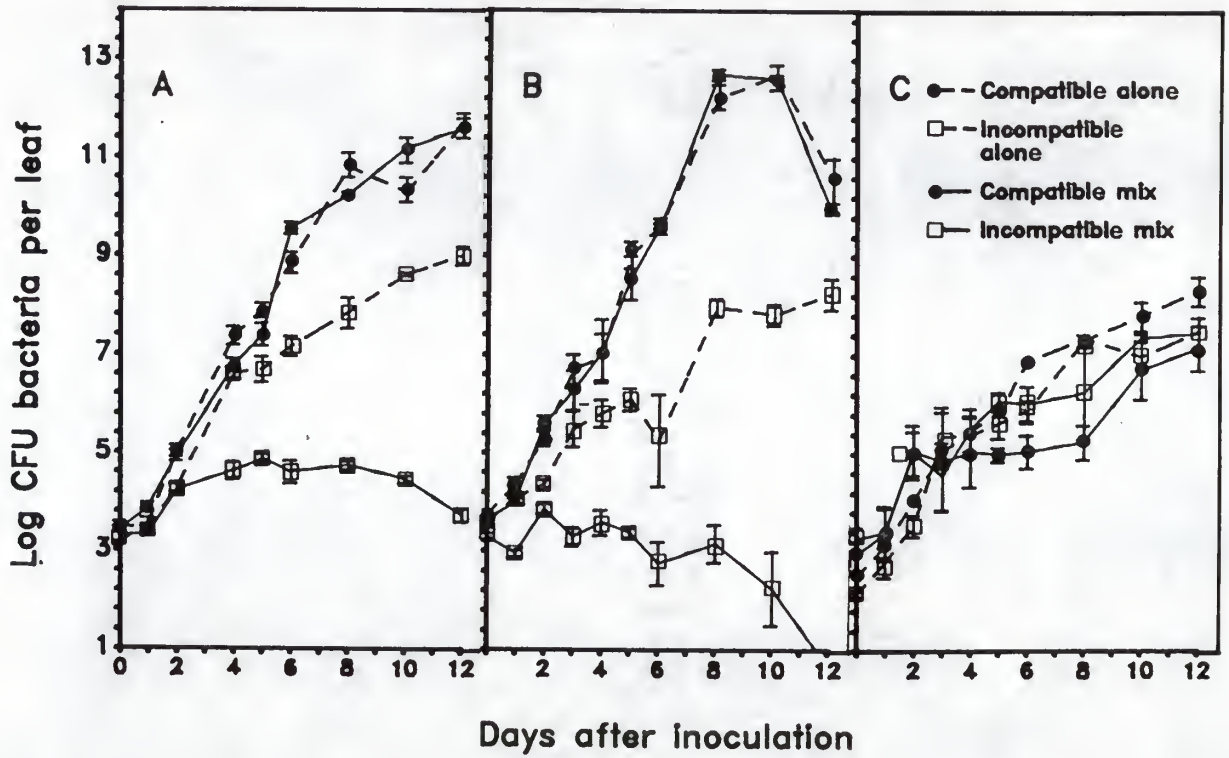
[a] = compatible; [b] = incompatible.

Table 2. Spread of Xanthomonas campestris pv. oryzae isolates PXO61^{Sm} (race 1) and PXO86^{Rif} (race 2) in adjacent leaf sections (A-F) of rice cultivar Cas 209. Progression out from the inoculation point was monitored in the upper 12 cm of the leaf at 2-day intervals by cutting 2-cm sections above and below the point of inoculation (section E).

Day	Race	Cfu/2-cm leaf sections						Total lesion length (cm)
		A	B	C	D	E	F	
0	1	0	0	0	0	$2x10^{4a}$	0	0
	2	0	0	0	0	$2x10^{4}$	0	0
2	1	0	0	0	0	$5x10^{4}$	0	0
	2	0	0	0	0	$3x10^{4}$	0	0
4	1	0	0	$5x10^{5}$	$7x10^{6}$	$3x10^{7}$	$1x10^{5}$	0
	2	0	0	0	$2x10^{4}$	$1x10^{6}$		0
6	1	$3x10^{6}$	$1x10^{7}$	$3x10^{7}$	$5x10^{7}$	$7x10^{7}$	$5x10^{7}$	0
	2	0	0	0	$3x10^{5}$	$7x10^{6}$	$3x10^{5}$	0
8	1	$5x10^{6}$	$5x10^{7}$	$3x10^{8}$	$3x10^{8}$	$3x10^{9}$	$5x10^{8}$	6.3
	2	0	$5x10^{5}$	$3x10^{7}$	$5x10^{7}$	$6x10^{7}$	$4x10^{7}$	0
12	1	$3x10^{7}$	$2x10^{7}$	$6x10^{7}$	$1x10^{8}$	$1x10^{8}$	$1x10^{8}$	19.5
	2	0	$2x10^{6}$	$2x10^{7}$	$3x10^{7}$	$6x10^{7}$	$1x10^{7}$	3.7

[a]Data are means of four replications. The experiment was repeated twice with similar results.

Fig. 1 Fig. 2 Fig. 3



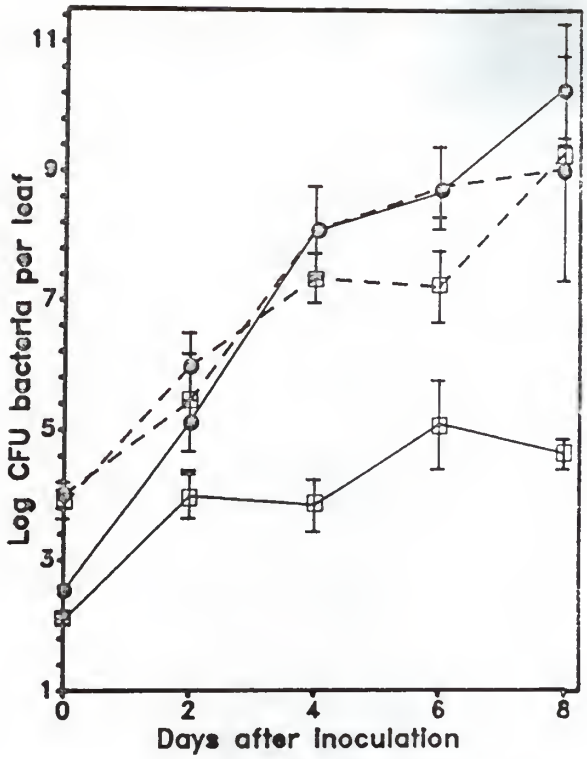


Fig. 4

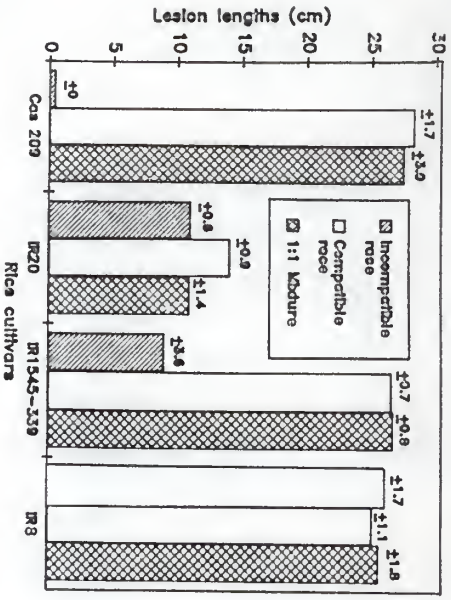


Fig. 5

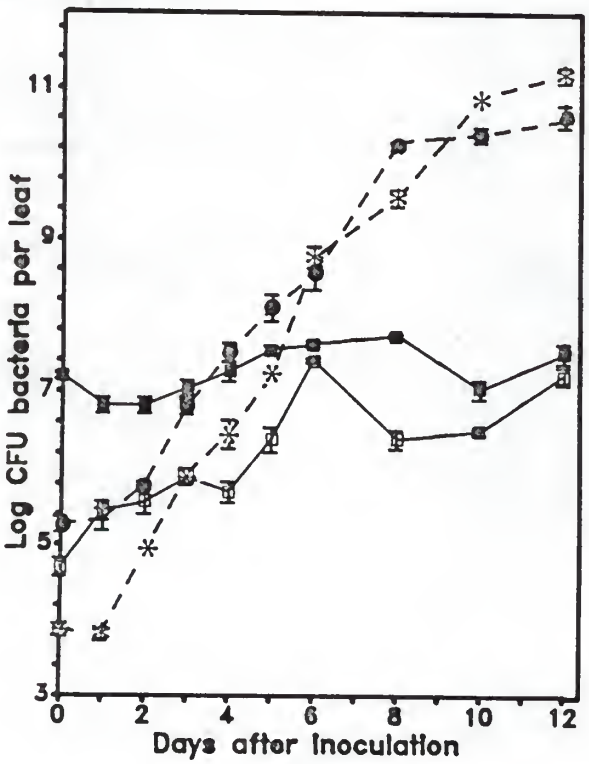


Fig. 6

Figure Legends

Figure 1. Multiplication of Xanthomonas campestris pv. oryzae (C6, compatible; I2, incompatible) per leaf of rice culativar IR1545-339. Rice leaves were inoculated individually with C6 or I2, or with a ratio of 1:1 mixture.

Figure 2. Multiplication of Xanthomonas campestris pv. oryzae (C6, compatible; I2, incompatible) per leaf of rice culativar IR1545-339. Rice leaves were inoculated individually with a ratio of 1:1 or 10:1 (I2:C6) mixture.

Figure 3. Multiplication of Xanthomonas campestris pv. oryzae (C6, compatible; I2, incompatible) per leaf of rice culativar IR1545-339. Rice leaves were inoculated individually with C6 or I2, or (A) with I2 (incompatible) four days before C6 (compatible), or (B) with C6 (comaptible) four days before I2.

Fig. 1

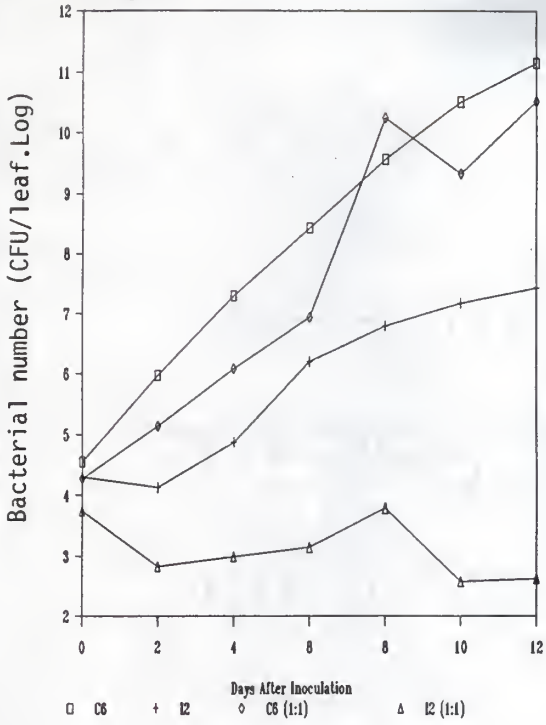


Fig. 2

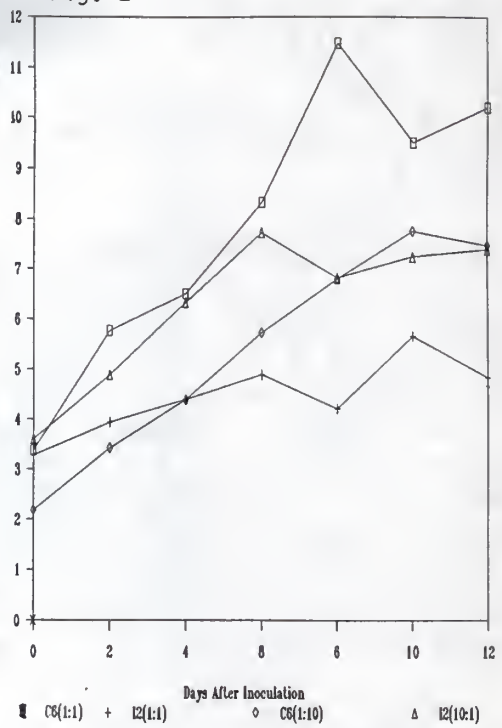
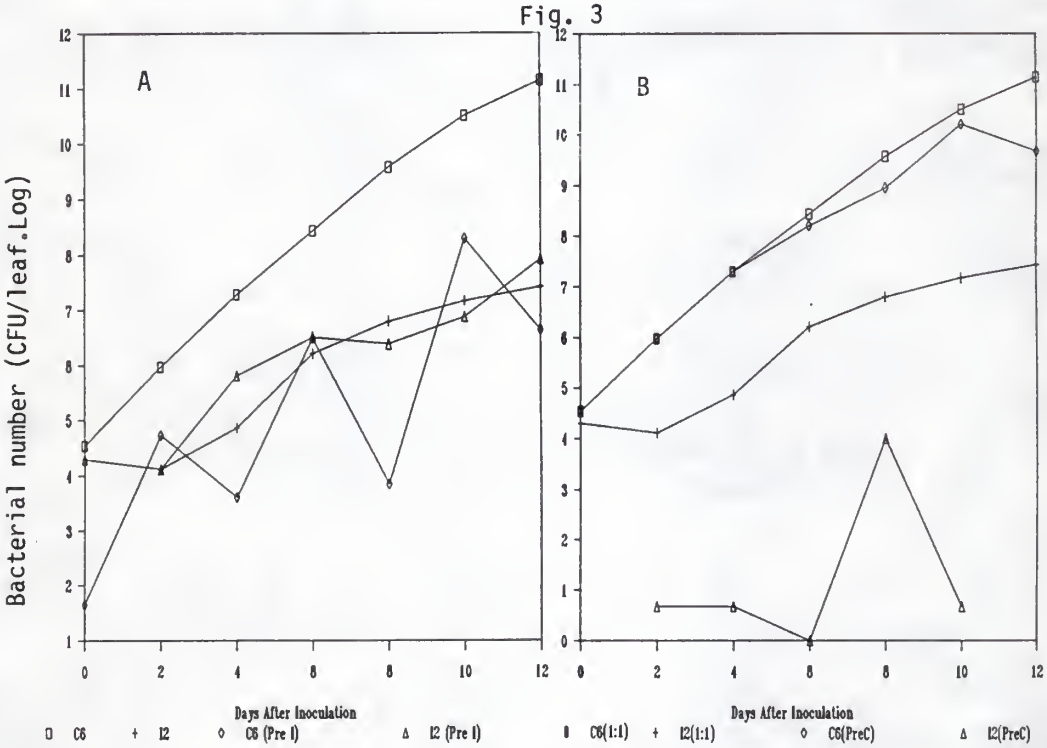


Fig. 3



INTERACTION OF RICE WITH ISOLATES OF COMPATIBLE AND INCOMPATIBLE *XANTHOMONAS CAMPESTRIS* PV. *ORYZAE* AND RACES

I. EXAMINATION OF RICE WATER PORES IN COMPATIBLE AND INCOMPATIBLE INTERACTIONS WITH SCANNING ELECTRON MICROSCOPY

II. CHARACTERIZATION OF BACTERIAL MULTIPLICATION DYNAMICS

by

AILAN GUO

B.S., Shandong Agricultural University

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Plant Pathology

KANSAS STATE UNIVERSITY
MANHATTAN, KANSAS

1988

ABSTRACT

Compatible and incompatible interactions between Xanthomonas campestris pv. oryzae and rice (Oryza sativa L.) were characterized with 1) scanning electron microscopy (SEM) and 2) bacterial growth dynamics and lesion development in rice. Individual water pores were examined by SEM for presence or absence of plugging exudate at 24 and 48 hr postinoculation. Water pore plugs were present following treatment with isolates of incompatible or compatible races and in water or untreated controls. No differences were observed in the percentage of plugs after any treatment. Therefore, water pore plugging is not specifically induced in X. c. pv. oryzae/rice interactions.

The growth rates of X. c. pv. oryzae isolates were initially the same in both compatible and incompatible combinations. Once 10^6-10^8 cfu/leaf were reached, distinguishable trends in multiplication were observed. In compatible combinations, bacterial numbers reached 10^{12}-10^{13} cfu/leaf, while in incompatible interactions, bacteria were maintained at 10^6-10^8 cfu/leaf. Lesions appeared earlier and were longer in compatible than incompatible combinations.

When rice leaves were inoculated with a 1:1 mixture of incompatible and compatible isolates, the growth curve of compatible isolates was similar to that of the compatible isolate when inoculated alone. However, the growth rate of incompatible bacteria in 1:1 mixed inoculations was reduced when compared with control inoculations (incompatible alone). Lesions resulting from inoculations with 1:1 mixtures were the same length as those in

compatible interactions. The growth pattern of the incompatible isolate in 1:1 mixtures suggested competition existed between X. c. pv. oryzae isolates in rice leaves.

If ratios of 10:1 (incompatible to compatible) or greater were used as inoculum, or if leaves were first inoculated with an incompatible isolate and challenged after four days with a compatible isolate, growth rates of both isolates were similar until the population of incompatible bacteria reached 10^6-10^8 cfu/leaf. Then, growth of both isolates slowed. Lesions resulting from these inoculations were the same length as those in incompatible interactions. Because initial growth rates of compatible and incompatible isolates were similar, inhibition of compatible isolate multiplication probably resulted from induction of resistance by incompatible isolates rather than from competition. This work supports the hypothesis that once induced, resistance is phenotypically dominant over susceptibility.