Bacterial Blight of Rice

International Rice Research Institute

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Foreword

Bacterial blight (BB) has been known for a century. Before high-yielding but susceptible varieties were introduced, the disease was found only in East Asia. In the 1960s, when the first high-yielding varieties were planted in South and Southeast Asia, BB occurrence was widespread. Since then, BB has become one of the major constraints to rice production in irrigated, rainfed lowland, and deepwater rice. In recent years, it has been reported on other continents; its appearance in the Americas and Africa has caused great concern about both its transmission and its dissemination.

In collaboration with national rice research programs, IRRI started breeding for BB resistance about two decades ago. The effort has made a significant contribution to lowering the disease pressure. Many breeding lines and popular modern varieties possess resistance to BB. But the presence of pathogenic races has initiated another phase of the problem. We cannot relax our efforts; new challenges emerge.

In response to the demand for new directions and to consolidate our strength, we must assess current problems, review past achievements, and face future challenges concerning the disease. Thus, IRRI decided to hold a workshop to assemble scientists actively working on both pathological and genetic aspects of rice BB. A few scientists who are experienced in bacterial diseases other than BB were invited to help identify research areas where collaboration could be strengthened and specific research efforts or methodologies that should be emphasized or applied.

The International Workshop on Bacterial Blight of Rice was held on 14-18 March 1988, followed immediately by a two-day teach-in on molecular techniques in *Xanthomonas:* The objectives of the workshop were

- to assess the threat of the disease on continents where it has been recently reported;
- to review the current status of epidemiology, ecology, methodology of varietal evaluation/screening for resistance, genetic sources for resistance, pathogen variability, and control measures for the disease;
- to strengthen ongoing collaborative research and to develop plans concerning fundamental and applied aspects of research into the disease; and
- to demonstrate and teach some molecular methods and techniques relevant and applicable to *Xanthomonas.*

This book represents the progress made, the information exchanged, and the reviews and recommendations arising from the workshop as provided by distinguished scientists.

We hope the recommendations formulated during the workshop will be helpful to institutions and individuals engaged in BB research.

I am very grateful to our cosponsors, the Administration Générale de la Coopération au Développement, Ministére des Affaires Etrangéres, Belgium, for their financial support.

I am also grateful to the members of the organizing committee for their efforts to develop the program: T.W. Mew (Chairman), G.S. Khush, D.J. Mackill, J.K. Ladha, H. bung, S.J. Banta, M.D. Pathak, and V. Segovia.

This volume was edited by S.J. Banta, with the assistance of E. Cervantes. T.W. Mew served as the technical editor.

Klaus Lampe
Director General

Welcome address

Welcome to IRRI, and to this workshop, a joint undertaking of the Administration Générale de la Coopération au Développement Ministere des Affaires Etrangéres, Belgium, and IRRI.

1 am pleased that the first workshop in which 1 am participating as IRRI's Director General is a joint one, because I strongly believe that cooperation is the only avenue for solving the problems that confront rice science today. This type of activity opens a window to IRRI's future. We are being asked to go into areas of "upstream research,"which requires strengthening of our collaboration with basic and strategic research institutions across the world. But this does not mean that we will ignore our partners in the Third World. Their functions and ours should be complementary, and IRRI should become the "research institute of last resort,'' concentrating on strategic research.

Upstream research is closely linked to collaboration with the industrialized countries. This collaboration is of special relevance to research areas that concern the spread of tropical pests and diseases. Shuttle research with institutions in Europe, Japan, and North America can minimize or even eliminate any risk factors linked to innovation. Perhaps another kind of North-South collaboration in research could be initiated, where elements of the research that might not be considered appropriate in the tropics could be done in temperate zones.

Public awareness of the advanced biological research we are undertaking is far greater than for most other fields with which IRRI deals. I see this as a positive sign. The world's citizens are beginning to exert influence on what is happening around them. Thus, the fate of the earth is no longer left in the hands of a few. IRRI has a dual responsibility in this respect: We must protect the sustainability of the environment while simultaneously increasing production.

We do not know enough about bacterial blight and its relationship to physiological processes in the rice plant. We do not know enough about the mechanisms that control genetic resistance to the disease. No one can explain why some rices are susceptible to bacterial blight, while others are resistant. Until we understand such process, we will never find the keys that will open the doors to new protection strategies and mechanisms.

We must find ways of controlling bacterial blight that are effective, harmless, and economically acceptable to resource-poor farmers. That is why we joined our Belgian colleagues with enthusiasm in organizing this gathering. This workshop will hopefully stimulate joint, worldwide programs, and IRRI will play its part.

Klaus Lampe
Director General

Recommendations

Many of the following lines of research are either in progress or are planned for the near future.

I. GENERAL

1. To improve our understanding of bacterial blight (BB) resistance mechanisms and to formulate better management tactics, the virulence of field races should be compared and the shifting of virulence monitored.
2. Because so much depends on the sensitivity of the methods used to detect the causal bacterium at low concentration (in seed, for example), more effort should be directed at improving new methodologies (e.g., DNA probes, plasmid and chromosomal restriction profiles, and monoclonal antibody analysis).

II. CLASSIFICATION AND IDENTIFICATION

1. A formal proposal should be initiated in the International Journal of Systematic Bacteriology for the elevation of both *Xanthomonas campestris* pv. *oryzae* (Xco) and X. *campestris* .pv. *graminis* from the rank of pathovar to that of species.
2. Contemporary immunological and molecular genetic techniques should be used to address the problem of differentiation of Xco strains (i.e., isolates or clones) differing in virulence and pathogenicity.
3. There should be more study of antigens other than extracellular polysaccharides (EPSs) (e.g., cell membrane proteins, enzymes, ribosomal proteins, and oligonucleotides linked to carrier proteins). Membrane protein antigens and ribosomes should be studied to determine their specificity levels.
4. A central, international serological reference laboratory should be set up in collaboration with national programs for preparing and distributing antisera used in typing.
5. An international standard serogrouping system should be established so that the results from independent workers can be evaluated and compared. However, much more work is needed on the reproducibility and limits of reliability of the test system.

6. Monoclonal antibodies to Xco should be prepared with a view to developing a typing scheme for epidemiological investigations, paralleling recent work on *X. campestris* pv. *campestris.*
7. A search should be made for race-specific antigens, particularly on the cell surface, from which monoclonal antibodies could be generated.
8. A search should be made for race-specific isozyme markers that might serve as specific antigenic determinants.
9. The EPSs of Xco races should be molecularly characterized. (But are the differences stable across cultural conditions?)
10. A reference culture collection of Xco strains should be established to provide a universally accepted series of standard races for comparative purposes. (J. Swings volunteered to maintain the collection.)

III. PATHOGENESIS

1. There should be more studies of mechanisms of basic pathogenicity in the Xco-rice interaction, a topic barely mentioned at the workshop. An approach that has been proven to work with other organisms consists of isolating mutants altered in pathogenicity, and then finding and analyzing genes from genomic libraries that complement the mutations. Since Xco libraries have been made and mutagenesis has been begun, this should not pose great problems.
2. Techniques should be developed for detailed physiological and ultrastructural study of the interactions between particular plants and Xco genotypes. Binary scoring systems such as compatible-incompatible are not adequate. The demonstration of consistent (although not absolute) physiological differences between Xco races (such as in carbon-source utilization) underlines the need for physiological study.
3. The interesting work on epiphytic colonization and possible hydathode specificity needs to be extended and made quantitative. If this aspect of the interaction were confirmed to be important in determining specificity, it would open the way for the development of rapid screening procedures. More work should be done to confirm the apparent entrapment of Xco in resistant cultivars at the level of the hydathode aperture, and to explain the role and nature of the exudate from water pores in the entrapment process.
4. More effort should be directed at understanding the role of EPS in the pathogenesis of BB of rice.
5. The role of avirulence genes in controlling compatibility and incompatibility relationships in the Xco-rice interaction, revealed for the first time at the workshop, should be fully investigated.

IV. EPIDEMIOLOGY AND ECOLOGY

1. The contradictory evidence on seed transmission of Xco creates a pressing need for more critical information on 1) production of pathogen-free seed, particularly for monocropped areas; 2) "curing" methods for seed (e.g., by hot water, chemical, or phage); and 3) the relative survival of Xco in seed of different

cultivars under different storage conditions (susceptible cultivars have been shown to differ markedly in the extent of seed transmission obtained from an infected crop).

2. Serological methods should be developed for detecting Xco in various parts of the rice plant (cf. I.2).
3. More research is needed to determine the exact role of wild rices and alternate weed hosts as factors contributing to the primary source of inoculum for the rice crop in a range of environments.
4. To develop rational and economical control measures, the extent of crop losses must be evaluated and related to the potential gain obtained from control practices. There is a need to develop better methodologies, technical capabilities, and a more workable system to monitor chronological crop losses.
5. Because existing screening techniques are inadequate to identify and exploit field resistance, more work should be done to evaluate field resistance and its mode of inheritance.
6. More effort should be spent on aspects of disease management suited to diverse rice ecosystems. Such management strategies need validation in farmers' fields before recommendations are made for large-scale adoption.
7. Quantitative knowledge about the components of the infection cycle should be accumulated so that pathosystems analysis can be done. Field work should be pursued to find out which of the models (exponential, logistic, Gompertz, or Richards) best describes the BB disease progress curve. Quantitative information is needed to improve our understanding of BB epidemiology. Without this knowledge it will be difficult, if not impossible, to develop sound disease management strategies.

V. HOST RESISTANCE AND PATHOGEN VARIATION

1. There should be further investigation of the relationship between the presence and concentration of inhibitory substances in vessel walls, and resistance to BB (cf. VII.1).
2. There should be more investigation of differences between resistant and susceptible cultivars at the level of the vessel wall.
3. More work should be done to confirm and extend the observation that water pores on rice leaves are involved in the specificity of the rice-Xco interaction. There is a need for greater understanding of hydathode structure and physiology in relation to the resistance or susceptibility of rice and weed hosts.
4. The quantitative relationship between dose and reaction should be further studied using the infectivity titration approach to compare aggressiveness in the pathogen with resistance in the host.
5. Some consideration should be given to the use of detached leaves On water agar as a means of assessing resistance (or as a means of detecting Xco in seed) following the recent work with dicotyledonous hosts.
6. Histological, anatomical, and biochemical analyses of lesion types in highly resistant lines are needed to help identify types of resistance.

7. The search for alternative methods for assessing resistance to characterize the resistance mechanisms found and to aid in distinguishing between otherwise similar levels of resistance should continue.

VI. GENETICS AND PLANT BREEDING

1. Although the major resistance (R) genes for Xco are likely to be nondurable, as judged by the many races already found, the present breeding approach should be continued. This consists of the introduction of R-genes into commercial varieties, provided that, in crosses with susceptible parents, only moderately susceptible entries are used.
2. To prepare for the situation in which R-genes no longer give sufficient protection because very complex races have evolved, emphasis should be placed on true quantitative resistance (not that which is based on R-genes with incomplete expression). This requires the use of well-defined and accurate assessment procedures, detailed cultivar-isolate studies, and detailed genetic studies.
3. Once this quantitative resistance is well recognized, it should be introduced into various suitable genotypic backgrounds for use as parental material by IRRI and national programs.
4. Highly susceptible lines should be constantly removed from breeding programs to slowly increase the level of quantitative resistance.
5. Strategies such as multilines, multiple major gene barriers, and rotation of cultivars with different R-genes are not specifically recommended, because they are too complicated to realize or exploit.
6. Cultivars and lines used in research should be homozygous, pure, and everywhere the same. Maintenance through continued single-plant reproduction is the only way to ensure this.
7. To support the study of quantitative resistance, isolates that differ in aggressiveness but are in other respects as related to one another as possible should be identified.
8. Once quantitative resistance and aggressiveness are well recognized, efforts should be made to find out what determines differences in them.
9. There should be more study of the mechanisms determining host range, i.e., the mechanisms involved in host recognition, since quantitative resistance may interfere with this mechanism rather than with pathogenicity itself.
10. A set of R-genes with the same genetic background (near-isogenic lines) and a set of avirulence genes with the same bacterial genetic background should be produced and made available to support pathogenicity studies (cf. VII.3).
11. In view of the reports at this Workshop concerning the relatively greater virulence of Xco races from Bangladesh, their study warrants greater emphasis. Some native rice varieties in Bangladesh have been shown to be highly resistant to the isolates from Bangladesh, suggesting the existence of new sources of resistance genes. There should be a collaborative research program involving IRRI, the Bangladesh Rice Research Institute, and Japan to study the

Bangladesh native varieties and Xco isolates so that races present in Bangladesh can be better understood and new sources of resistance identified.

VII. GENETICS OF *XANTHOMONAS CAMPESTRIS* PV. *ORYZAE*

1. The effect of phenolic lignin-related compounds (cf. V. 1) in rice on the Xco-plant interaction should receive more attention, in view of the fact that in several other plant-microbe interactions, these and related compounds have specific effects in controlling gene expression in the bacteria. For example, the presence in Xco of genes repressed or induced by these compounds could be easily studied with existing technology.
2. More avirulence genes should he isolated. Because the prevailing dogma is that dominant avirulence genes interact with dominant plant R-genes to produce incompatibility, the study should be extended to include Xco genes determining interaction type toward recessive *xa* resistance genes. In these cases the physiological mechanism might be different. As a biological model, the Xco-rice system is probably the best currently available for such studies, which demand near-isogenic plants and pathogenic lines.
3. Following VII.2, the use of cloned genes to construct near-isogenic bacterial strains should be pursued. Comparisons of the effects of mutation of certain genes are much more valuable if all strains have a common genetic background. Similar considerations apply to the use of near-isogenic rice lines (cf. VI. 10), although the technical problems in producing these are much more severe.
4. Since many plant-associated bacteria are found to have related genes involved in the interaction phenomenon, full advantage should be taken of heterologous probes from other bacteria (not only *Xanthomonas* pathovars) to isolate potentially interesting Xco genes from libraries.
5. The work on restricted fragment length polymorphism (RFLP) analysis to identify isolates is most encouraging and should be extended. As more avirulence genes are cloned, these will be obvious candidates to include in the program. The feasibility of using RFLP analysis in conjunction with standardized biotin-labeled probes (or other labeling systems) for diagnosis under semifield conditions should be investigated.
6. At present, serological tests (cf. II.6) seemed more suitable for field work than DNA hybridization. If genes are identified (cf. VII.5) as being of particular interest as diagnostic tools, it should be possible to overexpress gene products by genetic manipulation to prepare enough material for antibody production. Thus. the simplicity of serological detection would be combined with the more fundamental differentiation of the genes.
7. Little work is under way to study gene expression in rice following infection with different bacterial strains, and promising results have been obtained. This approach may also lead to the development of useful tools, and may give useful information for breeding programs.

An overview of the world bacterial blight situation

T. W. Mew

Bacterial blight (BB) of rice occurs throughout Asia, in several Latin American countries, and in northern Australia. Recently, it was reported in the Sahelian region and other parts of Africa and in the United States (Texas and Louisiana), where it seems to be indigenous. In the tropics, BB is usually more prevalent in both rainfed and irrigated rices in the wet season. In temperate countries, it is common during the rainy months. In China, BB occurrence depends on the climatic environment. BB occurs at all stages of the rice crop and shows either kresek or leaf blight symptoms. "Yellow leaf," a secondary effect arising from either symptom, appears due to a toxin produced by the bacteria. Breeding BB-resistant varieties has been a major goal of national and international rice improvement programs. However, the presence of pathogenic races of the bacterium has caused great concern. The issue of seed transmission remains controversial. A better understanding of the bacterium would enhance progress in these areas. Advances in molecular genetics and biotechnology will help.

Bacterial blight (BB) caused by *Xanthomonas campestris* pv. *oryzae* (Xco) is one of the most serious diseases of rice. It is also one of the oldest recorded rice diseases, having been known for over a century; the farmers of the Fukuoka area, Kyushu, Japan, first noted the disease in 1884 (Tagami and Mizukami 1962).

Known research on BB started around 1900 in Japan. BB also occurs in other rice-growing countries of Asia, but farmers easily confuse it with drought damage. In general, BB became known in other Asian countries only in the 1960s as a result of the introduction of improved but susceptible rice cultivars such as TN1 and IR8. By now, it has been recorded in almost all the rice-growing countries in Asia except those in the Middle East. Ou (1985) provided a detailed description of the disease in Asia.

OCCURRENCE

Besides its occurrence in Asia, which has been widely reported, the disease has also been found in northern Australia on cultivated rice and on the wild species *Oryza*

rufipogon and *O. australiensis* (Aldrick et al 1973). The spectrum of virulence there is narrow and indigenous. It is not known if the disease poses any serious threat to Australian rice production, since very few reports exist about rice diseases in that country. In recent years, BB has appeared in Queensland (Ramsey and Moffett 1989, this volume).

BB has recently been reported in Africa: in Mali (Buddenhagen et al 1979), the Cameroons (Notteghem and Bandin 1981), and Senegal (Trinh 1980). In Niger, it has caused concern (Reckhaus 1983). Rice culture in Niger has a long history along the Niger River, where farmers traditionally planted low-yielding local varieties. In 1978, BB was observed in some fields. During the 1982 growing season, it appeared for the first time as an economically important disease in the Niamey area. The most susceptible cultivar was Truntchen 22, a high-yielding, fast-growing cultivar preferred by farmers. The Sadia ricefields on the right bank of the Niger River near Niamey became severely infected, showing both kresek and leaf blight. About 50% of the irrigated rice in the Niamey area was badly damaged.

In Senegal, BB was first observed in a demonstration field in Guede Chantier. All the cultivars in the plot were moderately to severely infected. Surveys confirmed the presence of the disease in Guede Chantier, Mbantou, and Niangna. In Mbantou, the attack was serious in farmers' fields on cultivar Khuan She Shung, and an area of 7 ha was completely destroyed (Diop 1980). Farmers were advised to use disease-free seed from crops raised in the hot, dry season.

Following the reports of BB epidemics in Niamey and Sadia, Niger (Awoderu and John 1984), surveys were conducted in Gao and Mopti, Mali, and in Richard-Toll and Fanaye, Senegal, in 1982; and in parts of Mali, Niger, Senegal, and Burkina Faso in 1983. In Sadia, the disease was detected on cultivar Tchounchen 22 during the 1982 survey. Kresek symptoms were observed, and about 35% of the hills were killed at early tillering (Table 1).

BB was found in many ricefields in major rice areas in Niger. In Kirikisoi, Saga, Libore, and Tillabery, both kresek and blight symptoms were noted, while in Banfera, Karfiguela, and Niena Dionkele, only leaf blight was recorded. In Mali, leaf blight and kresek were both found in Mopti, but in Fanaye, Senegal, only leaf blight was observed. These surveys, conducted by scientists from the West Africa Rice Development Association (Awoderu and John 1984), confirmed the occurrence of BB in the Sahelian countries. The disease was observed mostly in irrigated and flooded ricefields on introduced dwarf and medium dwarf cultivars, causing speculation that it could be carried on seed from other countries. However, the fact

Table 1. intensity of bacterial blight in 4 Sahelian countries (Awoderu and John 1984).[a]

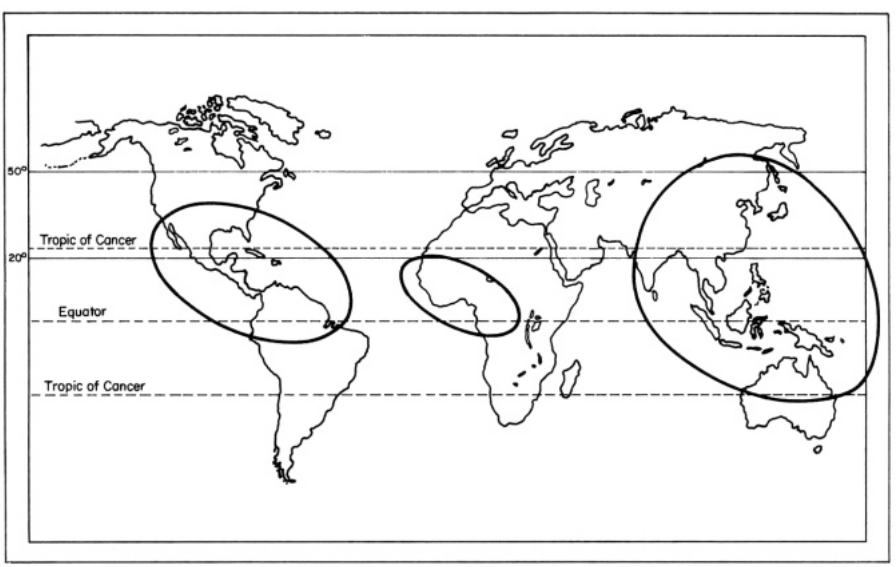
Symptom	Niger	Burkina Faso	Mali	Senegal
Kresek	+++	+	++	–
Leaf blight	+++	++	+	+

[a] –= absent, + = low, ++ = moderate, +++ = high.

that the introduced cultivars appear highly susceptible to the disease, and the low probability that these cultivars would have escaped heavy infection in their countries of origin, may indicate that BB is indigenous. Comparing bacterial strains would be useful both for disease management and for assessing the importance of seed transmission into the region.

Ou (1977) observed lesions on upland rice as well as on weeds both near to and far from ricefields in several Latin American countries. He suspected that the causative agent was similar to Xco, but this has not been confirmed by isolation, pathogenicity, or other bacteriological tests. Lozano (1977) isolated a bacterial pathogen from infected rice in the Caribbean region and identified it as Xco. No further reports of BB in the Americas emerged until July 1987, when a rice disease exhibiting symptoms similar to those of BB was observed in Texas and Louisiana in the United States (J. Leach, Kansas State University, pers. comm.). Bacterial isolates from infected tissues showed the cultural and bacteriological characteristics and pathogenicity of Xco. This was perhaps the first time the disease was reported in the U.S.

BB thus occurs globally, from Asia to Africa and the Americas (Fig. 1). Its distribution ranges from 20°S in Queensland, Australia, to 58° N in Heilang Jiang, China (Zhang Qi and Lin Shinchen, Chinese Academy of Agricultural Sciences, 1984, pers. comm.), and from sea level to the Tibetan Plateau. Usually it is more prevalent in the wet season (WS) than in the dry season (DS), and in lowland than in favorable upland environments. In subtropical regions that are double-cropped, it occurs in both rice crops. In temperate countries such as Korea and Japan, where most rice is monocropped under irrigation, the disease is common from July to



1. Global distribution of bacterial blight of rice.

October, during the rainy months, especially after heavy rainstorms. In China, where different cropping patterns are practiced, the disease behaves according to the climatic environment.

SYMPTOMS

BB is a vascular disease. The infection is, therefore, systemic. The pathogen normally enters the host through wounds or natural openings such as the water pores. In either case, the bacterium ends up in the xylem tissues, where it multiplies and moves throughout the plant (Mew 1987).

There are two symptoms on rice: kresek and leaf blight. Kresek is the more destructive manifestation of the disease. Leaves of entire plants turn pale yellow and wilt during the seedling to early tillering stages. Outbreaks usually lead to partial or total crop failure. Kresek was first observed in Indonesia and is very common in the tropics. It was later seen in Africa, China, and Korea. In China, it first appeared in Henan Province; between 1977 and 1979, it was widespread on hybrid rice in three major rice-growing provinces—Henan, Guangdong, and Guangxi (CAAS 1986). Several outbreaks in the past decade have been reported, but others may not have been recorded (Mew 1987, Ou 1985).

Leaf blight is the more common disease syndrome. Lesions on the leaf blades may extend to the leafsheath. The lesion enlarges in length and width, and may have wavy margins. It turns a whitish-straw color from its initial water-soaked grayish or yellowish hue in 1-2 wk. Bacterial ooze may be observed in humid and warm conditions. Leaf blight may occur at all growth stages, but it is common from maximum tillering until maturity.

"Yellow leaf" is also considered a syndrome of BB (Ou 1985). However, Mew (1987) considered this a secondary effect of kresek or leaf blight, apparently due to the effects of a toxin produced by the bacteria rather than to interruption of nutrients from roots to shoots.

DAMAGE

Economically, BB is so far important mostly in Asia. It can be very destructive in the tropics wherever rice is grown throughout the year. In West Africa, it has severely damaged rice cultivation in specific areas in Niger, Burkina Faso, and Mali. Its presence in the United States has caused concern to seed growers (J.E. Leach, pers. comm.).

Few estimates of yield losses caused by BB are available, but its epidemic potential is well documented. In China, when hybrid rice was attacked in the late 1970s, the 1,000-seed weight was reduced (CAAS 1986). If plants produced panicles, the sterility percentage was increased, as was the number of immature grains. Grain from diseased plants was easily broken during milling. If the infection was moderate, a 10-20% yield reduction was recorded, while in severely infected fields the reduction was as high as 50%. Plants in most infected fields produced no grains. In fields with kresek, the number of missing hills was increased, total crop failure was likely, and replanting was necessary.

EMERGING PROBLEMS

In the past two decades, national and international rice improvement programs have exerted tremendous efforts to breed BB-resistant rice. Consequently, many breeding lines and varieties have been developed and cultivated by farmers. Only later were some of these varieties found susceptible in some countries of South and Southeast Asia, or sometimes in one region but not others within a country (Mew 1987, Ou 1985). This is due to the presence of different pathogenic races. A preliminary survey has shown that there are six races in the Philippines (Vera Cruz and Mew 1989, this volume), more in South Asia, and several in other Southeast Asian nations. The virulence of the races is narrow in East and Southeast Asia, but broad in South Asia. Although it is unlikely that all the races will be present in one region at a given time, their presence in the different countries has suggested that different resistance genes are needed for cultivars in different countries.

Genetic exchange and dissemination have provided common sources of resistance to many breeding programs. Germplasm improvement efforts have thus unintentionally also promoted the selection of a common pathogenic race with similar virulence, thus making newly improved cultivars vulnerable to disease epidemics.

To improve our understanding of resistance mechanisms, and to formulate better management tactics, the virulence of field races should be compared, and the shifting of virulence monitored.

Although BB has become endemic in some regions and countries of South and Southeast Asia, its epidemic potential should not be ignored. In many countries, its importance has not been fully realized. In Burma, it is the most serious disease, and apparently many of the commercial cultivars are susceptible (R.K. Palis, International Rice Research Institute [IRRI], pers. comm.); but there is little information on or analysis of BB epidemics, or cultivar environment interactions. In the Philippines, the disease is apparently limited to specific areas. For instance, on the IRRI farm, it has not occurred naturally for nearly 10 yr despite the introduction of inoculum for screening, whereas in Mabitac—30 km to the south—it has occurred every year with a normal WS since we first surveyed the area 7 yr ago. The topography and landscape of ricefields obviously contribute to these discrepancies.

The presence of BB in West Africa implies that the pathogen has either been introduced through seed or is indigenous there. The concern is that, despite the severe infection and damage BB has caused to some popular cultivars there, little research has been conducted. Even though the total rice area in the region is small, BB epidemics have caused serious crop losses—up to 50% crop failure in major irrigated rice areas of Niger. If West African countries wish to develop their rice industry, BB has to be dealt with seriously.

Although seed transmission may not play an important role in the tropics, Xco can be seedborne. When the disease has already become established in a country, seed may not be a critical source of inoculum. But in countries where the disease has not been recorded, seed transmission may be very important. There is no reliable technique to detect the bacterium at low concentrations. DNA probes and monoclonal antibodies that are specific to *Xanthomonas* spp. and even to pathovar

oryzae will be useful to increase the efficiency of identification and assay. Genomic analysis has confirmed that Xco isolates from Texas are distinctly different from those from the Philippines or from other locations included in the study (J.E. Leach, pers. comm.). The DNA probe has become a powerful tool not only for pathovar identification but also for comparing strains from different origins. Both DNA probes and monoclonal antibodies will enhance the pathogenicity test on differential cultivars and on race evolution.

Taking a global view of the problems caused by BB, it is evident that in countries where resistant cultivars (if effective) have been deployed, the second-generation problem of virulence shifting has emerged. In countries where resistant cultivars have not been used, BB remains an important constraint. More significantly, however, in countries where BB has never been recorded, it has recently occurred, and in some regions it is destructive to rice production even though the scale of rice production may be relatively small.

Understanding BB as an important rice disease is an important goal, but with the advances in biotechnology and quantitative epidemiology, we should feel reasonably optimistic that in the next decade the "black box" of uncertainty will be clearer.

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Classification of the bacterial blight pathogen

J. Swings

The evolution and speciation of the genus *Xanthomonas* are discussed, as well as criticisms of the pathovar system of *Xanthomonas campestris.* DNA-rDNA hybridizations, sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein patterns, phenotypic analyses, and phytopathogenicity of *X. oryzae, X. campestris* pv. *oryzicola,* and "brown blotch" isolates have been used. *X. campestris* pv. *oryzicola* and *X. oryzae* can be distinguished by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the soluble cell proteins. None of the methods can distinguish among Philippine or Japanese virulence groups.

Classification, nomenclature, and identification are the three separate but interrelated areas of taxonomy. Classification is the arrangement into taxonomic groups (taxa). Nomenclature is the assignment of names to the taxonomic groups according to international rules. Identification is the process of determining that a new isolate belongs to one of the established, named taxa. Bacterial taxonomy, which began as a largely intuitive process, has become increasingly objective with the advent of modern techniques, e.g., numerical taxonomy and techniques for measuring evolutionary divergence in the structure of semantides, i.e., large, information-bearing molecules such as nucleic acids and proteins. There is general agreement among microbiologists that the complete DNA sequence would be the reference standard to determine phylogeny, and that phylogeny should determine taxonomy (Wayne et al 1987). At present the bacterial species is the only taxonomic unit that can be defined in phylogenetic terms, and recently an ad hoc committee defined as a given species those individuals with 70% DNA-DNA relatedness (Wayne et al 1987). A number of modern approaches to bacterial taxonomy, ecology, and epidemiology seem to be particularly promising:

Bacteriocin typing
Monoclonal antibodies
Polyclonal antibodies
Restriction fragment length polymorphism
Lipopolysaccharide analysis
Phage typing
Zymogram

Protein profile
Phenotypic analysis
Gas chromatography of cell fatty acids
DNA probe
Comparative enzymology
Chemotaxonomic markers
DNA-rDNA hybridization
rRNA catalog

Only a few bacterial genera contain species causing phytobacterioses. The genus *Xanthomonas* is one of the best known that does. It causes necrosis, gummosis, and vascular or parenchymatous diseases on leaves, stems, or fruit in at least 124 monocotyledonous and 268 dicotyledonous plants (Leyns et al 1984).

The genus *Xanthomonas* was originally proposed for yellow, slime-forming, plant pathogenic gram-negative rods, motile with one polar flagellum or nonmotile. Burkholder and Starr (1948) discussed the impossibility of distinguishing the different species of *Xanthomonas* by biochemical and physiological features alone without knowing their hosts, and they criticized the "new-host, new-species" concept. The fact that the different "species" were almost indistinguishable led to the reduction of the number of species to 5 within the genus *Xanthomonas* in the 8th edition of *Bergey's manual of determinative bacteriology* (1974), as well as in the latest edition of *Bergey's manual* (1984). These species are *X. albilineans, X. ampelina, X. axonopodis, X. campestris,* and *X. fragariae.* The evolution of the number of *Xanthomonas* species is given in Table 1.

Recent advances in Xanthomonas taxonomy can be summarized as follows:

- *X. ampelina* must be removed from the genus *Xanthomonas* (De Vos and De Ley 1983).
- *X. maltophilia,* originally classified as *Pseudomonas maltophilia,* clearly belongs in the genus *Xanthomonas* (Swings et al 1983). This species is the only one within the genus *Xanthomonas* that is not phytopathogenic. We are not aware of any host plant, and we do not know whether it is a "minor deleterious organism." It has been found associated with plants in high numbers.
- *X. populi,* which was originally described as *Aplanobacter populi,* also clearly belongs in *Xanthomonas* (Ridé and Ridé 1978).
- *X. oryzae* and *X. graminis* constitute new, separate clusters, different from all other species (Van den Mooter 1984).

Table 1. Evolution of the number of *Xanthomonas* species.

Reference	*Xanthomonas* species (no.)
Dowson (1939)	19
Bergey's manual (1957)	60
Bergey's manual (1974)	5
Bergey's manual (1984)	5
Van den Mooter (1984)	8

- A numerical analysis of 295 phenotypic features of 268 *Xanthomonas* or related strains used the S_{SM} coefficient and the unweighted pair-group method (Van den Mooter 1984). This analysis allowed an improved phenotypic description of the genus and the phena (species), as well as an improved phenotypic differentiation of the phena. It became clear that a number of traditionally used features are not of great value in *Xanthomonas* species differentiation, e.g., formation of slime, yellow pigment; or oxidase. Van den Mooter (1984) subdivided the genus *Xanthomonas* into eight species: *X. albilineans, X. axonopodis, X. campestris, X. fragariae, X. graminis, X. maltophilia, X. oryzae,* and *X. populi.*

In the latest edition of *Bergey's manual* (1984), the species *X. campestris* is further subdivided into 126 pathovars according to their host plants. In the past, the pathovar system was subject to serious criticism, which can be summarized as follows:

- The emphasis is on phytopathogenicity. Most of the isolates have been characterized only in a rudimentary way.
- Not all so-called pathovars belong in the species *X. campestris.* From Van den Mooter's (1984) numerical analysis of 268 *Xanthomonas* strains, it became clear that pv. *oryzae* and pv. *graminis* fell outside the species *X. campestris* and should be classified as separate species, viz., *X. oryzae* and *X. graminis.*
- Some individual pathovars are heterogeneous. A pathovar is not necessarily a homogeneous biological group. This became particularly clear through the work on *X. campestris* pv. *graminis* (Van den Mooter 1984, Van den Mooter et al 1986). Phenotypic and gel electrophoretic data as well as host plant specificity have shown that this pathovar should be split into four taxonomic entities. At least one of them should be considered as a separate species, viz., *X. graminis.* Several other pathovars are known to be heterogeneous: pv. *campestris,* pv. *hyacinthi,* pv. *phaseoli,* pv. *vesicatoria,* and pv. *pelargonii.*
- In a number of cases it has been shown that very low DNA homologies exist between pathovars. This could mean that a taxonomic level higher than pathovar (i.e., subspecies or species) should be given to the actual pathovar. This would give more credit again to the former species system.
- High host plant specificity, which is a characteristic of most *X. campestris* pathovars and which led to the new-host, new-species concept and later to the actual pathovar system, is clearly not found within each pathovar. This can be explained by the lack of extensive host range studies. A typical example is *X. campestris* pv. *graminis* (Van den Mooter et al 1986).
- The pathovar system is impractical for identification by phytopathologists. A *Xanthomonas* strain for which a host plant is not known or which is not phytopathogenic cannot be identified to the pathovar level.
- The trinomial pathovar system is intricate to use.

The taxonomic position of the causal agents of three bacterial rice diseases (bacterial blight, leaf streak, and brown blotch) have been examined extensively (Vera Cruz et al 1984). A collection of 35 *X. oryzae,* 14 *X. campestris* pv. *oryzicola,*

and 6 "brown blotch" strains have been separately examined by 133 phenotypic features, gel electrophoregrams of soluble cell proteins, % G+C determinations, and DNA-rDNA hybridizations. From this study it became clear that

- both *X. campestris* pv. *oryzicola* and *X. oryzae* belong in the genus *Xanthomonas* in the second rRNA superfamily, whereas the "brown blotch" pathogen does not;
- *X. oryzae* and *X. campestris* pv. *oryzicola* display clearly distinct protein patterns in SDS-PAGE and can also be differentiated from each other by four phenotypic tests;
- there is no correlation between the Japanese or Philippine virulence groups on one hand and the clustering obtained after a numerical analysis of the phenotypic features on the other;
- *X. oryzae* strains constitute a homogeneous group of bacteria regardless of their geographic origin; and
- *X. oryzae* strains form in Van den Mooter's (1984) global numerical analysis of phenotypic features a clearly distinct phenon. He has concluded that *X. oryzae* sp. nov. should be reclassified as a new species.

As far as *X. campestris* pv. *oryzicola* is concerned, we can now, with great certainty, confirm that taxonomically it has 85% DNA-DNA homology with *X. oryzae,* confirming that it should be classified in the species *X. oryzae,* either as a subspecies or as a pathovar (M. Gillis, pers. comm.).

Recently, a number of modern techniques have been applied on *X. campestris* "pathovars" and seem to present great hope for the differentiation of the different "pathovars" in the future:

- SDS-PAGE profiles of total cell proteins (Kersters and De Ley 1980, Vera Cruz et al 1984). Personally, I prefer this method for the practical identification of *X. oryzae.* We should stress, however, that this technique does not allow determination of the virulence of a strain or a further subdivision into "pathogenic" races.
- SDS-PAGE profiles of total membrane proteins (De Weger et al 1986, Minsavage and Schaad 1983)
- restriction fragment length polymorphism (Lao et al 1987)
- plasmid restriction fragment profiles (Lazo and Gabriel 1987)
- monoclonal antibodies
- gas chromatography of cellular lipids

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Serology of *Xanthomonas campestris* pv. *oryzae*

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Highly specific direct or indirect immunofluorescence techniques using rabbit polyclonal antisera to whole bacterial cells have been successfully adapted for detecting and identifying *Xanthomonas campestris* pv. *oryzae* (Xco) from cultures, diseased rice leaf tissue, rice seed, rice and weed leaf surfaces, and irrigation water. With agar gel diffusion tests using the same types of antisera, serovar groupings of Xco isolates from China, Japan, Indonesia, India, Thailand, Vietnam, and the Philippines have shown at least three serovars. These serological data suggest the possibility of developing universal antibody probes for the serodiagnosis of Xco and establishing an International Standard Serogrouping System for the same. With the exception of purified extracellular polysaccharide (EPS), purified malate dehydrogenase, and crude extracellular glycoprotein preparations, other chemical constituents of the Xco cell have not been used as antigens. Nevertheless, data on agar gel diffusion tests with polyclonal antisera to purified EPS, glutaraldehyde-fixed cells, and heated cell or cell extract antigens have shown an Xco-specific precipitin band. The composition and structure of the determinant(s) of the antigenic molecule(s) responsible for such specific reaction, however, remain to be determined. Practically nothing has been done on the serological detection of Xco races. However, limited data on the use of monoclonal antibodies for the serodiagnosis of Xco races indicate the possibility of developing Xco race-specific antibody probes.

Serology is a useful tool for detecting and identifying plant pathogenic bacteria from cultures, host plant tissues, and materials from the immediate environment of host plants. As a taxonomic tool, however, serology has been useful so far only in grouping strains of a bacterial nomenspecies into *serovars* (syn. = serotype), *Serovar* is an infrasubspecific rank, and grouping of bacterial strains in this rank is based solely on distinctive antigenic properties. Like the other infrasubspecific ranks (e.g., pathovar, phagovar, biovar, morphovar), serovar has no official standing in bacterial nomenclature (Staley and Krieg 1984). Infrasubspecific ranks, however, have great practical usefulness for studying bacterial pathogens, particularly in the areas of ecology and epidemiology. On the other hand, serology as an approach to

investigating the molecular architecture of bacterial cells, and, therefore, relationships between bacteria, has the same objective as chemotaxonomy, although the methodologies used in these two approaches are different. In chemotaxonomy, chemical and physical techniques are used to elucidate the chemical composition of whole bacterial cells. In serology, antisera to purified chemical constituents (e.g., purified enzymes as antigens) of a group of bacterial strains are used to detect the degree of serological similarity or the serological cross-reactions of homologous antigens in crude extracts of other bacterial strains.

SEROLOGICAL TECHNIQUES AND STUDIES OF TAXONOMICAL VALUE

Serological techniques depend on the immunogenic capacity—the ability to induce antibody production in a vertebrate—of the chemical constituents of a bacterial cell. The antibodies used in serological studies are produced by plasma cells and are found in the blood serum. These antibodies will bind specifically with the antigenic determinants that elicited their production. The antigen-antibody reaction is the fundamental basis of all serological tests.

Bacterial cell surface constituents, including the slime layer, capsule, flagella, pili, cell wall, and cytoplasmic membranes, have been used to elicit antibodies for detecting similarities and differences between bacteria. Whole cells, killed or live, have also been commonly used as particulate immunogens in serological studies to aid taxonomy. Purified enzyme immunogens have been used in assessing structural similarities between homologous proteins from different bacteria (Jones and Krieg 1984).

Serological methods used for detecting, identifying, and classifying plant pathogenic bacteria (i.e., serovar groupings) include agglutination, immunodiffusion, immuno-electrophoresis, precipitin tests, complement fixation, and labeled antibody techniques (immunofluorescence, enzyme-linked immunosorbent assay [ELISA], and radio immunoassay [RIA]). Detailed descriptions of these methods can be found elsewhere (e.g., Langone and Van Vunakis 1981, 1982; Van Vunakis and Langone 1980). In plant pathogenic bacteria, agglutination and immunodiffusion (double agar diffusion or Ouchterlony double diffusion test) techniques have been popular for serogrouping (Schaad 1979), while immunofluorescence (De Boer 1984, De Boer and Wieczorek 1984, Lin et al 1987, Schaad 1979) and ELISA (Alvarez and Lou 1985, Civerolo and Fan 1982, Elango and Lozano 1980, Lazarovits et a1 1987, Yuen et al 1987) have been used to detect and identify bacteria in natural situations. RIA has also been used in grouping strains of some plant pathogenic bacteria (Alvarez et al 1985). Both ELISA and RIA, because of their sensitivity, are useful in making critical analyses of structural similarities or differences and in elucidating antigenic structures of immunogens.

PURIFIED IMMUNOGENS

Cell surface antigens

The bacterial cell envelope, which includes the cell wall and its associated components (flagella, pili, slime layer, capsule, and cytoplasmic membrane), is made

up of an array of chemical compounds, many of which are antigenic and are valuable in taxonomy. Very little is known about the chemical constituents of the cell envelope of *Xanthomonas campestris* pv. *oryzae* (Xco). However, studies of the cell envelope of other gram-negative bacteria indicate that it is structurally and chemically complex (Costerton et al 1974).

The cell wall proper of gram-negative bacteria contains lipopolysaccharides, lipoproteins, polysaccharides, and proteins, while the cell membrane is made up of proteins (including a number of enzymes involved in biological oxidations), lipids, and traces of carbohydrates. Some components of the cell walls of *X. campestris,* particularly the lipopolysaccharides and polysaccharides, have been analyzed (Hase and Rietschel 1976; Moss et al 1973; Volk 1966, 1968a,b).

Of the several chemical constituents of the cell envelope of Xco, only the extracellular polysaccharides (EPS) have been used as immunogens. The antiserum to purified EPS is specific to Xco but not to the races of Xco (Parry 1985). Whether or not antibodies to other purified cell envelope components would be of value in Xco taxonomy remains to be seen.

Intracellular proteins

Cytoplasmic proteins (proteins in the cytoplasm, in contrast to membrane-bound or cell wall-bound proteins), particularly enzymes and ribosomal proteins, are another group of immunogens that can be used in serology, but none has been utilized yet for Xco.

Purified enzyme immunogens have been used in serological studies of some nonplant pathogenic bacteria, and available data indicate serological homology at the species level (London and Kline 1973, Sgorbati 1979, Sgorbati and Scardovi 1979). In some bacteria, very high correlations exist between the serological relationships of certain enzymes and genetic relatedness based on DNA/DNA homology at the species level (Kandler and Schleifer 1980). Whether or not this approach will distinguish Xco from other pathovars of *X. campestris* or will be useful in Xco race differentiation remains to be seen.

Nucleic acids

Another group of compounds that can be used in serological studies of Xco are the nucleic acids. Nucleic acids themselves do not stimulate significant antibody production. However, when they or their components (e.g., nucleotides and oligonucleotides) are linked with carrier proteins or synthetic polypeptides, they become good immunogens (Beiser et al 1967, Plescia 1967). It is now possible to produce specific antibody reagents for each of the normal bases of DNA and RNA, for modified bases, and for base sequences. Some detailed protocols for producing antibodies to nucleic acids were described by Stollar (1980). With biochemical and recombinant DNA techniques (e.g., DNA hybridization), it might be possible to detect and isolate unique (e.g., Xco-specific) DNA fragments and use these (after linking them with carrier proteins or synthetic polypeptides) as immunogens.

One significant problem in dealing with proteins and nucleic acid antigens is the extensive secondary and tertiary folding of these molecules that may mask (i.e., make inaccessible to antibodies) the many bases (in nucleic acids) or amino acid

sequences (in proteins) that constitute specific antigenic determinants. Nevertheless, serological tests utilizing these molecules to assess structural similarities between homologous proteins or nucleic acids might be useful in classifying Xco. It might also provide some insights into the possible phylogenetic relationships of Xco with other phytopathogenic bacteria including pathovars of *X. campestris.*

ANTIGENIC STRUCTURE

The ultimate goal in serological studies of bacteria in aid of taxonomy is to identify the antigenic structure or structures that differentiate species, a subdivision of a species (e.g., pathovar), or a division within the infrasubspecific rank (e.g., race). The definition of antigenic structure could be determined with the use of purified immunogens. If purified immunogens are specific (e.g., Xco-specific or Xco race-specific), we should explore the biochemical structure of the molecule and the production of monoclonal hybridoma antibodies using the purified immunogen. This approach is required for producing homogenous antibodies directed against specific determinants. Such homogenous antibodies could be used for isolating the antigenic molecule and possible antigenic fragments produced by protease or chemical digestion of the antigenic molecule; the antibodies, of course, can also be used to develop diagnostic assays. The combined tools of immunology, immuno-chemistry, and biochemistry would be necessary to explain the chemical and structural characteristics of antigenic molecules.

SERODIAGNOSIS OF Xco

Goto (1970) was the first to use serology to detect and identify Xco in ricefield water. Using a direct immunofluorescent procedure involving polyclonal rabbit antisera to live Xco cells, he was able to detect 10^5 Xco cells/ml in ricefield water from the IRRI experimental farm. However, he did not evaluate the specificity of the technique. Lee et al (1982) also used polyclonal antisera to live Xco cells in an indirect fluorescent antibody stain (IFAS) to positively identify 88 Xco cultures from rice seeds and leaves gathered in 11 provinces and regions in China. With the stain they were also able to detect Xco from rice seed artificially inoculated with 90 Xco cells/ml. In specificity tests, very weak reactions were observed with *X. campestris pathovars campestris, citri, malvacearum, oryzicola,* and *pruni,* and with *X. leersiae* var. *oryzae* (?); negative reactions (i.e., no fluorescence) were observed, however, with these organisms when the antiserum was absorbed by cultures of pv. *oryzicola.* Cultures of saprophytic bacteria from rice leaves (43 isolates) and seeds (17 isolates) showed "negative" reactions (no fluorescence or extremely weak reactions). Washing precipitates (i.e., after centrifugation) of glumes from rice seed collected in 1976-80 yielded fluorescent cells. In samples from a "slightly diseased field there could be as few as 19 cells/ 50 view fields (vf) with weak fluorescence," whereas in seeds "collected strictly from diseased heads of rice, large amounts of bacteria (517 cells/50 vf) could be found"(Lee et al 1982). It is evident from this work that there were nonspecific reactions with IFAS utilizing antisera to live Xco cells.

Quimio and Mew (1987, unpubl. data), using a direct fluorescent antibody stain (FAS) procedure utilizing antisera to glutaraldehyde-fixed Xco cells (PXO 86 race 2), found a very high degree of stain specificity with Xco. This was based on tests with 117 isolates of Xco collected in 1972-86 from various parts of the Philippines and representing the 6 Philippine Xco races, 25 naturally infected rice leaf samples from different geographic locations of the country and representing 4 of the 6 Xco races, 6 Philippine Xco race differential cultivars artificially inoculated with representative isolates of the6 races, 14 other pathovars of *X. campestris* (pathovars *arrhenatheri (?), begoniae, campestris, cerealis, citri, graminis, oryzicola, glycines, hyacinthi, malvacearum, phleipratensis, poae* (?), *phaseoli, and vesicatoria),* 13 other cultures of bacteria from 8 bacterial genera (*Alcaligenes* spp., *Azospirillum brasilense, Bacillus subtilis, Erwinia herbicola, E. chrysanthemi, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, P. fluorescens, P. fuscovaginae, P. marginalis, P. solanacearum, and Serratia marcescens*), and 80 bacterial contaminants from isolation plates of rice seed washings and ricefield water. The FAS procedure was also effective in detecting Xco from seeds of infected plants, symptomless rice and weed leaves, and necrotic lesions that were atypical of bacterial blight. In controlled experiments where Xco was mixed with pure cultures of other bacteria, rice seed and leaf washings, or ricefield water with a background bacterial count of 10^6-10^8 colony-forming units (cfu)/ml, the practical detection (sensitivity) limit of the FAS procedure using a 0.01-ml sample/smear was 10^4 Xco cfu/ml.

Wang et al (1980) used the reverse indirect hemagglutination test utilizing antisera to live cells to detect Xco from leaves and stubble of infected rice. They observed no reaction with cultures of *X. campestris* pathovars *oryzicola* and *panici, Erwinia herbicola,* and *Pseudomonas translucens* (?).

The available information on serodiagnosis of Xco indicates that Xco-specific polyclonal antisera can be easily produced and used for the qualitative detection of Xco in natural situations (e.g., rice seed and leaf washings and ricefield water) and for specific identification of Xco in cultures and in plant tissue extracts. Polyclonal antisera to live cells, and glutaraldehyde-fixed cells are not race-specific. Antisera to heat-killed or sonicated cells (Addy and Dhal 1977, Choi et al 1980b), extracellular glycoproteins (Quimio and Mew, 1987, unpubl. data), and purified EPS (Parry 1985) used in immunodiffusion tests are also not race-specdic.

SEROVAR GROUPINGS OF Xco

Addy and Dhal (1977), using unpurified antisera to live Xco cells, found only one serovar among 45 Indian Xco isolates on the bases of agglutination reactions and gel diffusion tests. They also found isolates of varying pathological reactions within the said serovar. They did not, however, evaluate the specificity of the antisera outside of Xco.

Wang et al (1980), using indirect hemagglutination tests and antibodies—purified by $(NH_4)_2SO_4$ precipitation and diethylaminoethyl cellulose column chromatography—to live cells, observed 2 serovars—serovar 1 (97.5%) and serovar 2 (2.5%), among 202 Xco isolates from China. The antiserum had no cross-reactions

with *X. campestris* pathovars *oryzicola* and *panici, Erwinia herbicola,* or *Pseudomonas translucens* (?).

In a study of 160 Asian Xco isolates (63 from Japan, 8 from Indonesia, 5 from India, 29 from the Philippines, 48 from Thailand, and 7 from Vietnam), Choi et al (1980b) found 3 serovars on the basis of gel diffusion tests utilizing unpurified antisera to live and heat-killed cells of 2 isolates, namely Q7472 and 47502. In this classification, 132 isolates, designated serovar A, produced precipitin bands with both Q7472 and Q7502 antisera. The remaining 28 isolates, which they designated serovar B, produced bands with anti-Q7502 but not with anti-Q7472 serum; they divided this serovar into serovar B-I (20 isolates), with heat stable antigens, and serovar B-II (8 isolates), with heat-labile antigens (antigenicity was lost when cells were boiled for 1 h). Again, Choi et al (1980a) observed in specificity tests using the Ouchterlony diffusion technique that Q7472 (serovar A) serum reacted only with intact cells of isolates belonging to the homologous serovar A, while anti-Q7502 (serovar B-I) serum and anti-N5837 (serovar B-II) serum cross-reacted (produced bands) with *X. campestris* pathovars *citri, hordei, vesicatoria,* and *vitians.* Antiserum to sonicated cells of Q7472 (serovar A) also cross-reacted with *X. campestris* pathovars, but not with species belonging to the genera *Bacillus, Corynebacterium, Pseudomonas,* and *Rhizobium.* However, when heated antigens were used, the antiserum to sonicated Q7472 cells showed specific reactions to Xco isolates regardless of serovar grouping. These observations indicate general nonspecific reactions of antisera to live or heat-killed Xco cells.

Quimio and Mew (1987, unpubl. data) investigated the serological groupings of Philippine strains of Xco using antisera to glutaraldehyde-fixed cells and crude extracellular glycoprotein of a Philippine race 2 isolate (PXO 86) and the Ouchterlony double diffusion test; they used $(NH_4)_2SO_4$-precipitated antibodies (50% saturation cut-off). With glutaraldehyde-fixed cell antiserum, only one serogroup, designated serovar-1 (S-l), was observed. Within this group, two subgroups were recognized, namely S-la, with two precipitin bands, and S-1 b, with three precipitin bands. S-la represented 59.7% and S-lb represented 38.3% of the 139 isolates examined. A minor class (3 of 139 = 2.2%) that did not react with the antiserum was temporarily designated serovar 2. The antiserum, showing a very high degree of specificity, did not cross-react with antigens of 14 other pathovars of *X. campestris* or those of 13 other bacteria belonging to 8 genera. Only one serogroup was observed with extracellular glycoprotein antiserum. This serogroup, designated serovar A (S-A), had two major and two minor subgroups. Subgroup S-A_1 was characterized by 2 precipitin bands and constituted 58% of the 90 isolates tested; subgroup S-A_2 had 3 bands and constituted 40%. The minor subgroups S-A_3 and S-A_4, which had only one isolate each, were variants of S-A_2 and S-A_1, respectively. The antisera cross-reacted with other *X. campestris* pathovars when nonheated antigens were used. With heated antigens, only one precipitin band, that of Xco, was noted. There was some clustering of certain races with the serovar subgroups, but in both antisera, specificity could not be related to pathogenicity characters of the races.

The foregoing discussion indicates at least two major serovars of Xco on the basis of Ouchterlony double diffusion agar tests utilizing antisera to whole cells.

Differences in the specificity reaction observed by different workers were obviously due to the different approaches they used in preparing antisera and perhaps to other factors related to antibody production.

APPRAISAL

Rapid detection and identification of Xco by direct and indirect immunofluorescent procedures have been demonstrated using highly specific rabbit polyclonal antisera to glutaraldehyde-fixed cells (Quimio and Mew, 1987, unpubl. data) or saline-washed cells that were absorbed with cells of cross-reacting *X. campestris* pv. *oryzicola* (Lee et al 1982). Other antigens such as the membrane protein complex (Thaveechai and Schaad 1986a,b), and ribosomes (Schaad 1976) that have shown specificity at the pathovar level in *X. campestris* pathovars *campesrris* and *vesicatoria,* and other cellular components remain to be studied in Xco. Nevertheless, a universal antibody probe for Xco detection and identification can probably be developed using rabbit polyclonal antisera.

Studies on serogroupings of Xco (Addy and Dhal 1977; Choi et al 1980b; Quimio and Mew, 1987, unpubl. data; Wang et al 1980) have indicated that there are very few serovars (probably fewer than 5) of the pathogen. The implication is that serovar cultures can be easily identified and deposited in a reliable culture collection for future reference. Specific sera from these cultures can be prepared by a central Xco laboratory (e.g., Department of Plant Pathology, International Rice Research Institute [IRRI], Los Baños, Laguna, Philippines) and distributed throughout the rice-growing world to those who need them for research or extension work (e.g., phytosanitary certification services). The cooperation of scientists from national rice research programs is needed for such a project, whose basic requirements should include standard methodologies regarding

- antigen preparation; obviously an antigen that is easily prepared and exhibits high specificity and affinity is preferred;
- rabbit immunization;
- collection, partial purification, and preservation of sera;
- working conditions; these are defined conditions that include sample antigen preparation for the serology test(s); and
- specific serology method(s) (e.g., agglutination, double agar gel diffusion, immuno-electrophoresis, passive hemagglutination, immunofluorescence, ELISA); simple but accurate serology methods are preferred.

In addition to these basic requirements, an international standard serogrouping system should be adopted so that results from independent workers can be evaluated and compared. In standardizing the system, reproducibility of the serogrouping scheme must be established. Whether the serogroup changes in culture, in storage, in passage through the rice plant, or in different varieties of rice is a basic question that should be addressed to determine the limits of reliability of the system.

The challenge of identifying and elucidating the unique antigenic structures that make Xco different from the other pathovars of *X. campestris* should be addressed. Such an approach might provide evidence for the return of the infrasubspecific rank of pv. *oryzae* to the original rank of species, i.e., *X. oryzae* (Uyeda and Ishiyama)

Dowson (Dowson 1943). The use of purified immunogens and monoclonal antibody techniques is a basic strategy for such studies.

So far, serological reports on Xco have indicated that antisera to live cells, heat-killed cells, glutaraldehyde-fixed cells, sonicated cells, crude extracellular glycoproteins, and purified extracellular polysaccharides are not race-specific. The challenge, therefore, is the serological detection of Xco races. It seems that specific antisera for Xco races would be difficult to determine. This difficulty arises from the fact that in the *X. campestris* nomenspecies, and in most plant pathogenic bacteria for that matter, races as subdivisions of the infrasubspecific rank pathovar are defined solely on the basis of their ability to induce specific graded disease patterns on differential host cultivars. The implication is that the number of races detectable would depend on the number of differential cultivars available. In fact, in a given rice-growing country where races of Xco have been studied to some extent, a specific number of such races (pathogenic or bacterial groups, or pathotypes) exists. For example, in Indonesia (Yamamoto et al 1975) and also in Japan (Yamada et al 1979), at least five pathotypes, based on the reactions of five cultivars, have been identified; in the Philippines, six races are known to occur. Comparative studies on virulence of Xco isolates from different Asian countries on the same sets of differential cultivars have shown great variation in the spectrum of virulence (Buddenhagen and Reddy 1972, Reddy and Ou 1976). Horino et al (1980, 1981). in detailed studies of pathotypes from Japan and the Philippines using six Philippine (IRRI) and five Japanese differential cultivars, clearly demonstrated the existence of different pathotypes in different countries. Apparently, these pathotypes or races have evolved and have been selected as a result of host-pathogen-environment interactions through the years. This means that serological groupings of Xco races for practical purposes have to be location-specific.

As far as I know, there has been no published report on the serological detection of Xco races. However, A. Alvarez (University of Hawaii, 1987, pers. comm.) was able to isolate hybridoma clones secreting specific antibodies to Philippine races 1 and 2 based on a limited test with about 10 isolates. It would be interesting to find out if the race specificity of the antibodies could be confirmed against several Xco isolates from different geographic locations in the Philippines. Since Alvarez used whole-cell antigens, the race-specific antigenic determinants (if race specificity of the antibodies can be established) could well be located on the cell surface. But Xco races are defined by a gradation of symptoms on differential cultivars and, therefore, several genes are probably involved in their specificity. If so, antibodies from more than one hybridoma clone will be needed to identify an Xco race.

A major challenge to Xco race serology is the isolation of race-specific antigenic molecules from the cell. These molecules could be cell surface-bound as intimated by Alvarez' preliminary work, or intracellularly located, or even diffusible cell products that are essential in host-parasite interactions. Whatever they are, the problem is how to isolate and identify these race-specific antigenic molecules.

Purified extracellular polysaccharides (Parry 1985). crude extracellular glycoprotein and glutaraldehyde-fixed cells (Quimio and Mew, 1987, unpubl. data), and live cells, heat-killed cells, and sonicated cells (Choi et al 1980b) have shown Xco-specific precipitin bands near the antigen well in double diffusion agar tests.

Results of Parry's (1985) tests indicate that the Xco-specific band is due to EPS. However, there has been no attempt as yet to determine if EPSs of the different races are made up of the same or different molecular species. Kuo et al (1970) found three of the four molecular species of Xco-extracellular polysaccharides separated by Sephadex G-200 column chromatography antigenic. However, they did not evaluate the race specificity of the said EPS molecular species. If there are race-specific molecular species of EPS, they can be isolated and used as antigens to determine the race specificity of their antibodies. The composition of EPS, however, may be dependent on the substrate in which the cells are growing; therefore, variations in its antigenic makeup or at least in the accessibility of its antigenic determinants to the antibody(ies) may affect the serological specificity of the test.

The Xco-specific immunoprecipitates mentioned above can also be a good starting material for isolating race-specific immunogens. Xco-specific immuno-precipitates with antisera to other purified cellular components—if their specificity can be demonstrated—can also be used as immunogens. There are biochemical and immunological preparative procedures that can isolate the antigen from such immunoprecipitates. Race-specific molecular species in these antigens, if there are any, can be demonstrated by immunological techniques.

The powerful tools of molecular genetics provide another means for the possible isolation of race-specific antigens. In particular, messenger RNA (mRNA) preparations isolated from sucrose gradient centrifugation and enriched by electrophoresis in acrylamide and agarose can be obtained, and race-specific molecules, if there are any, can be used as immunogens. It might also be possible to hybridize single stranded complementary DNA copied from the mRNA of one race with mRNA of another race(s); a single-stranded nucleic acid that does not hybridize in such a system because it has a unique sequence (i.e., race-specific) can be isolated by hydroxyapatite chromatography and used as an immunogen. This hypothesis is an attractive one. Staskawicz (cited by Panopoulus and Peet 1985) found, for example, that a portion of a large DNA fragment of *Pseudomonas syringae* pv. *glycines* was unique to a race from which the race-specificity gene was cloned, while other portions were common to all races.

The prospects of using specific protein isolates, particularly enzymes, as immunogens for producing race-specific antibodies should be explored. In a recent study of isozymes of Philippine Xco races, Quimio and Mew (1987, unpubl. data) found that race 6 could be easily distinguished from the 5 other races by the zymograms of malate and malic dehydrogenases. The differential zymograms of these two enzymes did not change with pH changes in the electrophoreses buffer, with medium, with storage conditions, or with the passage of isolates through the rice plant. Frequent subculturing of isolates also did not alter the differential zymograms characteristic of the two enzymes. However, polyclonal antibodies to purified malate dehydrogenase of race 6 cross-reacted with purified malate dehydrogenase of race 2 and vice versa, indicating relatedness between races. Nevertheless, it would be interesting to study the isozyme race markers as immunogens for producing race-specific antibodies in a monoclonal antibody system, since such a system is more suited to detect specific antigenic determinant(s) than polyclonal antibody procedures.

Serological research on purified cell surface (i.e., cell envelope) antigens has been limited to EPS (Parry 1985). Practically nothing is known about the bacterial surface that actually makes contact with the host. Whether there are in fact unique race-specific determinants on the cell surface remains to be determined.

Extracellular proteins, including enzymes, can be used as immunogens. Presumably these proteins are directly or indirectly involved in the initial phases of infection, and different races may have unique arsenals of these proteins for which race-specific antibodies can be produced.

If a serotyping system is to be established for Xco, it might be worthwhile to know if certain biological properties can be correlated with epidemiological types, if there are such. Are certain serovars more pathogenic? Are certain serovars more resistant to bactericides? Do certain serovars prefer particular locations? Are certain serovars more fit to spread faster and survive better under certain environmental conditions? These questions are difficult to answer. There must be a reason, however, for the preponderance of certain races and the limited distribution of others. In the Philippines, for example, race 6 has been found only at IRRI in Los Baños, Laguna. Race 5 has been found only in two provinces in Northern Luzon (Ifugao and Benguet), while race 4 has been encountered only in Laguna (specifically, at IRRI) and Palawan. In general, race 2 is more prevalent than races 1 and 3. While these observations, which are based on isolations made from 1972 to 1986, have been limited by the lack of extensive surveys. the available information indicates epidemiological types of Xco.

CONCLUSIONS

Immunology and immunochemistry provide very powerful tools for studying Xco. Unfortunately, there have been very few studies on the use of such tools in understanding the unique properties of Xco, particularly its races or pathogenic groups. The prospect of developing a universal antibody probe for pv. *oryzae* is at hand. There is also no reason why serotyping isolates and an international Xco serogrouping scheme cannot be established.

The challenge of detecting and isolating race-specific antigens should be addressed. The successful detection and identification of races, including ecotypes within a race, if any, by serodiagnosis have wide implications for ecological and epidemiological studies of Xco. Presumably these races evolved as a result of their interactions with their hosts under a disease-favorable environment. There are factual differences among races with regards to their pathogenic behavior. There is also theoretical background pointing to race-unique antigenic molecules that can be explored for Xco-race detection and identification. But whether these are extracellular, cell-surface bound, or intracellular molecules remains to be discovered. Identifying and describing Xco-specific and Xco-race specific antigenic structure(s) are research challenges to both taxonomy and serodiagnosis. The tools of immunology, immunochemistry, molecular genetics, and biochemistry in general are available for such research.

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How rice plants are infected by *Xanthomonas campestris* pv. *oryzae*

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Published information and results of our recent investigations on infection of hosts and nonhosts by *Xanthomonas campestris* pv. *oryzae* (Xco) are reviewed. Special emphasis is placed on transmission and scanning electron microscopy of the contact between the bacterium and plant tissue, and on the movement of the bacterium from the outside to the inside of the tissue. Xco enters its hosts primarily through hydathodes and wounds. Within 24 h after spray-inoculation, the bacteria multiplied outside the hydathodes of susceptible rice cultivars and gained entrance through them, but they were trapped and embedded in a thin layer of exudate secreted from the water pores of resistant cultivars. Bacteria of virulent EPS^+ strains were surrounded by electron-transparent zones in a matrix of fibrillar material (FM) in xylem vessels 3 d after inoculation by the clipping method. Bacteria of avirulent EPS^- strains were not protected by electron-transparent zones, were embedded in FM, and lost their structural integrity. Xco was able to multiply for a while on seedling leaf surfaces of maize, a nonhost, suggesting that nonhosts may serve as alternate inoculum sources of the pathogen for nearby host plants.

Successful infection of a host plant by a bacterium involves movement of the bacterium toward the host, contact between the two, penetration of the host by the bacterium, and proliferation of the bacterium inside the host immediately following entrance. Characteristics of both the bacterium and the host, such as bacterial motility and chemotaxis of bacteria toward plant exudates and leachates, affect bacterial movement. Surface structures of both organisms affect the contact and the subsequent recognition of each other. Environmental factors such as relative humidity, nutrition, temperature, and light also affect the infection process. In general, penetration of the host plant by bacteria is accomplished either through wounds or natural openings such as stomata, hydathodes, or nectaries. For a general account of the infection process, see reviews by Billing (1982), Goodman (1976, 1982), and Huang (1986). This paper focuses primarily on interactions between *Xanthomonas campestris* pv. *oryzae* (Xco) and both hosts and nonhosts, with

special emphasis on transmission and scanning electron microscopy of the contact between the bacterium and plant tissue, the movement of the bacterium from the outside to the inside of the plant, and the host-parasite interaction immediately following penetration.

INFECTION OF HOSTS

Xco enters the plant chiefly through hydathodes and wounds.

Penetration through hydathodes

Hydathodes have structures similar to stomata except that the guard cells associated with a hydathode do not regulate the aperture opening. Hydathodes usually occur on the serrations of leaves or at the leaf tips of plants belonging to the Gramineae, where water is guttated (excreted). Guttation occurs in the morning hours when the soil is abundantly supplied with water and when transpiration is reduced because of either high humidity or the closing of stomata under reduced light.

Water droplets secreted by the hydathodes are brought to the surface by the terminal tracheids of the veins and pass through the intercellular spaces of the loosely packed parenchyma called epithem. These water droplets are in continuous contact with the water in the plant's vascular system and may be drawn back into the leaf as the stomata open and transpiration accelerates. Thus, bacteria suspended in the water droplets may gain entrance through the hydathodes into the vascular tissues. In addition, the water droplets secreted from hydathodes contain trace amounts of organic and inorganic substances that may serve as chemical attractants as well as nutrient sources for plant pathogenic bacteria (Feng and Kuo 1975).

The black rot disease organism, *X. campestris* pv. *campestris,* was the first bacterial plant pathogen reported to penetrate plant tissue through hydathodes (Cook et al 1952, Meier 1934, Staub and Williams 1972). Recently, electron microscopy has indicated that hydathodes of rice plants are portals of entry for Xco (Horino 1984, Mew et al 1984).

Mew et al (1984) used scanning electron microscopy to investigate the multiplication of Xco strains on rice cultivars and the penetration of the bacteria through the water pores of the leaf blades. They observed no marked difference in bacterial number on the leaf surface 1 h after spray-inoculation on all cultivar-strain combinations. The number of bacterial cells of strain PXO 61, which is virulent to rice cultivars TN1 and Cas 209, increased significantly 24 h after inoculation. They were densely distributed on or around the water pores, and some have gained entrance through the openings by 72 h after inoculation. Bacteria of PXO 101, a strain that has lost its virulence, did not multiply significantly on the leaf surface and appeared to be embedded either in a thin layer of exudate secreted by the water pores or in the walls of hydathodal guard cells. Bacteria of PXO 86, which is virulent to TN1 but avirulent to Cas 209, multiplied on the water pores of TN1 but were trapped in the exudate at 48 h after inoculation. These results demonstrate that in incompatible host-parasite combinations, bacterial cells may be immobilized and bacterial multiplication may be inhibited at the water pores. Mew et al (1984) also

noted that bacterial cells of these three strains were not observed to multiply on stomata of either TN1 or Cas 209; this observation is in agreement with the reports of Tabei (1967, 1977), who introduced Xco into stomata and found no external symptoms. He reported that bacteria were localized in the apertures of the stomata. These reports thus suggest that hydathodes, but not stomata, serve as portals of entry for Xco and also that bacterial strain-rice cultivar interactions are specific.

The physical structure of the water pore has been related to resistance to hydathodal invasion by Xco (Horino 1984). The outer ledges on the upper side of hydathodal guard cells in resistant *Leersia japonica* are well developed, consequently reducing the aperture opening between the 2 outer ledges to about 0.9 μm. This opening is too small for the bacterium (average size 0.61 X 1.79 μm) to pass through freely. On the other hand, the hydathode apertures in susceptible *Oryza sativa* are much larger (average 2.9 μm) due to the reduced growth of the outer ledges. allowing Xco to pass through freely (Horino 1984).

Infection through wounds

Many plant pathogenic bacteria that enter the plant through natural openings can also enter through wounds, In many cases, infection seems more successful through wound sites than natural-openings. Wallis et al (1973) were unable to induce lesion development on cabbage by using a hydathode inoculation method wherein bacterial suspensions of *X. campestris* pv. *campestris* were introduced into guttation drops at the margins of young cabbage leaves. However, they were able to obtain typical disease symptoms of vein blackening, necrosis, and desiccation of interveinal areas by inoculating the bacteria at wounding sites made in tertiary veins. Goto (1962) reported that the minimal doses of *X. campestris* pv. *citri* necessary for infection through stomata and wound tissues were 10^5 and 10^2-10^3 cells ml, respectively.

Outbreaks of bacterial blight (BB) on rice have been correlated with wounds caused by rainstorms (Ou 1972). This is in line with the observation that blight on young soybean leaves caused by *Pseudomonas syringae* pv. *glycinea* frequently occurred after strong winds and rainstorms. Little disease occurred in the absence of storms or during rain without wind, even though inoculum was present. Lesions resulting from storms were often associated with obviously injured tissues (Daft and Leben 1972).

Horino (1981) used a double-needle prick method to inoculate seedlings of moderately susceptible variety Kogyoku and highly resistant varieties IR28 and Tetep with Xco strain T7174. On the 3d day after inoculation, electron microscopy revealed that bacterial cells were normal and multiplying in the xylem vessels of Kogyoku. Subcellular organelles in the vascular parenchyma cells adjacent to vessels appeared to be intact. In the vessels of IR28 and Tetep, bacterial cells were irregular in shape and enveloped by abundant fibrillar material (FM), which was not observed in the vessels of Kogyoku and in healthy leaves. On IR28 and Tetep leaves. disruption of subcellular organelles was also observed. Horino concluded that the development of FM may be associated with the expression of resistance of a variety to BB.

Horino (1976) used electron microscopy to investigate compatible and incompatible interactions involving two rice cultivars and several bacterial strains. He concluded that in incompatible combinations, most bacteria were enveloped by FM near the vessel walls 3 d after inoculation (DAI). Enveloped bacteria were irregular in shape and apparently dead. In compatible combinations, bacteria multiplied actively in vessels, and no FM was observed. At 20 DAI, bacteria in incompatible cultivars were enveloped by FM and had lost their structural integrity. Bacteria in compatible combinations were surrounded by an electron-transparent layer, possibly bacterial capsular material. Although FM was observed and alteration of the bacterial fine structure was noted, most bacteria retained their structural integrity in the compatible tissue. Horino concluded that FM was produced by the host, and the rapidity of its appearance might be associated with resistance to Xco. Although the chemical nature of FM had not yet been determined, he believed it was derived from the inner vessel walls.

Bacterial extracellular polysaccharides (EPS) have been implicated as determinants of virulence in a number of plant pathogenic bacteria, including *Erwinia amylovora* (Goodman et al 1974), *P. solanacearum* (Sequeira and Graham 1977), P. *syringae* pv. *phaseolicola* (El-Banoby and Rudolph 1980), and various pathovars of *X. campestris* (Kuo et al 1970, Lesley and Hochster 1959). The toxigenic nature of EPS is suggested by the observation that isolates devoid of EPS are also completely avirulent. However, comparative studies of EPS^+ and its single-gene EPS^- mutant have not been carried out. Consequently, the exact role of EPS in recognition between bacteria and plant cells, specificity in host-parasite interaction, and toxigenicity in plant pathogenesis remains to be illustrated.

We have recently used a Tn5 transposon mutagenesis technique to obtain mutants varying in EPS-producing capability from strains of Xco. In this experiment, *Escherichia coli* strain SM10, which is resistant to kanamycin because of the presence of the Tn5-containing plasmid pSUP1011, was the donor. Recipients were streptomycin-resistant (St^r) derivatives of EPS^+, and virulent Xc0 strains PXO 40a, PXO 61, PXO 71, PXO 99, and ISA128a. Bacteria of donor and recipient strains were mixed and trapped on 0.22 mm Millipore membranes and incubated on soft nutrient agar plates for 16 h. Bacteria grown on membranes were washed into saline solution and plated on a medium containing kanamycin and streptomycin. Transconjugants thus obtained were tested for virulence and assayed for EPS production.

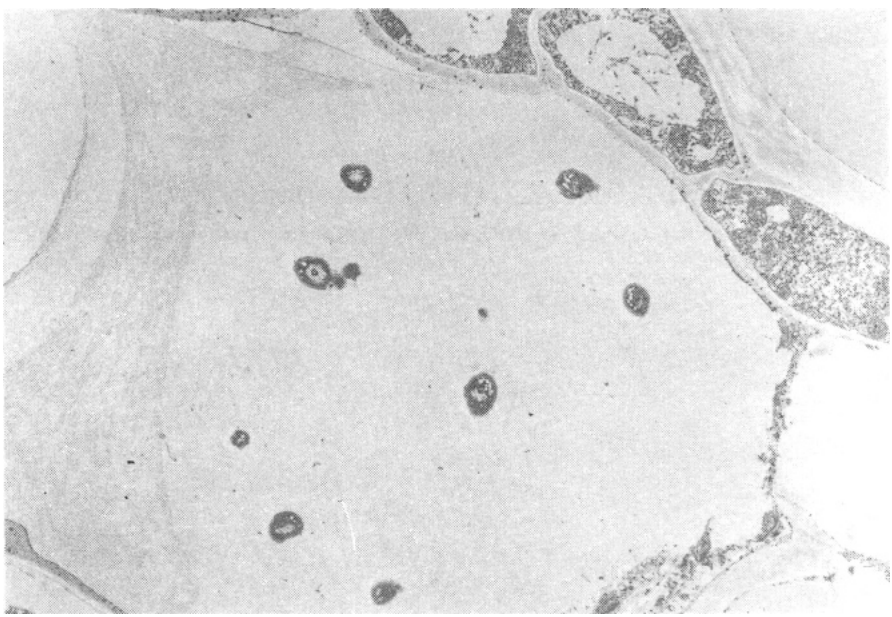
The St^r wild types and those transconjugants with moderate virulence had large, mucoid colonies on agar plates and produced large quantities of polysaccharides in liquid medium. Those weak in virulence or avirulent transconjugants had small, nonmucoid colonies and produced small amounts of polysaccharides in liquid medium. The relationship between the degree of virulence and the amount of EPS produced by St^r wild types and their Tn5-induced mutants was determined by inoculation on clipped leaves of rice cultivar TN1 (Kauffman et al 1973). The virulence of each mutant on the leaves was rated moderate, weak, or avirulent by measuring the lesion length at 14 DAI.

We initiated an electron microscopic study to determine the importance of EPS as a virulence factor and its role in the infection process in Xco-rice interactions. We

Table 1. Virulence and extracellular polymccharides (EPS) produced by a wild type of *Xanthomonas campestris* pv. *oryzae* and its Tn5-induced mutants.

Isolate	Lesion length on TN1 rice leaves [a] (cm)	Virulence	EPS (μg/ml) [b]
Wild-type ISA128aSt^r	9.4	Highly virulent	1620
Tn5-induced mutants			
1128a-8	2.4	Moderately virulent	1035
1128a-3	0.5	Weakly virulent	190
1128a-13	0	Avirulent	160

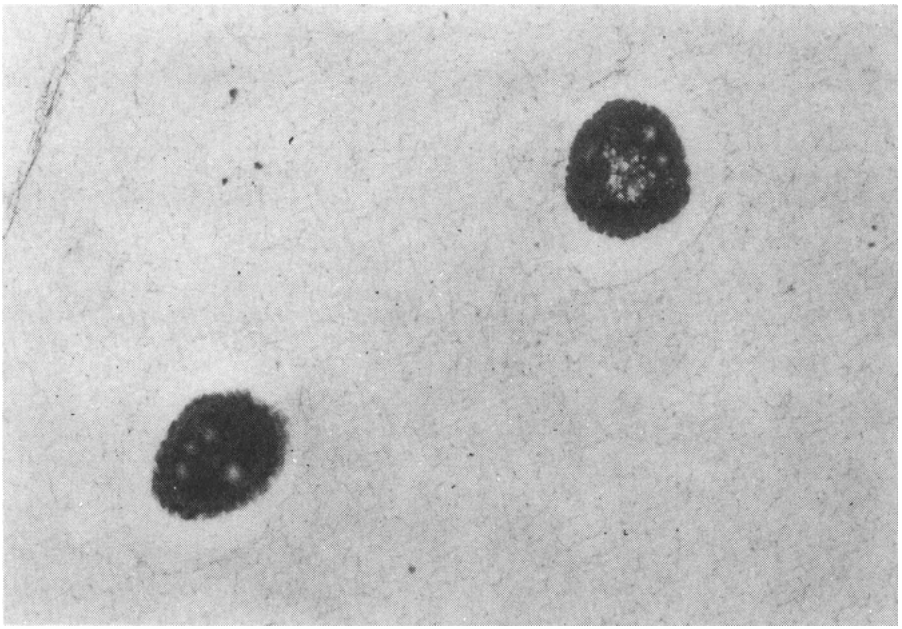
[a]Rice leaves were inoculated by the clipping method (Kauffman et al 1973). Lesion length was measured at 14 d after inoculation (DAI). [b]EPS were determined at 5 DAI in a liquid medium prepared according to the procedure of Huang (1980).



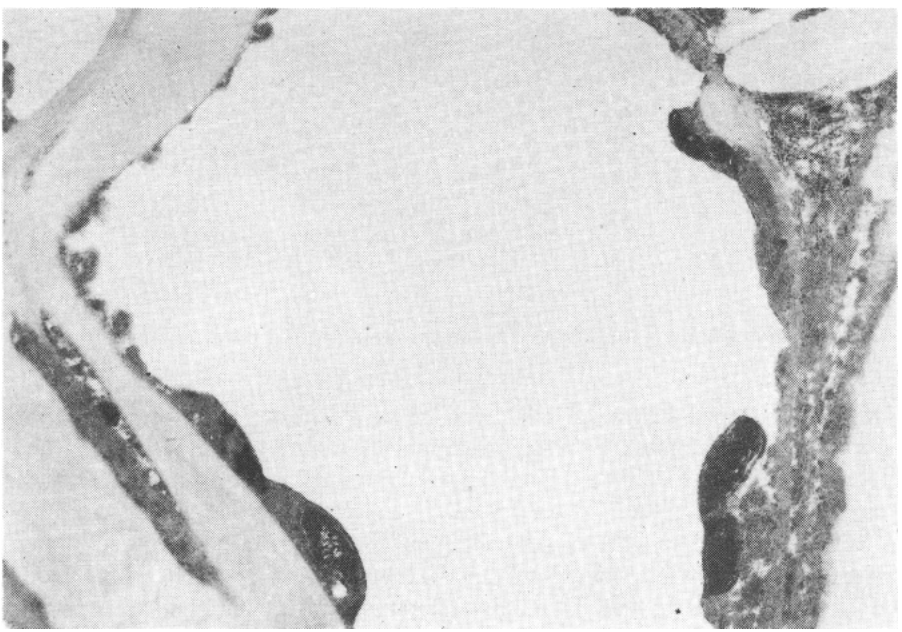
1. Bacteria of virulent strain ISA128aSt^r of Xco in xylem vessel of TN1 rice at 3 DAI (12000X).

inoculated clipped leaves of TN1 with highly virulent wild-type ISA128aSt^r, moderately virulent mutant 1128a-8, weakly virulent mutant 1128a-3, and avirulent mutant 1128a-13 (Table 1). Leaves were prepared for transmission electron microscopy (Huang et al 1986) at 3 DAI.

Cells of both the highly virulent wild type and the moderately virulent mutant were found only in the xylem vessels and were surrounded by electron-transparent zones in a FM matrix (Fig. 1). At high magnification, bacteria appeared normal, since cell walls, ribosomes, and nuclear regions were recognizable (Fig. 2). Xylem parenchyma cells also appeared normal. Bacteria of weakly virulent mutants and



2. Bacteria of virulent strain ISA128aSt^r surrounded by electron-transparent zones in xylem vessels of TNI rice at 3 DAI (44500X).



3. Attachment of avirulent bacteria of mutant 1128a-13 to vessel walls of TNI rice at 3 DAI (27800X).

4. Immobilized avirulent bacteria of mutant 1128a-13 in xylem vessel of TN1 rice at 3 DAI (66600X). Note the loss of integrity of bacterial fine structure.

avirulent mutants were attached to vessel walls (Fig. 3) and lost their structural integrity (Fig. 4). These observations suggest that EPS forms a barrier that protects virulent bacteria from entrapment by FM.

INFECTION OF NONHOSTS

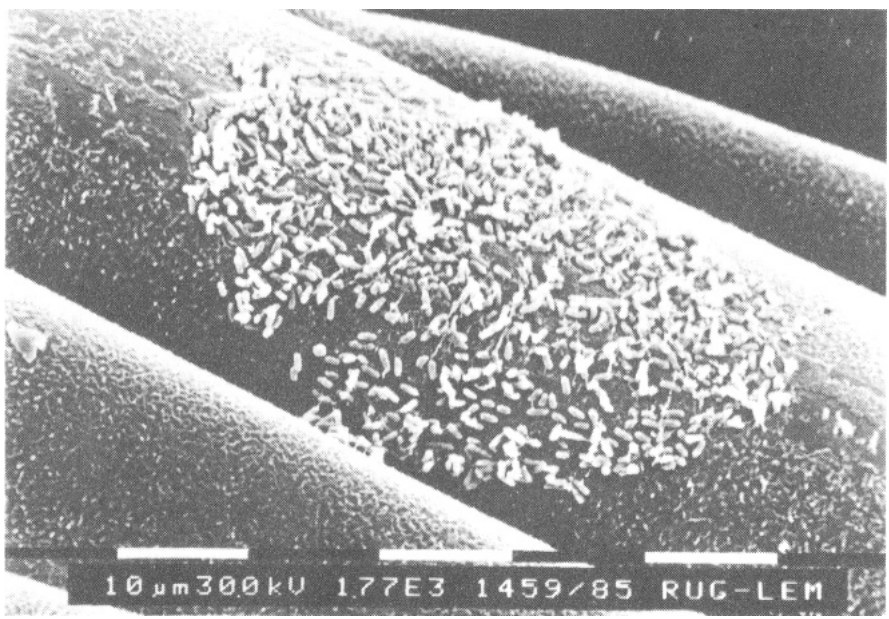
Recent studies by Robinson and Callow (1986) on the multiplication and spread of various pathovars of *X. campestris* in host and nonhost plants revealed that homologous interactions were characterized by higher multiplication rates and larger population increases than heterologous interactions, except for pv. *oryzae,* which increased as much as pv. *poae* in leaves of *Poa.*

To determine whether similar results could be observed in other bacteria-plant interactions, scanning electron microscopy (SEM) of the ingress and progress of

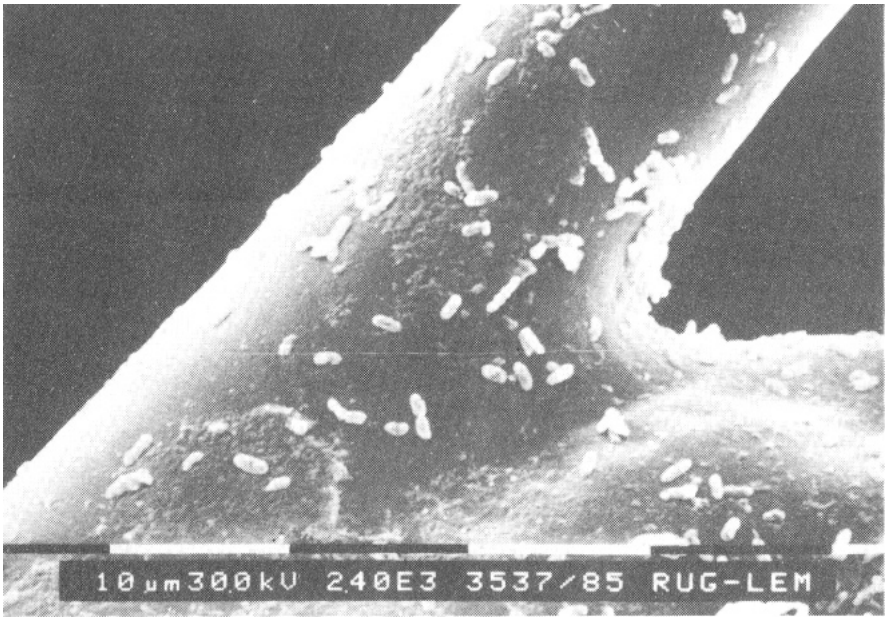
three pathovars of *X. campestris* on two different plants was initiated. Bacterial suspensions of pv. *oryzae* (strain IRN 452), pv. *graminis* (LMG 621), or pv. *oryzicola* (LMG 658) were gently and uniformly sprayed on the upper sides of the 3d leaves of 5-wk-old Italian ryegrass (*Lolium multiflorum* Lam) and maize (*Zea mays* L.) under a pressure of approximately 1 bar. Inoculated plants were kept in a growth chamber with a temperature of 22 °C, a photoperiod of 16 h, and a relative humidity of 95-100%. SEM revealed that bacteria, regardless of pathovar, were distributed over the leaf surface of Italian ryegrass at 1 DAI (Fig. 5); there was no particular concentration around the stomata. No disease symptoms were expressed. The highest detectable amount of bacteria was observed at 3 DAI. At 6 DAI, the bacterial number of pv. *graminis* was reduced, and most of the bacteria of pv. *oryzae* and pv. *oryzicola* disappeared.

On maize leaves, bacteria of pv. *graminis* were regularly observed on the porus of the stoma. Bacteria of pv. *oryzicola* were concentrated on prickle hairs, on microhairs, and on or at the bases of cushion hairs. Bacteria of pv. *oryzae* were sparsely distributed on the epidermal cells of the nerves and on the trichomes of the leaf edges at 1 DAI (Fig. 6). At 3 DAI (Fig. 7) and 6 DAI (Fig. 8), masses of bacterial cells covered the whole surface area, including the trichomes, but not the stomata. No FM could be noticed.

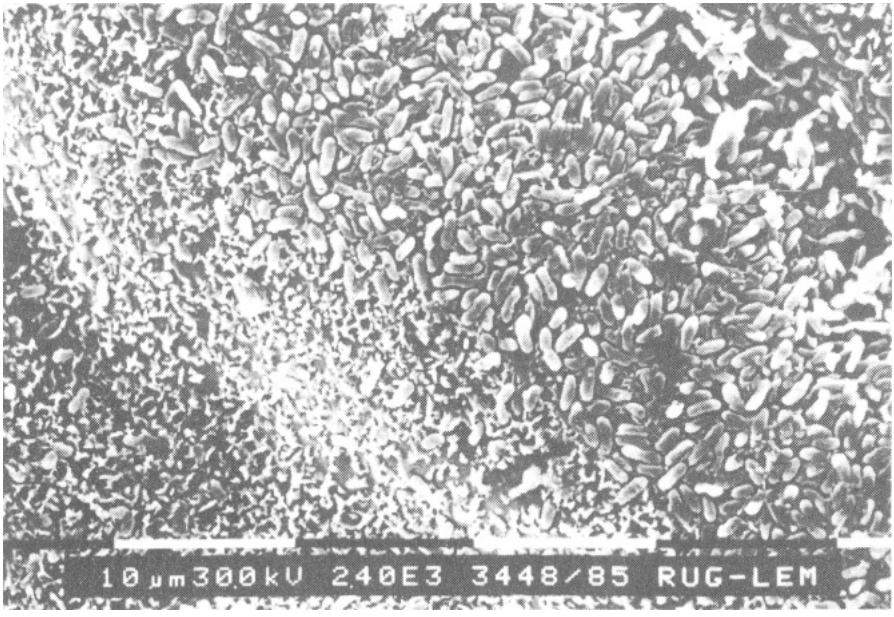
These observations indicate that bacteria of pv. *graminis,* pv. *oryzae,* and pv. *oryzicola* reached maximal number on Italian ryegrass at 3 DAI. The number of bacteria dropped back to the level of 1 DAI. No particular concentration around the



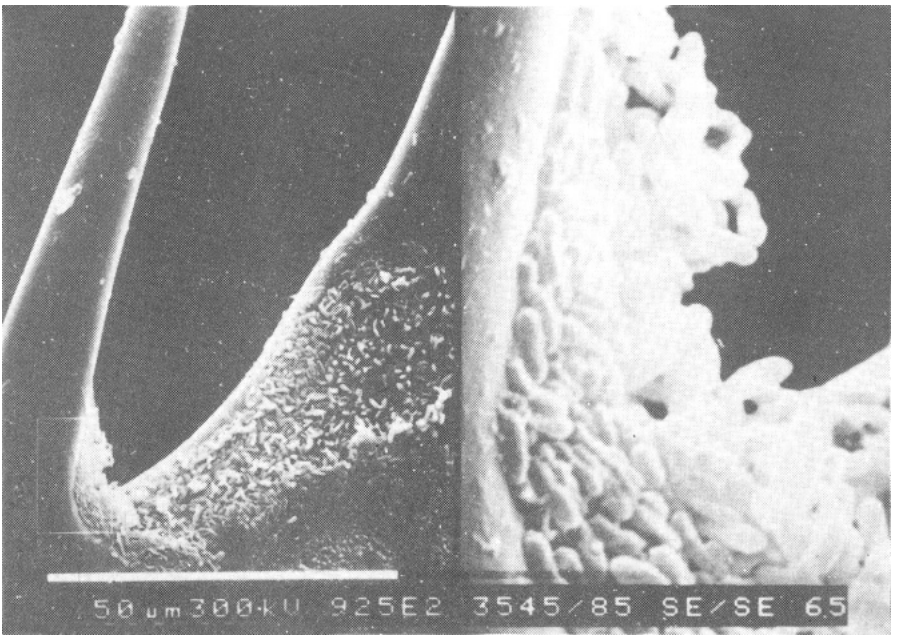
5. Scanning electron micrograph of unwounded Italian ryegrass at 3 d after inoculation with Xco. Note bacteria lying on epidermal cells. Bar = 10 µm.



6. Scanning electron micrograph of a trichome on an unwounded leaf of maize at 1 d after inoculation with Xco. Bar = 10 mm.



7. Scanning electron micrograph of an unwounded leaf of maize at 3 d after inoculation with Xco. Bar= 10 mm.



8. Scanning electron micrograph of an unwounded leaf of maize at 6 d after inoculation with Xco. Note leaf surface, including a trichome, covered by masses of bacterial cells. Bar = 50 µm.

stomata was observed. Pv. *oryzae* and pv. *oryzicola* were detected on the leaf trichomes, while pv. *graminis* was not. On maize leaves, the attachment of bacteria of pv. *oryzae* and pv. *oryzicola* to the trichomes was much more pronounced than on ryegrass. In addition, pv. *oryzae* spread further into the leaves of maize than did the other 2 pathovars and could be detected in masses at 6 DAI. It is possible that hairy maize leaves constitute a good habitat for pv. *oryzae,* and the bacterium may become an epiphyte on the nonhost plant.

These results suggest that nonhosts may function as alternate inoculum sources of the BB pathogen for nearby rice plants. Epiphytic survival of Xco on leaves of some graminaceous weeds for up to 140 d has been reported (Thri Murty and Devadath 1981).

CONCLUSIONS

Although the stoma is one of the most common portals of entry for plant pathogenic bacteria, Xco enters plant tissues mainly through hydathodes and wounds.

Hydathodes are involved in the specificity of rice cultivar-bacterial strain interaction. Cells of Xco multiply rapidly outside the water pores on susceptible cultivars but are embedded by exudates secreted from the water pores of resistant cultivars. The mechanisms responsible for the observed differences are not known.

FM was constantly observed in rice xylem vessels following inoculation with Xco. This material appears to be capable of covering and immobilizing avirulent EPS^- bacteria, but not virulent EPS^+ ones. Virulent bacteria were always surrounded by electron-transparent zones. The electron-transparent zone protects the virulent bacterial cell from the immobilizing effect of FM. Similar observations have been reported in *Erwinia amylovora*-apple petiole and *Pseudomonas solanacearum*-tobacco interactions in which bacteria of avirulent but not virulent strains were embedded by FM (Huang et al 1975, Sequeira et al 1977). The FM is believed to originate from host cell walls. Its chemical nature, however, has not been determined.

Multiplication of Xco on maize, a nonhost, as revealed by SEM, underlines the ability of this bacterium to survive epiphytically and the importance of field sanitary practices in the control of rice BB.

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Notes

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Multiplication of *Xanthomonas campestris* pv. *oryzae* in rice leaves

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

Populations of *Xanthomonas campestris* pv. *oryzae* (Xco) were monitored over time to determine their growth kinetics in rice leaves. Both compatible and incompatible populations increased equally in rice leaves for about 2-4 d (from 10^3-10^4 colony-forming units (cfu)/leaf to 10^7-10^8 cfu/leaf). Thereafter, in some rice cultivars, growth of incompatible bacteria slowed compared with that of compatible bacteria. The time needed after inoculation to observe this difference was dependent on the concentration of the inoculum, indicating that a threshold level of bacteria was required before the host responded and bacterial multiplication ceased. The spread of Xco in rice leaves was more rapid and extensive in compatible than in incompatible interactions. Multiplication of compatible Xco isolates in rice leaves co-inoculated with incompatible isolates was suppressed if ratios of 10:1 (incompatible: compatible bacteria) or greater were inoculated into the leaves.

Pathogenic specialization of *Xanthomonas campestris* pv. *oryzae* (Xco), the causal agent of bacterial blight (BB), has been demonstrated on rice cultivars that have specific genes for resistance to the disease (for review, see Mew 1987). Because race-specificity exists in this interaction, studying specific cultivar-race combinations will allow us to more readily address the mechanisms of compatibility and incompatibility. One means of addressing these phenomena is to monitor the population dynamics of pathogen races in resistant and susceptible hosts. In the rice-Xco interaction, the classic hypersensitive response is absent (Parry and Callow 1986; Leach et al, unpubl. data), and resistance is defined by a difference in lesion length (Mew et al 1981, Ogawa 1983). To complement lesion length data and to provide a quantitative measure of the effects of the host's response on bacterial populations in compatible or incompatible interactions, we investigated the growth kinetics of the BB pathogen in rice leaves. We will discuss here our findings on the population dynamics of various Xco races in resistant and susceptible rice cultivars in relation to the work of others.

MULTIPLICATION AND SPREAD OF Xco IN RICE LEAVES

When populations of an isolate of one race are compared on resistant and susceptible dicotyledonous hosts, incompatibility is generally reflected in lower pathogen populations (Borkar and Verma 1985, Keen et al 1981, Stall and Cook 1966). In the rice-Xco interaction, the literature encompassing bacterial population dynamics in compatible and incompatible interactions is contradictory and inconclusive. Mohiuddin and Kauffman (1975), monitoring Xco multiplication in leaves from resistant and susceptible rice cultivars, found no bacterial population differences in compatible or incompatible combinations if leaf samples were taken only at the inoculation point. At 1-3 cm below the inoculation point, lower rates of multiplication and spread were observed in the incompatible interaction, and differences in bacterial numbers approaching 100-fold were observed. In contrast, Parry and Callow (1986) found little difference in the rate at which Xco-compatible or -incompatible bacteria multiplied or in the overall populations in the top 10 cm of infected rice leaves. In addition, they observed no difference between the spread of compatible and incompatible bacteria. They concluded that resistance expression in rice is not reflected in reduced populations of incompatible bacteria, but is characterized predominantly by reduced symptom expression in the host.

To critically define the effect of host resistance on bacterial multiplication in rice leaves, we monitored the growth kinetics of several Xco races in three differential rice cultivars. The Xco isolates described herein and the pattern of their interactions on specific differential cultivars are summarized in Table 1. Leaves of rice cultivars IR20 (carrying the resistance gene *Xa-4*), Cas 209 (*Xa-10*), and IR1545-339 (*xa-5*) were inoculated with suspensions (5×10^9 colony-forming units [cfu]/ml) of streptomycin (Sm)- or rifampicin (Rif)-resistant Xco isolates PXO 61^{Sm} (race l), PXO 86^{Rif^r} or PXO 93^{Rif^r} (race 2), or PXO 99^{Sm} (race 6), using a double-needle technique (Muko and Yoshida 1951). The double-needle technique delivers about 10^3-10^4 cfu bacteria/site. We are aware that compatibility and incompatibility are interaction phenomena; however, for simplicity, those Xco isolates that, in association with a given rice cultivar, result in host resistance are herein called incompatible bacteria. Those isolates involved in interactions that result in host susceptibility are called compatible bacteria.

Table 1. Disease reaction of Philippine *X. campestris* pv. *oryzae* races on differential rice cultivars (adapted from Mew 1987).

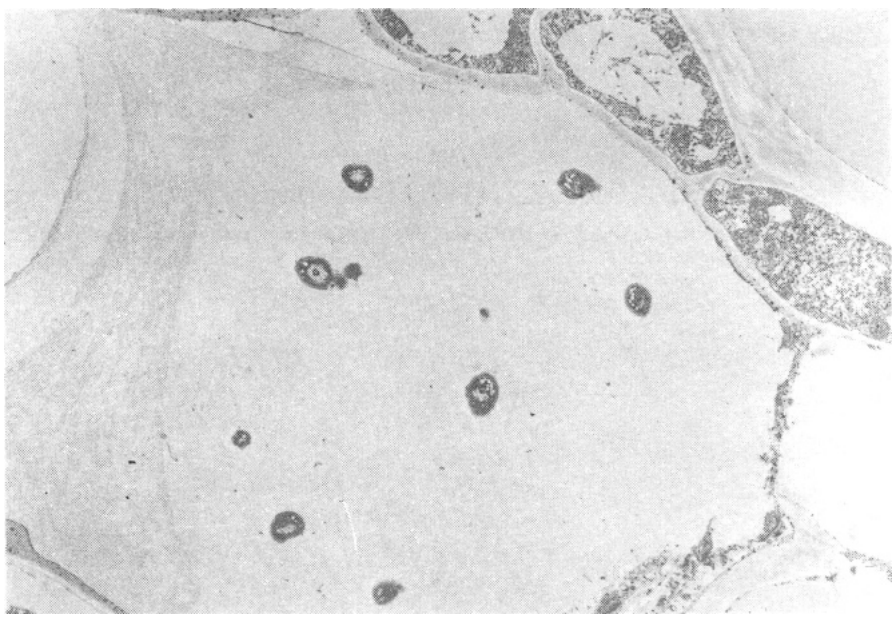
Differential cultivar (resistance gene)	Disease reaction[a]			
	Race 1	Race 2	Race 4	Race 6
IR8 *(Xa-11)*	C	C	C	C
IR20 *(Xa-4)*	I	C	C	C
Cas 209 *(Xa-10)*	C	I	C	C
IR1545-339 *(xa-5)*	I	I	C	C

[a] C = compatible, I = Incompatible.

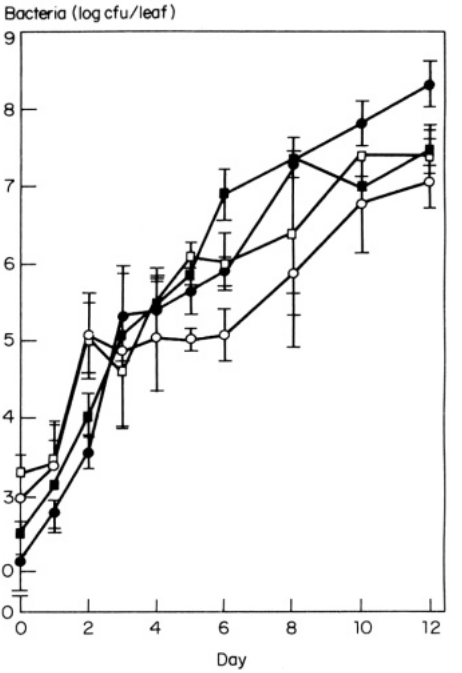
In Cas 209 and IR1545-339, compatible and incompatible populations increased steadily and equally in rice leaves for 24 d after inoculation (DAI) to about 10^7 cfu/leaf (for examples, see Figs. 1-3). Thereafter, bacterial growth in the incompatible combination with these hosts slowed compared with the compatible combination. Bacterial populations in compatible interactions reached 10^{11}-10^{13} cfu/leaf. The response in IR20 was less definitive—large differences in bacterial populations were not observed.

Symptoms on leaves began to appear at 7-10 DAI. Average lesion length differed among cultivars. For example, in the compatible interactions on Cas 209, lesions ranged from 9.0 to 12.4 cm, with an average of 10.5 cm, whereas in IR20, compatible combinations resulted in lesions ranging from 7.5 to 10.3 cm, with an average of 9.1 cm. In incompatible interactions with Cas 209 and IR20, lesions ranged from 0 to 6.0 cm, with an average of 2.7 cm, and 6.1 to 10.2 cm, with an average of 8.0, respectively. Lesion length data from IR1545-339 were similar to those of Cas 209 in that lesions were shorter in incompatible responses.

The time needed to observe the differences in bacterial numbers between incompatible and compatible interactions was dependent on the initial inoculum concentration. For example, when Cas 209 leaves were infiltrated with high



1. Populations of *Xanthomonas campestris* pv. *oryzae* (race 1, isolate PXO^{Sm} . and race 2, isolate PXO 8h^{Rif}) per leaf of rice cultivar Cas 209. Rice leaves were inoculated individually with each isolate (PXO 61^{Sm}● or PXO86^{Rif} ■), or with a ratio of 1:1 incompatible to compatible (incompatible, PXO86^{Rif} ▽ or compatible, PXO 61^{Sm} o) using the double-needle technique. Four leaves from each treatment were sampled individually on the indicated day.

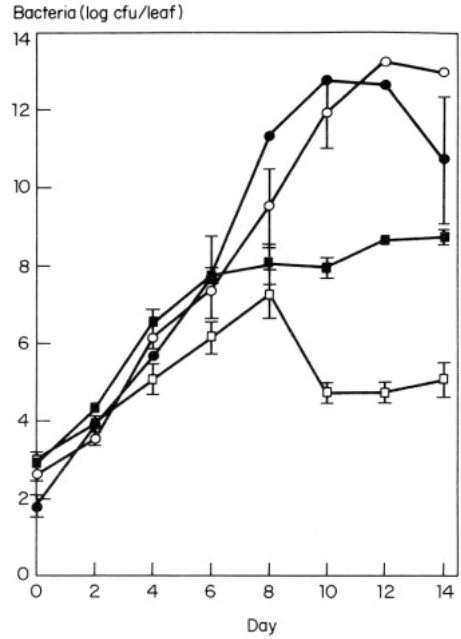


2. Populations of *Xanthomonas campestris* pv. *oryzae* (race 1, isolate PXO 61^{Sm}, and race 2, isolate PXO 86^{Rif}) per leaf of rice cultivar IR20. Rice leaves were inoculated Individually with each bacterial isolate (PXO 61^{Sm} ■ or PXO 86^{Rif} ● or with a ratio of 1:1 incompatible to compatible (incompatible, PXO 61^{Sm} □ ;compatible, PXO 86^{Rif} o) using the double-needle technique. Four leaves from each treatment were sampled individually on the indicated day.

concentrations of Xco (using a Hagborg apparatus; Hagborg 1970), bacteriostasis of the incompatible race occurred earlier. In fact, if 10^7 cfu/site of incompatible bacteria was infiltrated, the bacteria did not multiply (Fig. 4).

To monitor the spread of bacteria from the inoculation point, Cas 209 leaves inoculated 10 cm from the tip with compatible or incompatible bacteria were cut into 2-cm sections from the top to 2 cm below the section containing the inoculation site (Table 2). Bacterial number was determined in each individual leaf section every 2 d through day 12. Both compatible and incompatible bacteria multiplied and moved outward from the inoculation point. In the incompatible combination, however, bacteria multiplied less rapidly and moved to adjacent sections much more slowly. In addition, symptoms were never observed in advance of the bacteria; rather, bacterial populations in a given section approached 10^8 cfu before symptom expression. Because lesions were never seen in advance of the pathogen, it is unlikely that a bacterial-produced soluble toxin plays a significant role in pathogenesis.

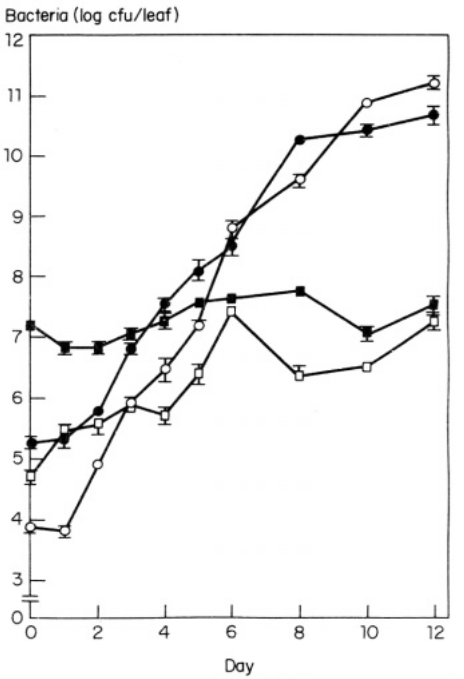
Our investigations thus indicate that incompatibility in the interaction between Xco cultivars Cas 209 and IR1545-339 was reflected in lower pathogen populations, delayed movement of incompatible bacteria within the leaf, and reduced lesion development. The differences between compatible and incompatible bacterial populations were more substantial (10^4-10^5 cfu/leaf) than those previously reported



3. Populations of *Xanthomonas campestris* pv. *oryzae* (race 6. isolate PXO 99^{Sm}, and race 2, Isolate PXO 93^{Rif}) per leaf of rice cultivar IR1545-339. Rice leaves were inoculated individually with each bacterial isolate (PXO 99^{Sm} ● or PXO 93^{Rif} ■);or with a ratio of 1:1 Incompatible to compatible (incompatible, PXO 93^{Rif} □ : compatible, PXO 99^{Sm} o) using the double-needle technique. Four leaves from each treatment were sampled individually on the indicated day.

(Mohiuddin and Kauffman 1975). Resistance in IR20 was not reflected in lower populations of incompatible bacteria; nor did we observe significant reduction in lesion length. Others have reported that resistance in IR20 is not complete (summarized in Mew 1987). However, bacterial populations in compatible interactions with IR20 do not reach the high levels observed in other cultivars, suggesting that some level of resistance to even the compatible pathogen exists. Further, in comparison with Cas 209 and IR1545-339, compatible interactions in IR20 result in reduced lesion development (Horino et al 1982; Barton-Willis et al, unpubl. data). Thus, IR20 contains some means by which to inhibit bacterial growth and restrict lesion development; whether or not it is a feature of *Xa-4* gene action is not clear.

Parry and Callow (1986), using Cas 209 and IR20 in combinations with isolates PXO 86 (race 2) and PXO 71 (race 4), found no population differences between compatible and incompatible interactions. The sectioning experiments (Table 2) suggest that they may not have sampled enough leaf tissue to observe large population differences in Cas 209. Ou (1985) reported that Xco accumulated in the stems and internodes of rice plants. Because of the difference in total bacterial numbers in the sectioning data (Table 2) and whole leaf data (Fig. 1), we suggest that bacteria may also accumulate in the lower portion of the leaf.



4. Effect of inoculum level on populations of *Xanthomonas campestris* pv. *oryzae* (race 1, isolate PXO 61^{Sm}, and race 2, isolate PXO 86) per leaf of rice cultivar Cas 209. Rice leaves were infiltrated using a Hagborg apparatus with starting inocula of $5x10^{10}$ (PXO61^{Sm} ●, PXO 86^{Rif} ■) and 5 x 10^{8} o, PXO 86^{Rif} □) cfu/leaf. Four leaves from each treatment were sampled individually on the indicated day.

Table 2. Populations of *Xanthomonas campestris* pv. *oryzae* race 1 (isolate PXO 61) and race 2 (isolate PXO 86) in adjacent Cas 209 leaf sections (A-F). Progression outward from the inoculation point was monitored in the upper 12 cm of the leaf at 2-d intervals by cutting 2-cm sections above and below the point of inoculation (section E).

Day	Race	2-cm leaf section						Total lesion length (cm)
		A	B	C	D	E	F	
0		10	0	0	0	$2x10^4$	0	0
	2	0	0	0	0	$2x10^4$	0	0
2		10	0	0	0	$5x10^4$	0	0
	2	0	0	0	0	$3x10^4$	0	0
4		10	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

INFLUENCE OF TEMPERATURE ON RESISTANCE AND SUSCEPTIBILITY IN IR20

Resistance to BB conferred by IR20, although not complete, has been well documented (for reviews, see Mew 1987, Ou 1985). In fact, in the greenhouse, we have observed substantial differences in lesion length and bacterial populations in compatible and incompatible interactions with IR20 (Barton-Willis et al 1986). Parry and Callow (1986) measured differences in lesion length between compatible and incompatible interactions, but were unable to detect differences in bacterial populations. It is possible that the environmental conditions (growth chamber, 32 °C night, 12-h photoperiod, 70% relative humidity) used to obtain the data shown in Figure 2 did not favor expression of resistance.

Horino et al (l982) reported that gene *Xa-4* for BB resistance, carried by IR20, is influenced by temperature. At high temperatures (33 °C day, 25 °C night), lesions in incompatible interactions (race 1, isolate PXO 61) developed up to 5 times longer than lesions at low temperatures (29 °C day, 21 °C night). We monitored incompatible. bacterial populations and lesion lengths in IR20 under low temperatures (29 °C day, 21 °C night) and high temperatures (33 °C day, 22 °C night). Our preliminary results indicate that incompatible bacterial populations at both temperature regimes increase at equal rates for the first 12 d (to 10^8-10^9 cfu/leaf). After this, populations at the low temperature regime were about 10 times lower than those at high temperature. Although bacterial numbers were substantial at the low temperature, lesions were about 5 times shorter when plants were incubated at the low temperature than at the high temperature. Therefore, we speculate that environment plays a strict role in the expression of resistance by IR20. Parry and Callow (1986) suggested that resistance in rice may be reflected by symptom expression in the plants rather than by reduced incompatible pathogen populations; perhaps in IR20 this is true.

MIXED INOCULATION EXPERIMENTS

Results of mixed inoculation experiments using isolates of two Xco races are also controversial. In other host-bacterial pathogen interactions, mixed inoculation experiments have provided valuable insight into the induction of resistance. For example, when soybean leaves are inoculated with a 1:1 mixture of compatible and incompatible *Pseudomonas syringae* pv. *glycinea* isolates, multiplication of compatible bacteriain the leaves is restricted, presumably because the expression of hypersensitivity inhibits growth of both isolates (Long et al 1985). Thus. once elicited, resistance is physiologically “dominant.” Fugii (1976) monitored Xco populations using a mixture of a virulent isolate and an avirulent (not virulent on any cultivar) mutant of that isolate. The avirulent mutant alone multiplied very slowly in leaves and caused no visible symptoms on the host. Multiplication of the virulent isolate was reduced both in vitro in liquid cultures and in rice plants if equal amounts of the avirulent mutant were added to the inoculum. The avirulent isolate in the 1:1 mixture grew more rapidly and spread further in the leaf than if inoculated alone. Lesion lengths after inoculation with the mixture were approximately 30% shorter than those after inoculation with the virulent isolate alone. These

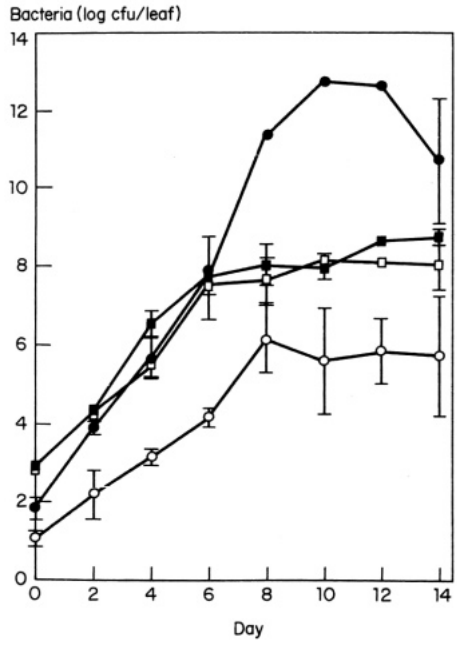
experiments indicated that the avirulent mutant protected rice leaves against the virulent isolate.

In contrast, Reddy and Kauffman (1973) found that lesion lengths in leaves inoculated with a 1:1 ratio of compatible to incompatible isolates were equal to those in the compatible control, indicating no induction of resistance. Parry and Callow (1986) found that an inoculum of a 1:1 mixture of compatible and incompatible isolates resulted in lesions not significantly different from those produced by inoculation with the compatible isolate on one cultivar (Cas 209), and lesions intermediate in length to those resulting from the control compatible and incompatible reactions on another cultivar (IR20). Thus, the effect of co-inoculation on host response seemed to vary with cultivar. The effect on the bacteria themselves is unclear, because populations were not monitored.

We investigated the effects of co-inoculating compatible and incompatible races onto Cas 209, 1R20, and IR1545-339. Leaves were double-needle inoculated with each bacterial isolate individually or with a mixture of the isolates in a 1:1 (incompatible race:compatible race) (IR20, Cas 209, or IR1545-339), 10:1 (Cas 209 or IR1545-339), or 100:l ratio (IR1545-339). If the mixture contained equal numbers of bacteria from both compatible and incompatible races, multiplication of bacteria representing compatible races was not restricted by comparison with growth in singly inoculated leaves in all cultivars (see Figs. 1-3, 5). However, multiplication of bacteria representing incompatible races was inhibited, and bacterial numbers dropped over time. Because we observed the same phenomenon in IR8 (Barton-Willis et al, unpubl. data), which carries no known genes for resistance to these isolates, inhibition of incompatible bacterial growth was likely due to competition between the isolates. With in vitro mixed liquid cultures, no inhibition of one isolate by another was observed. However, the race 2 isolate used in the in vitro studies (PXO 86^{Rif}) multiplied at a slower rate alone and in the mixtures than did the race 1 isolate (PXO 61^{Sm}). Lesion lengths were not significantly different on day 12 between the compatible bacteria inoculated alone and the 1:l mixture in IR1545-339 or Cas 209.

With higher proportions of incompatible to compatible bacteria (10:1, see Fig. 5; or 100: 1, data not shown), resistance was physiologically "dominant" in IR1545-339 and Cas 209; that is, populations of the compatible bacteria were suppressed similarly to those of incompatible bacteria. In the 10:1 mixtures, lesion lengths were very similar to those of the incompatible combination alone. In addition, if the incompatible bacteria were inoculated 4 d before inoculation of the same IR1545-339 leaf with compatible bacteria, populations of compatible bacteria were restricted (data not shown). It is still possible that inhibition of incompatible isolate multiplication was due to competition. Therefore, we are constructing single-gene race-change mutants of the pathogen (mutants altered to elicit a compatible rather than an incompatible response in a particular rice cultivar) to address these questions more critically.

Like Parry and Callow (1986), we did not observe significant differences in the protective response (as measured in lesion lengths) with inoculum ratios of 1:1 (incompatible to compatible) in Cas 209 or IR1545-339. Our work also substantiates



5. Populations of *Xanthomonas campestris* pv. *oryzae* (race 6, isolate PXO 99^{Sm}, and race 2, isolate PXO 93^{Rif}) per leaf of rice cultivar IR1545-339. Rice leaves were inoculated individually with each bacterial isolate (PXO 99^{Sm} ● or PXO 93^{Rif} ■), or with a ratio of 10:1 incompatible to compatible (incompatible, PXO 93^{Rif} □; compatible, PXO 99^{Sm} 0) using the double-needle technique. Four leaves from each treatment were sampled individually on the indicated day.

their observations of a reduction in lesion length in combinations involving IR20 (compatible alone, 14 cm; incompatible alone, 11 cm; 1:1 mixture, 11 cm). Again, it is important to note that substantial lesions do develop in incompatible combinations with this cultivar.

When a higher ratio of incompatible bacteria was introduced (10:1 or higher), lesions in the compatible combinations were reduced to a length similar to those of the incompatible interaction. In the mixed inoculation experiments with Cas 209 and IR1545-339 (10:1 incompatible:compatible), the reduction of symptoms (lesion length) is reflected by a reduction in bacterial population and overall lesion length in compatible combinations by comparison with other cultivars.

SUMMARY

In some rice-Xco interactions, reduced lesion length and suppression of incompatible bacterial multiplication are characteristic expressions of resistance. Compatibility, on the other hand, is characterized by rapid bacterial multiplication and spread in the host leaf. In these ways, the population dynamics observed in the rice-Xco interaction are similar to those observed in dicotyledonous plant-bacteria interactions. We and others (Parry and Callow 1986) have not observed a typical

hypersensitive response in rice challenged with incompatible bacteria. In fact, if infiltrated with high numbers of bacteria (as in Fig. 4), the host response to compatible bacteria (water soaking) was observed at 24-48 h. No response was observed in the incompatible combinations until 48-72 h. Thus, although population dynamics are somewhat similar in the incompatible responses between rice and some dicotyledonous hosts, we have not observed the rapid collapse and desiccation of host tissues characteristic of the hypersensitive response in rice.

Mixed inoculations (compatible + incompatible bacteria) were complicated by competition among field Xco isolates. However, if ratios of incompatible to compatible bacteria of 10:1 or greater were used, the reductions in bacterial population and lesion length suggest that incompatibility is physiologically "dominant" over compatibility. This supports the hypothesis that the rice-Xco interaction follows a gene-for-gene type of resistance.

The *Xa-4* resistance gene carried by IR20 is of particular interest, especially if the temperature influence observed by Horino et al (1982) is a trait of that particular gene and not of the host background. A temperature-influenced gene such as *Xa-4* would be useful to investigate the influence of resistance genes not only on bacterial population and lesion length, but also in molecular studies of rice-Xco interactions.

The results reported herein are the groundwork for an ongoing comprehensive investigation in our laboratory of compatibility and incompatibility. We are developing the genetic tools in Xco necessary to more critically address the mechanisms by which the pathogen interacts with its host, rice. Thus, the prospects for understanding this important disease and host disease resistance are promising.

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Notes

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Does rice seed transmit the bacterial blight pathogen?

T. W. Mew, N. Unnamalai, and M. R. Baraoidan

Xanthomonas campestris pv. *oryzae,* the causal organism of rice bacterial blight, has been reported to be seed-transmitted based on detection methods that were only suggestive. Recent studies have indicated that the bacteria may be detected by direct isolation only in immature seeds or seeds from severely infected plants. Seed transmission may thus play a role only in areas free of the disease. In the tropics, the seed may not serve as an important inoculum source.

Thurston (1973) reviewed tropical plant diseases of potential international importance, dividing them into those of high, intermediate, and limited threat. To be highly threatening, a disease "should be characterized by the ability to spread rapidly, cause serious losses, and be difficult to control." Among the five diseases listed as highly threatening was rice bacterial blight (BB) caused by *Xanthomonas campestris* pv. *oryzae* (Xco). BB is severe in both temperate and tropical rice-growing countries. Epidemics in Japan affected thousands of hectares in the 1950s and 1960s, with losses ranging from 20 to 30% (Mizukami and Wakimoto 1969). In tropical Asia the disease has affected rice production every year unless resistant varieties have been planted (Mew 1987), and it continues to pose a serious threat to some countries (Mew 1989, this volume). Much of the recent occurrence has been attributed to seed transmission (Awoderu and John 1984, Lozano 1977).

CONTROVERSY OVER SEED TRANSMISSION

BB is considered highly threatening not only due to its epidemic potential but also because it is thought to be seedborne. But there is controversy in the literature about its transmission by seed (Goto et al 1988, Ou 1985). Some authors have stated that infected rice seed can carry the pathogen until the following crop season, depending on the infection in the field as well as storage conditions (Ou 1985). However, the seed seems to be a weak point in the life cycle of the pathogen in the tropics. Seedling infection rarely occurs in nurseries, and disease incidence through infected seed is much lower, if at all, than from other sources such as straw, stubble, ratoons, or

volunteer rice (Mizukami and Wakimoto 1969). The controversial claims of Xco seed transmission have been caused by the following:

- *Disease history.* Disease history is often used to deduce BB occurrence through seed transmission (Fang et al 1982). In the early 1950s, in regions where BB was prevalent, using seed from disease-free areas minimized the disease (CAAS 1986). On the contrary, in regions where the disease had not been recorded, introduction of seed from regions where the disease was severe was sometimes followed by disease outbreaks. Both situations pointed to seed transmission. However, sowing seed from a diseased field into a disease-free field did not always lead to a disease outbreak; and infection could have been explained by an indigenous strain. So disease outbreaks are not direct proof that Xco is seedborne.
- *Lack of direct isolation.* Most of the experimental evidence demonstrating Xco seed transmission was obtained through the bacteriophage technique. Direct isolation of Xco from seed is difficult because the bacterium grows slowly on laboratory media, and because of contamination by a variety of fast-growing bacteria, many of which form yellow colonies on agar. Direct isolation from seed carrying the bacteria, especially at low concentration, is not easy. However, Hsieh et al (1974) isolated the bacteria from seed using a streptomycin-resistant mutant.
- *Rate of detection.* Some reports cited evidence, using the blotting paper test, that bacteria were carried by the seed and could cause seedling infection (Singh and Rao 1975). Unfortunately, the infection rate was abnormally high for seed transmission.

Persistence of Xco in rice hulls for several months after harvest has been demonstrated (Mizukami and Wakimoto 1969). In contrast to bacterial diseases caused by pseudomonads, seedborne Xco causing infection of rice plants either in the nursery or in the field has not been positively proved (Murty and Devadath 1984, Tagami et al 1963).

Many reports have indicated that seedlings are infected through seed (Koroleva et al 1985, Reddy 1983, Singh et al 1983, Srivastava and Rao 1964); but most experiments used too little seed, or the rate of seedling infection was unusually high. More importantly, such results should be repeated and confirmed.

DETECTION METHODS

Several indirect methods have been widely used to detect the BB pathogen in nature, but their degree of precision is not always mentioned. Most of the results obtained by such methods, unless proved by direct isolation, the efficiency or specificity of the technique, or bacteriological confirmation, are merely suggestive (Goto et al 1988). The following techniques have recently been critically reviewed (Goto et al 1988):

- bacterial streaming,
- bacterial exudation (especially employing the blotter test),
- phage technique,
- isolation and pathogenicity test.

RECENT STUDIES

Fang et al (1982) used the bacteriophage technique to study seedborne Xco over 10 yr. Their methods and results were all published in Chinese.

A high number of phages had been repeatedly isolated from infected seed coming from diseased fields, causing speculation that the existence of phages in seed samples might be directly related to seedborne transmission of the bacterial pathogen. But the mere detection of a large number of phages in a seed sample does not necessarily indicate that the bacterium is also present. Fang and his colleagues used anti-phage serum or Fe salt to inactivate the free phages. The corresponding platings with bacterial indicator suspensions were those phages being absorbed by living bacteria in the seed samples. The data suggested that bacteria were carried by the seed, but the majority of bacterial cells invaded by the phages might have been lysed, especially during nursery planting in the following season. The significance of seed transmission could again be negligible.

At IRRI, we have examined seed transmission for several years. We have repeated most of the techniques various scientists have reported, and the results have not been conclusive. The methods seem not sensitive or reliable enough to detect low numbers of bacterial cells.

One experiment used two sets of IR36 plants. At flowering, one set was artificially inoculated by injection and spraying with Xco isolate PXO 79; the other set served as the check. At maturity, the seeds were examined separately for the presence of the BB pathogen by several methods:

- *Direct isolation.* A suspension made-from crushed hulls and brown rice washings from whole seeds was streaked onto peptone-sucrose agar (PSA) and onto a semiselective medium.
- *Phage method.* A phage specific to PXO 79 was added to the suspension for incubation at 28 °C for 0 and 10 h. A significant increase of phage number by plaque count would indicate the presence of bacterial cells in the samples.
- *Growing-on test.* Rice seeds were sown in sterile soil, on moist blotting paper in a petri dish, and in N-enriched solution in beakers. Observations were made for BB lesions and bacterial ooze on the germinating seedlings from 24 h after sowing until 21 d after sowing (DAS) for those sown in sterile soil, and until 7 DAS for the other 2 treatments. Pathogen isolates from seedlings showing apparent infection or ooze would be tested for pathogenicity.

By direct isolation, many yellow bacterial colonies formed on the PSA plates and were isolated. One of them appeared to be Xco. Some were like *Erwinia herbicola.* Xco was isolated from panicle branches (Table 1, 2). Direct isolation of Xco was positive with healthy seeds mixed with fine dry powder of infected leaves (Table 3).

We also tested direct phage isolation methods. Seed lots of naturally infected rice plants from fields in different localities in the Philippines were collected and assayed for free phages, absorbed phages, and direct isolation. For each sample, whole seeds, hulls, grains, and seed washings were checked for the presence of bacteriophages. The indicator bacterial isolates were those isolated from infected leaves at the respective sites. The phage counts were obtained and compared. Seeds together with

Table 1. Detection of *Xanthomonas campestris* pv. *oryzae* from different parts of the panicle of IR36 rice plants infested by the injection method.

Trial	Part of panicle	Direct isolation[a]	Phage test[b]	Pathogenicity to rice[c]
1	Panicle axis	+	NT	+
	Primary branch	+	NT	+
	Secondary branch	-	NT	-
	Pedicel	-	NT	-
	Grain	-	NT	-
2	Panicle branches	+	+	+
	Grain	-	-	-

[a] + = isolated, - = not isolated. [b] NT = not tested, + = positive result, - = negative.
[c] + = pathogenic, - = not pathogenic.

Table 2. Detection of bacteria by the growing-on test from IR36 grains obtained by the injection method.

Treatment	Seeds sown (no.)	Abnormal seedlings (no.)	Direct isolation[a]	Pathogenicity test[b]
100 ppm N	2,500	23	+	-
Blotting paper in plates	4,644	1 17	+	-
Sterile soil	10,200	22	+	-

[a] + = isolated, - = not isolated. [b] - = not pathogenic.

Table 3. Isolation of *Xanthomonas campestris* pv. *oryzae* from healthy seeds mixed with the fine powder of 66-infected leaves on a semiselective medium.[a]

Weight of leaf powder added (g)	Colonies isolated (cfu/ml)
14	3.4×10^6
7	4.95×10^5
5	7.15×10^5
3	6.8×10^4
1.5	2.25×10^4
0.5	2.5×10^4

[a] Each sample had 1,000 seeds weighing approximately 25 g and was brought to a liquid suspension volume of 250 ml with peptone-sucrose agar. All showed phage sensitivity to PXO 86 and direct immunofluorescence. cfu = colony-forming units.

the panicles at different stages of development with various degrees of infection were also collected and tested. Disease incidence at the sampling sites was also assessed.

Of 38 naturally infected samples, only 16 showed positive increases in adsorbed phages after 10 h of incubation (Table 4). The other 22 samples showed a positive increase in free phages. The free phages survived longer without the host bacteria,

but in an earlier study with phages and bacteria introduced together into panicles, the phages could not survive alone. The implication is not clear. Perhaps in those seed samples showing an increase in free phages alone, the bacteria were present and were lysed by phages during the operation.

Isolation of phages from different parts of the seed also showed that bacteria were present inside the seed. The number of phage particles in washings of the crushed seeds and husks was higher than that from the grain (endosperm and

Table 4, Isolation and multiplication of *Xanthomonas campestris* pv. *oryzae*-specific free phage (in plaque-forming units) from naturally infected seeds collected from IRRI, Nueva Ecija, Palawan, and Mabitac, Laguna, ricefields.

Seed lot[a]	Surface washings		Crushed whole seeds		Husks		Grains	
	0 h	10 h	0 h	10 h	0 h	10 h	0 h	10 h
1	0	1.1 x 10^2	0	2.5 x 10^5	0	1.6 x 10^5	0	1.0 x 10^4
2	0	0	0	68	0	43	0	3
3	0	6.0 x 10^6	34	6.8 x 10^9	0	1.4 x 10^6	3	6.5 x 10^4
4	0	0	0	4.2 x 10^4	0	1.2 x 10^3	0	1.0 x 10^3
5	0	0	0	4.85 x 10^4	0	1.0 x 10^7	0	0
6	0	0	0	63	0	43	0	25
7	0	5	0	64	0	58	0	70
8	0	0	0	8.4 x 10^4	0	7.2 x 10^4	0	0
9	0	0	0	4.2 x 10^4	0	4.8 x 10^5	0	3.0 x 10^5
10	0	0	0	6.2 x 10^9	0	4.82 x 10^9	0	0
11	0	0	0	1.75 x 10^5	0	1.14 x 10^5	0	0
12	0	0	0	4.8 x 10^6	0	2.2 x 10^4	0	7
13	0	0	0	7.2 x 10^4	0	1.8 x 10^4	0	2.18 x 10^2
14	0	0	0	2.84 x 10^2	0	1.13 x 10^2	0	14
15	0	3.89 x 10^2	0	10^6	0	1.44 x 10^5	0	4.74 x 10^2
16	0	49	0	4.0 x 10^4	0	81	0	29
17	0	4.2 x 104	0	8.2 x 10^6	0	3.0 x 10^5	0	3
18	0	0	0	4.0 x 10^4	7	1.83 x 10^5	3	1.06 x 10^5
19	0	0	13	9.2 x 10^8	14	3.85 x 10^7	0	0
20	0	0	1	8.4 x 10^8	24	1.19 x 10^5	0	0
21	0	0	0	0	0	0	0	0
22	0	0	0	0	0	0	0	0
23	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0
25	0	0	0	0	0	0	0	0
26	0	0	0	0	0	0	0	0
27	0	26	4	3.4 x 10^8	0	1.5 x 10^4	0	35
28	0	0	0	5.0 x 10^3	0	50	0	0
29	0	0	0	1.4 x 10^7	0	1.2 x 10^8	0	14
30	0	0	0	2.42 x 10^7	0	8.0 x 10^7	0	0
31	0	6.3 x 10^6	1	4.6 x 10^5	0	1.5 x 10^{10}	0	2.3 x 10^4
32	0	5.5 x 10^4	0	1.2 x 10^{10}	0	3.8 x 10^8	0	54
33	0	0	0	0	0	0	0	0
34	0	0	0	2.0 x 10^4	0	6.4 x 10^8	0	0
35	0	0	226	2.9 x 10^{10}	0	1.0 x 10^{10}	0	0
36	0	0	0	8.3 x 10^6	7	1.14 x 10^7	0	0
37	7	1.51 x 10^5	19	3.32 x 10^9	1	3.6 x 10^7	0	0
38	0	1.2 x 10^6 C	(10^3)	4.7 x 10^{10}	7	2.3 x 10^{10}	0	2.3 x 10^4

[a]45-50 seeds/sample for seed lots 1-36. 100 seeds/sample for seed lots 37 and 38.

Table 5. Detection of *Xanthomonas campestris* pv. *oryzae* by phage isolation from seeds of different maturity grades.[a]

Seed maturity	Seeds (no.)	Supernatant (pfu/ml)	
		0 h	10 h
Immature	50	(103)[b]	3.3×10^{10}
Mature, discolored	50	0	0
Unfilled	50	0	1.14×10^{5}
Mature, healthy looking	50	36	0
Mature, healthy looking	100	12	3.4×10^{8}

[a]All pellets showed no plaque-forming units (ptu). [b]Clear plates.

Table 6. Isolation of free phages and absorbed phages incubated at 28 °C for 10 h after removing the free phages from seed samples collected from 4 locations in the Philippines where the mother plants were infected with BB,1986.

Collection site	Grains (no.)	Total samples (no.)	Samples (no.) showing presence or absence[a] of phages in							
			Crushed whole seeds		Surface washings		Husks		Grains	
			Supernatant	Pellet	Supernatant	Pellet	Supernatant	Pellet	Supernatant	Pellet
IRRI	45-50	26	17+ 2±	8+ 3±	4+	3+ 2±	16+ 4±	9+ 1±	7+ 4+	2+
Nueva Ecija	45-50	10	9+	2+ 1±	2+ 1±	2+ 2±	8+ 1±	2+ 1±	4+	2±
Mabitac, Laguna	100	1	+	–	+	–	+	–	+	–
Palawan	100	1	+	–	+	–	+	–	+	–

[a]+ = positive increase in plaques, ± = little increase in plaques, – = no plaques.

embryo) and the surface of the whole seed. The adsorbed phages were fewer than the free phages. Adsorbed phages were detected in two samples, and free phages in eight samples. Detection was greater from immature panicles than from mature panicles (Table 5). Both free and adsorbed phages were detected in freshly harvested seed from farmers' fields and from experiments where severe BB occurred (Table 6). The detection was positive until 4 wk after seed storage at both 6 and 28 °C, regardless of sample size (maximum 1,000 seeds) (Table 7). No free phages were detected in seed that was harvested from plants with a disease score of 3 or less (Table 8).

DISCUSSION

The available information implies that the bacteria diffuse into water during imbibition prior to germination, but the bacterial cells die before the first leaf develops, just failing to infect seedlings (Goto et al 1988). It is not clear why the

Table 7. Detection of *Xanthomonas campestris* pv. *oryzae* by phage isolation in supernatant[a] in number of plaque-forming units per milliliter at 0 h and 10 h from seeds of different maturity grades stored at 6 and 28 °C for different periods.[a]

Storage temperature (°C)	Seed maturity[b]	4 wk of storage		6 wk of storage		8 wk of storage	
		0 h	10 h	0 h	10 h	0 h	10 h
6	Immature	0	8.4×10^5	0	7.3×70^{10}	106	3.9×10^{8c}
	Mature, healthy looking	0	0	0	0	0	0
	Mature, discolored	0	2.8×10^4	0	0	0	0
	Unfilled	0	1.12×10^9	0	2.8×10^8	0	0
28	Immature	0	7.2×10^6	0	2.6×10^6	0	0
	Mature, healthy looking	0	0	0	0	0	0
	Mature, discolored	0	0	0	0	0	0
	Unfilled	0	1.5×10^8	0	2.9×10^8	0	0

[a]All pellets showed no plaque-forming units. [b]100 seeds/sample. [c]Small plaques (1 mm diam.).

Table 8. Isolation of phage specific to *Xanthomonas campestris* pv. *oryzae* from seed samples collected from BB-infected rice crops at IRRI and their disease index.

Disease (BB) index	Isolation of phage[a]	
	Free phage	Adsorbed phage
7	+	+
1	±	±
7	+	+
7	+	+
1	+	-
1	±	-
1	±	-
7	+	+
7	+	+
7	+	+
1	+	-
1	+	+
1	+	-
1	+	±
7	+	±
7	+	+
1	+	±
1	+	±
7	+	+
1	+	+
1	-	-
1	-	-
3	-	-
3	-	-

[a]Based on crushed whole seed technique. + = positive increase in plaque count. + = doubtful increase. –= no plaques.

bacterial cells in seed hulls decline so fast. It could be due to lower tolerance for adverse soil environments including antibiotics, or lower competitiveness than other soil microorganisms. Bacteriophages may also be involved (Kauffman and Reddy 1975) but may not be critical. Xco may also be present as an epiphyte on the plant surface. The resident phase of the bacterial pathogen could also result from germination of infected seed, and the bacterial cells carried by seed might "migrate" during imbibition to the surface of the seedlings and eventually to the main field in transplanting. No evidence has confirmed this speculation, but it is an area worth studying.

Xco could be detected in seed, especially immature panicles. The frequency and number were much lower in mature panicles. In temperate environments, the bacteria were detected and shown to survive for about 5 mo during winter (Mizukami 1961). The length of survival in seed is critical to determine the possibility of seedborne transmission. A long period of survival of 9 mo was reported by Singh et al (1983) and Singh and Rao (1975). Our results with phages indicate that free phages could be detected in the immature seed up to 1.5 mo after collection. In mature seed, both free and adsorbed phages were detected up to 2 wk after harvest. The bacterial cells living in seed were estimated to be approximately 10^2 cells (Wakimoto 1953). We could not detect phages, an indirect means of detecting bacteria, in seed stored at 6 and 28 °C for 2 wk. It seems clear that, wherever seed is stored, the bacteria can live only a short while, i.e., a few weeks. We used a sample size of 1,000 seeds. The sample size is very important and needs to be verified.

The disease index of seed lots collected from IRRI fields was correlated with the isolation of adsorbed phages. The adsorbed phages were isolated from seeds collected from severely infected plants. It appears, therefore, that Xco may be seedborne; however, the seedborne inoculum may not play an important role in disseminating the disease, especially in areas where the disease has occurred. But in regions where the disease has not been recorded, seedborne inoculum may become essential for disease transmission.

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Notes

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Survival of *Xanthomonas campestris pv. oryzae*, the causal organism of bacterial blight of rice

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This paper reviews and discusses the mode of reproduction of *Xanthomonas campestris* pv. *oryzae,* the causal organism of bacterial blight disease of rice, in different plant materials, along with methods for its detection. In Japan, rice seed, plant debris, and naturally infected graminaceous weed hosts play a significant role in the survival of the pathogen and in infecting the ensuing rice crop. In China and India, rice seed is the primary source of the disease in the field; epidemics have occurred in both countries, most probably due to the transport of seed of susceptible cultivars from one part of the country to another and to the large-scale cultivation of such varieties over many years in the same locality. The role of infected straw and stubble in providing the initial inoculum is negligible. The bacterium can survive in soil for only a few days. Wild rices perpetuate the pathogen and infect cultivated rice. All these sources constitute the inoculum in double-cropped areas, whereas seed alone may initiate infection in rice monocrop regions. Entry of the bacterium into the plant system and its multiplication are also discussed.

Bacterial blight (BB) of rice caused by *Xanthomonas campestris* pv. *oryzae* (Xco) has been reported since the early 1900s in Japan. Reports of its occurrence have also come from most of the rice-growing countries of Asia, Latin America, and Africa, and from Australia (Aldrick et al 1973, Lozano 1977, Ou 1985, Reinking 1918, Srinivasan et al 1959). However, disease intensity varies with time and location, possibly due to the rice genotypes under cultivation, cultivation practices, or the presence of bacterial inoculum at vulnerable crop growth stages. The intensity of infection may also depend on the crop growth stage at which it is initiated in the field in rice varieties differing in susceptibility.

Apparently, the source of inoculum is the most essential factor in the buildup of the disease. There are conflicting views on the nature of pathogen survival in different sources—seed, straw, stubble, rhizosphere, or soil—its transmissibility in the subsequent crop, and its ability to induce disease. Hence, the survival of Xco and its consequent effects on the rice crop need thorough review and discussion. This might help in formulating crop cultivation strategies for minimizing disease incidence in the field by reducing the inoculum likely to infect the crop.

DETECTING THE PATHOGEN

The presence of the BB pathogen can be detected by observation, isolation, inoculation, bacteriophage concentration, the paper towel method, and serology.

Isolation

Samples thought to possess the BB pathogen can be subjected to normal surface sterilization before plating on artificial media. Pale yellow, slimy bacterial colonies appear on the media within 4-5 d of incubation at 28 ± 2 °C.

Inoculation

Plant parts thought to be infected are cut into small bits or ground into powder (in the case of seed) and suspended in sterile distilled water for a few hours so that bacterial cells ooze out into the water. The suspension is centrifuged at 7,500 g for 10 min. The concentrated suspension is inoculated into the leaves of healthy, susceptible plants either through a needle prick (Isaka 1970, Muko and Yoshida 1951) or the clipping method (Kauffman et al 1973). Symptom expression indicates the presence of the bacterium in the sample.

Direct observation

Conventionally, seed collected from an infected crop is considered infected seed, since discernible disease symptoms do not normally occur on seed. However, Durgapal et al (1930a) observed that infected panicles and seed could be detected by bacterial streaming at the panicle base and on the seed pedicels. A method has been standardized to produce infected seed by inserting bacterial inoculum into a fine slit (1-1.5 cm long) made at the panicle base. This is further pricked with a fine (no. 20) needle from outside, around the culm circumference. Fully developed infected seed can be obtained by inoculation, preferably at the milk stage.

Crown inoculation method

Xco can be detected in seed, soil, or water by crown-pricked rice seedlings. Essentially, 4-wk-old seedlings raised from suspected seed are uprooted, thoroughly washed, and pricked with a fine (no. 20) needle at 5-6 points along the circumference at the crown (shoot base). The seedlings are allowed to grow in the substrate for 10-15 d, after which they are microscopically examined for signs of infection. Bacterial streaming in vascular strands at the crown denotes the presence of the BB pathogen. Crown-pricked seedlings can also be used to detect the presence of the bacterium in soil and water (Durgapal et al 1980b).

Bacteriophage method

Suspected plant material is macerated and mixed with liquid potato-sucrose-peptone (PSP) medium. Bacteriophage specific to Xco is then added in known concentration and thoroughly mixed. A small quantity of this mixture is centrifuged, and the supernatant is plated with bacterial suspension on PSP medium to obtain the control or zero-hour value. The remaining mixture is incubated for 24 h

at 28 ± 2 °C, and the supernatant is again prepared and plated to obtain the "test" value. Greater plaque number in the test value is indicative of the presence of the pathogen in the sample (Wakimoto 1954).

Paper towel method

Singh and Rao (1977) standardized a simple technique for detecting Xco in rice seed. Seeds are equally spaced between 2 paper towels (45 × 28 cm) previously soaked in tap water, The towels are rolled and incubated at 30 ± 2 °C for 5 d in an upright position in a plastic tray covered with plastic to provide humidity. Bacterial streaming can be microscopically observed in small pieces of coleoptile, leaf sheath, and leaf from infected germinating seedlings. Pieces showing bacterial ooze are crushed in sterile distilled water and inoculated into healthy seedlings of cultivar TN1. The authors observed typical BB symptoms within 72 h after inoculation.

Serological methods

Guthrie et al (1965) detected *Pseudomonas phaseolicola,* a bean pathogen, in bean seed extracts by serological techniques such as the slide agglutination, tube precipitin, and gel diffusion tests. Serological techniques have yet to be standardized for detecting the rice BB pathogen in suspected plant parts. However, they have been used to differentiate the isolates of Xco into different serotypes. Lin et al (1969) reported that virulent and weakly virulent strains could be easily distinguished by the gel diffusion test. Addy and Dhal (1977) found only 1 serotype among 45 Indian isolates based on agglutination and gel diffusion tests. In China, 278 isolates were grouped into 2 serotypes (Wang et al 1981). Serological tests have also revealed that most *Xanthomonas* spp., a few pseudomonads, and *Erwinia* spp. share some antigens with Xco (Ou 1985).

SURVIVAL AND TRANSMISSION OF THE PATHOGEN

The pathogen survives in dry and growth forms. The bacteria in dry form is commonly found in vascular vessels and xylem parenchyma of dried diseased plants and in exudates from infected leaves (Wakimoto 1956). The exudates dry up and fall into the ricefield water. Also, bacteria survive in dry form on seed from infected plants. Dry form bacteria normally become activated by moisture. Bacteria in dried leaf tissues lying on the soil surface may pass into the soil after wetting, whereas at least some of the bacterial cells in seed may reach living plants upon germination.

Growth form bacteria are normally found in stubble and ratoon plants and in some susceptible grasses, especially *Leersia* spp.; they may provide an inoculum for a rice crop (Mizukami 1961).

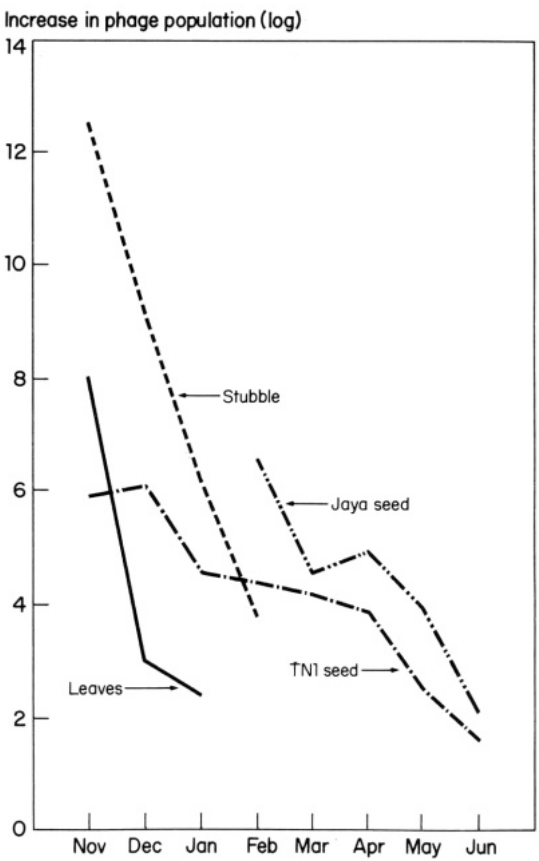
Seed

Several workers have demonstrated seed infection and survival of the BB pathogen in infected seed for periods extending to the next sowing season (Dharam Singh et al 1983, Fanget al 1956, Kauffman and Reddy 1975, Reddy 1983, Srivastava and Rao 1964, Wakimoto 1955, Yoshimura and Tagami 1967). However, Mizukami (1961)

felt that seed is not the important source of infection, because bacteria decrease rapidly in June and die in the course of seed soaking before sowing (Tagami et al 1963). Studies conducted in Hyderabad, India, showed that glumes of seeds readily become infected, as observed by streaming of bacteria through the cut ends of glumes. Infected seed stored under temperatures of 25-35 °C harbored viable bacteria for 2 mo, after which bacteria could not be detected. Immediately after harvest, Xco could be isolated in as many as 90% of the husks of seeds showing bacterial streaming; it was 70% after 1 mo and 40% at the end of 2 mo of storage. However, transmission of the pathogen and expression of symptoms were not observed in freshly harvested nor in stored seed. The failure to transmit the disease to the seedlings was due to increased multiplication of bacteriophages, which decimated the bacterial population during presowing soaking (Kauffman and Reddy 1975). Similarly, Pal et al (1982) could not detect the bacterium in infected seed 3 mo after harvest of Pusa 33 and TN1. Eamchit and Ou (1970) in the Philippines also failed to demonstrate disease transmission through infected seed. However, Hsieh et al (1974) demonstrated the presence of Xco in approximately 15% of seed from inoculated panicles by using a streptomycin-resistant strain of the pathogen. This neither proved nor disproved seed transmission of the disease.

Positive evidence of seed transmission of rice BB was reported as early as 1956 by Fang et al, who observed that the organism was present not only in the vascular system of glumes but also occasionally in the endosperm; they considered the seed the source of infection. Fang and Hsu (1978) reported epidemics of BB throughout China except in Qinhai, Gansu, Xinjiang, and Inner Mongolia during the 1960s, when seed transport was very intensive. Isaka (1970) demonstrated disease transmission through infected seed by raising a nursery under shaded conditions with heavy fertilizer application (pers. comm. cited by Srivastava 1972). Seed samples from Indonesia and the Philippines were also found infected with Xco. The bacterium formed distinct, slimy yellow colonies on D-5 selective medium of Kado and Heskett and on Difco peptone agar (Supriman and Tantera 1972). In India, Srivastava and Rao (1964) demonstrated disease transmission from infected seed to seedlings raised under high humidity. The bacterium was successfully isolated from roots, stems, and leaves of infected seedlings. Their studies further revealed that infected seed collected from Sambalpur, Orissa, showed 100% infection, whereas seed from Kanpur, Uttar Pradesh, showed 80% infection. Chakravarti and Rangarajan (1967) isolated the pathogen from infected seed of cultivar NP130. Subsequently, Rao (1970) and Reddy (1972) proved the survival of pathogenic bacteria in infected grains of susceptible varieties TN1 and Jaya up to 8 mo after harvest through the phage multiplication method (Fig. 1). Singh and Rao (1977) observed the bacterium to survive in infected seed up to 11 mo after harvest of the wet season (WS) crop. They recorded 21% of seedlings exhibiting bacterial streaming immediately after harvest, which fell to 11% in May and June, the period for nursery sowing. Reddy (1983) further demonstrated transmission of the pathogen from seed to seedlings, causing wilting. Profuse bacterial streaming was observed under the microscope in sections of coleoptiles, leaf sheaths, and leaves. However, bacterial oozing decreased in the top portions of tubular leaf sheaths, and

the topmost leaf showed only feeble owing. The bacterium was not detected in the vascular bundles of roots. These observations indicate that bacterial cells present in seed are activated by moisture, multiply, and move upward along with the transpiration stream, finally resulting in seedling wilt. Typical leaf blight symptoms could be observed only in 60-d-old plants to the extent of 10% in cultivar Karuna, 3% in TN1, and 7% in IR8. Interestingly, susceptible rice cultivars differed markedly in the extent of disease transmission. The highly susceptible TN1 did not develop the symptoms of wilting, although it expressed symptoms of leaf blight at maximum tillering and heading. This may be because TN1 possesses larger quantities of phenolic prohibitins toxic to the pathogen (Reddy and Sridhar 1975) than IR8 or because of the presence of a higher number of bacteriophages in seed (Reddy 1972). The later development of the disease may be due to the escape of a few remaining cells of the bacterium into the vascular system, which invariably takes longer for symptom expression. On the other hand, IR8, Karuna, and Parwanipur 1 from



1. Detection of *Xanthomonas campesiris* pv. *oryzae* surviving in seed, leaves, and stubble by the phage multiplication method (Reddy 1972).

Nepal showed efficient disease transmission (5, 23, and 85% wilted seedlings, respectively), Hence, cultivars differ widely in expressing effective seed transmission of the pathogen. Murty and Devadath (1984) found that the pathogen survived for 170-180 d after harvest of the kharif crop (June to December), but only 120-130 d in rabi (January to April)-harvested seed. Seed infection was found to be 54% in kharif-harvested seed and 45% in rabi-harvested seed. Although the infected seed failed to produce symptoms on seedlings, the researchers felt that the seed may serve as a source of inoculum from season to season. Besides, even chaff collected from a severely infected crop has been shown to contain Xco, serving as a source of inoculum for nearby self-sown rice seedlings. This was further confirmed when seedlings raised in soil mixed with infected chaff produced symptoms (Devadath and Premalatha Dath 1985).

Plant pathogenic bacteria, in general, do not have any recognized survival structures. Seeds form "protected positions" for a few bacterial cells to survive the off-season (Leben 1973). Bacteriophages in seed may reduce the number of bacterial cells to an undetectable level, thus inhibiting symptom development. However, Goto (1973) stated that Xco and its phages can coexist in a closed system at bacterial concentrations lower than 10^4-10^5/ml without any effect on each other. It is thus possible for the bacteria to survive by hibernating in infected seed. Multiplication of the cells may start when they gain entry into the vascular system of the growing seedlings. Symptom expression may depend on the initial number of bacterial cells and the nutrition available to the growing seedlings. However, the success of seedborne bacteria is dependent on their location on the seed, the anatomical structure of the seed and its germination type survivability. and the bacterial species itself. Seed with hypogeal germination (gramineae) can prevent transmission of bacteria to the aerial parts. On the other hand, epigeal germination (e.g., beans) favors transmission of seedborne bacteria to the aerial parts (Schuster and Coyne 1974).

Straw and stubble

Repeated cultivation of susceptible rice cultivars on the same plot of land increases the possibility of perpetuating the pathogen in plant tissues, particularly in the straw that is often left lying in the field. Investigations by several workers on the survival and transmission of the BB pathogen in infected rice leaves and straw have yielded varying results. Goto et al (1953) reported that Xco overwinters in rice straw and seed. Inoue et al (1957) agreed that the pathogen can survive for 5 mo on diseased parts of rice. However, Tagami (1958) reported that, although the pathogen may survive in a dried state in stored rice straw protected from moisture and rain, the organism perishes completely within 2 mo when such straw is applied to the soil or exposed to moist conditions. Similarly, Reddy (1972), using the bacteriophage multiplication technique, observed that the bacterium survives for only 3-4 mo in straw and stubble. Rice stubble that survives the winter has been found to harbor the bacterium at the base of the stem and roots until the following spring (Ou 1985). Nwigwe (1973) found that the bacterial population declines faster in lesions of susceptible than resistant varieties. Three-week-old lesions contained very few or no

bacteria. The pathogen could not be isolated from the terminal bleached portions of infected leaves. In infected rice stubble, the organism was not recoverable from the outer dead leaf sheaths beyond 1 wk, or from the inner leaf sheaths beyond 3 wk. In West Bengal, India, Chattopadhyay and Mukherjii (1974) studied the survival pattern of Xco in dead and living tissues and stubble in the field. In general, dry tissues—namely, leaves, inside tissues in dry stubble, sheath tissues on stubble, and dead roots collected from drier plots—showed a very low percentage of average infection (1.2%). On the other hand, the living tissues—leaves of self-sown plants and ratoon plants, tissues inside roots, and living roots of such stubble—showed a high percentage of average infection (14.3%), making these substrates possible sources of inoculum. Similarly, Watanabe (1979), while working in Sri Lanka, could detect neither phage nor Xco from stubble stored for 7 mo under dry laboratory conditions, whereas freshly collected stubble induced severe kresek, indicating that the causal bacteria were inactivated during 7 mo of storage and leading to the assumption that the infected stubble may not become the source of inoculum for the next crop season. However, the BB pathogen has been shown to survive in stubble of kresek-infected plants in China until the next May without losing infectivity, forming a source of primary inoculum for the subsequent sown or transplanted crop (Guo et al 1980).

Hsieh and Buddenhagen (l975) comprehensively investigated the pattern of Xco survival in relation to substrate, temperature, and humidity. In general, Xco survived longer under low relative humidity (RH) and temperature. Diseased rice leaves in soil with high water content decomposed rapidly at high temperatures, and Xco in these tissues soon lost viability. Thus, in constantly humid and warm tropical climates, the chances of survival without a living host appear to be small. Xco can survive in diseased rice leaves for more than 800 d at 0% RH and for 110 d at 100% RH in temperate zones where the temperatures are below 10 °C. The bacterium could be isolated from diseased leaves at 100% RH for 185, 110, 40, and 10 d at 1-4, 10, 20, and 30 °C, respectively. In flooded soil and soil with 40% moisture content, the bacterium disappeared completely within 12 d at 30 °C and within 20 d at 20 ºC. In leaves buried in soil at 20% moisture content, bacteria survived for 40 d at 30 °C, 60 d at 20 °C, and still longer in soils close to 0% moisture content (Table 1, 2). However, Xco surviving for along time under arid conditions in some monocropped areas in India has been suggested as a source of inoculum. Farmers in most

Table 1. Survival of *Xanthomonas campestris* pv. *oryzae* in ooze and diseased rice leaves at various temperatures and relative humidity (RH) regimes (Hsieh and Buddenhagen 1975).

Location	Period of survival (d)										
	1-4°C and 0% RH	10°C and 0% RH	20 °C at RH (%) of					30 °C at RH (%) of			
			0	20	54	76	100	0	30	68	100
Ooze	760	760	330	330	90	60	5	150	210	90	5
Diseased leaves	800	800	360	340	125	110	40	260	210	90	5

Table 2. Xco survival in soil (Hsieh and Buddenhagen 1975).

	Diseased leaves buried in soil at		Xco in soil at			
	20°C	30°C	1-4°C	10°C	20°C	30°C
Flooded soil	30	12	62	31	12	4
40% moisture	25	15	92	80	32	4
20% moisture	60	40	170	130	48	15
0% moisture	360	180	190	150	48	30

monocropped areas in northwestern India grow wheat soon after harvesting rice. The fields are plowed and irrigated, during which the pathogen disappears from the diseased rice leaves in the fields. Under these cultivation practices, BB appeared in epidemic proportions during 1980 WS and 1981 WS (Reddy 1980, 1981).

Hence, Xco lives and multiplies only in living parts of rice plant tissue and does not maintain an appreciable saprophytic existence on dead tissues. Diseased rice tissues do not appear to constitute a significant means of survival and perpetuation of the BB pathogen.

Rhizosphere and soil

There is no evidence regarding the perpetuation of pathogenic bacteria in soils of infected ricefields (Seki and Mizukami 1955, Wakimoto 1956, Yoshimura 1963). The organism may live in soil for 1-3 mo depending on the soil moisture and acidity (Mizukami 1961), the humidity, and the antagonistic effects of soil microflora (Mizukami and Wakimoto 1969). Thus, the soil is not considered an important source of inoculum (Ou 1985). Hsieh and Buddenhagen (1975) reported that the BB pathogen in a free state could survive only 4 d in soils with 40% water content and 15 d at 20% water content. Pandey (1970) failed to demonstrate bacterial survival in the rhizosphere of several nonhosts. However, bacteria swarm to the surface of the roots, apparently due to high metabolic activity in the root tips and root hairs; rice roots have an "activating function" in enabling the bacteria to become infective (Mizukami 1961). Thus, the overwintering nature of Xco on roots and rhizomes of various graminaceous plants has been confirmed using the phage technique (Wakimoto 1956, Watanabe and Kurita 1958). Singh (1971b) observed that Xco could not survive even for 1 wk in unsterilized soil or farmyard manure, and therefore soil did not appear to form a potential source of inoculum. However, when seeds and seedlings were grown in soil artificially contaminated with the pathogen, plants became infected (Premalatha Dath and Devadatb 1983). This could not be simulated for natural field soil that had supported the rice crop infected with the BB pathogen, since the susceptible cultivar IR8 did not develop the disease when grown in soil collected from a field on which the crop suffered severe kresek.

Weed hosts and wild rices

Xanthomonads have generally very specific pathogenicity under natural conditions. Under artificial inoculation, *Leersia oryzoides* var. *japonica, L. oryzoides, Zizania*

latifolia, and *Phalaris arundinacea* can become severely infected with BB. Of these, *L. oryzoides* var. *japonica* is commonly found infected in nature. Overwintering of the bacteria on roots and rhizomes of these plants was confirmed by the phage technique. Lesions developed much earlier on *L. sayanuka* than on rice; hence the former was considered to function as an active natural host in Japan (Goto et al 1953, Inoue et al 1957, Yoshimura et al 1959). Natural occurrences of BB on *Cyperus rotundus* and *C. difformis* were reported by Chattopadhyay and Mukherjii (1968), while Pandey (1970) tested 32 common weeds and concluded that none of them could be considered active hosts. Under artificial inoculation, *L. hexandra* was found susceptible by Rao and Kauffman (1970), and *L. hexandra* and *Paspalum scrobiculatum* by Reddy and Nayak (1974). However, natural infection on any of these grasses was not observed in India, and hence their contribution of primary inoculum to the rice crop is considered negligible. Wild rices *Oryza sativa* f. *spontanea* (Kulkarni and Thombre 1969) and *O. perennis,* commonly found in and around ricefields in coastal areas of India, have often been found infected with the disease and may form a potential source of inoculum for the neighboring rice crop. Besides, a number of wild rice species were also found susceptible under artificial inoculation (Devadath et al 1974). Buddenhagen (1987) examined BB occurrence in rice in Australia, Africa, Latin America, and Asia and concluded that the pathogen survives and evolves along with wild rices. The domesticated rice crop gets the infection from the neighboring wild rice plants. It is presumed that the disease has many centers of origin in the rice-growing world and that each evolved separately with wild rices present on the different continents, as was seen in *O. glaberrima, O. barthii,* and *O. longistaminata* in Mali, Cameroon, and Niger, and *O. rufipogon* and *O. australiensis* in northern Australia. Further studies are needed to determine the exact roles of such alternate hosts as contributing factors to the primary source of inoculum for rice.

Irrigation water and ricefield water

Rice leaves infected with Xco produce exudates in the form of milky or opaque dewdrops that can be easily observed in the morning hours. They dry up during the day to form small, yellowish beads that drop into the ricefield water, spreading the disease from field to field along with the water. Srivastava (1972) attributed the increased occurrence of kresek to the use of high N-responsive rice varieties and to staggered sowing and transplanting in areas with assured irrigation. During the early crop growth stages, inoculum builds up and contaminates the seedlings grown for the next season. Hence, overlapping of crops of different ages was considered the main factor for spreading the disease, heavy fertilization being only a complementary factor. It is often said that uprooting the seedlings causes wounds in the root system and paves the way for the entry of bacterial cells into the plant system from the water in nursery beds. However, disease development was not reported when uprooted seedlings were dipped in nursery water and planted separately in pots, despite the presence of high phage populations in such waters. Kresek could be readily developed when the same water was artificially supplemented with sufficient bacterial inoculum (Watanabe 1975). A high phage population in ricefield water, channels, and tanks need not always be due to the presence of the BB pathogen

alone, since most of the phages encountered in irrigation water are polyvalent. The BB pathogen has been reported to survive only for 15 d in ricefield water (Singh 1971a), and for less than 6 d at 30°C, l2 d at 20°C, 37 d at 10°C, and 60 d at 1-4°C (Hsieh and Buddenhagen 1975). However, Premalatha Dath and Devadath (1983) observed symptom development when the lower leaves were made to touch water artificially contaminated with Xco, indicating that contact of rice leaves with water is one of the prerequisites for disease initiation in the field. Large-scale epidemics of the disease occurred on the plains of Punjab and Haryana, India, during 1980 and 1981 despite the fact that the fields were irrigated through tubewells without any possibility of water stagnation or overflowing of water from field to field.

MULTIPLICATION

Xco multiplies primarily in the vascular system of the rice plant. The path of entry of the bacterium into the system has remained a debating point. The important entry points for infection are water pores (hydathodes) on the leaf blades, growth cracks caused by emerging roots at the base of the leaf sheath, and wounds caused by various means (Ou 1985). Fresh wounds are more conducive to infection than old. The minimum concentration of bacterial inoculum required to initiate infection through a wound is about 1×10^4 cells/ml (Mizukami 1961). The bacterium attains the logarithmic growth phase after 2 d and reaches the vascular tissues, through which it spreads inside the plant.

In the case of entry through water pore, the bacterium multiplies in the epithem into which the vessel opens. After sufficient multiplication, it invades the vascular system, out of which some may ooze from the water pores (Tabei and Muko 1960). The bacteria are also attracted by roots broken during uprooting, swarming into them (Mizukami 1957).

Multiplication and movement of Xco in resistant BJ1 and susceptible TN1 were demonstrated by Reddy and Kauffman (1973) through the pin-prick inoculation method. Multiplication of the pathogen started at the inoculation site 2 d after inoculation (DAI) and continued until 14 DAI in both varieties. However, the multiplication trends 1 cm away from the inoculation point were very different. Although multiplication was observed at 5 DAI in both varieties, the rate of increase of population was 100 times more in TN1 than in BJ1 by 10 DAI, and 10 times at 11-14 DAI. The bacterium moved rapidly from the inoculation site toward the leaf base in TN1, resulting in a lesion length of 13.0 cm, whereas it was only 1.6 cm in BJl at 14 DAI.

In Sri Lanka, Watanabe (1975) showed that the kresek phase of the disease is caused mainly by infection at the basal part of the stem or by root infection. Tabei (1977) observed that kresek infection came from the leaves, while Zaragoza and Mew (1979) demonstrated the importance of root injuries due to kresek development.

Hsieh (1979) showed that the BB organism multiplies and moves downward or upward from inoculated leaves or roots to the growing points of the plant by about 10 DAI. At 17 DAI, vascular bundles in the meristem region are filled with bacteria, and the plant begins to wilt.

Table 3. Populations of *Xanthomonas campestris* pv. *oryzae* in different parts of inoculated plants of cultivar Karuna.

Days after inoculation	Root	Crown	Leaf sheath portion				Leaves	
			1	2	3	4	2d from top	Top
0	-	-	-	-	-	-	-	-
3	-	1.0×10^2	-	-	-	-	-	-
6	-	1.5×10^3	4.0×10^2	-	-	-	-	-
9	2.0×10^3	1.5×10^3	1.6×10^4	5.0×10^3	-	-	-	-
12	1.5×10^3	7.5×10^3	8.5×10^4	6.5×10^4	3.5×10^3	2.5×10^2	-	-
15	2.0×10^3	8.0×10^4	3.2×10^5	1.4×10^5	9.0×10^4	4.0×10^4	2.0×10^4	8.0×10^3

[a]A dash (–) indicates not detectable.

Plants of a susceptible cultivar inoculated by root-dipping revealed that Xco initially colonized the roots and moved upward gradually into the plant. The bacterium could be detected in the roots at 3 DAI; it reached the crown at 6 DAI, and the incipient stem and leaf sheaths at 9 DAI. Vigorous bacterial multiplication was recorded at 12 DAI, followed by initial symptoms of wilting in the second leaf from the top at 15 DAI. The top leaf did not show any symptoms, although it harbored the pathogen(8.0×10^3cells/2-cm leaf sample) (Reddy and Shukla 1986). These results show clearly that the bacterium moves upward from the root tips to the top leaves and multiplies, causing wilting of the leaves (Table 3). According to Watanabe (1975), in certain incompatible host variety-bacterial isolate combinations, bacterial multiplication is restricted to a few vascular bundles at the basal part of the stem, whereas in kresek-inducing combinations, abundant bacterial populations have been detected throughout the plant system. Even in susceptible cultivar-virulent isolate combinations, all the inoculated plants may not die due to kresek. Hence, symptom production appears to depend on the amount of initial inoculum that can get into the root system and multiply in any plant. If the initial inoculum builds up quickly in a large population, it may cause wilting. In cases when a small number of bacterial cells gain entry into the root vascular system, they may take a long time to multiply, and in the process they might get transported to the terminal water pores and slowly multiply in the apical part of the leaf, causing lesions progressing downward along the veins.

However, in the field, a few plants at random develop the initial symptoms and transmit the disease to neighboring plants, forming a diseased patch. Each such disease patch was observed to originate from a single infected plant. This suggests that only a few seeds were able to harbor a sufficient inoculum, out of which some cells might get transmitted into the growing seedlings, finally leading to the expression of BB symptoms at tillering or booting.

CONCLUSIONS

The importance of the primary source of inoculum is more relevant in rice monocrop areas, whereas any one or all of the above sources may contribute

inoculum in double-cropped areas. This is because the intervening period between the 2 seasons is hardly 2 mo, during which time the inoculum might be in the active multiplication stage in living tissues such as stubble, ratoon plants, or self-sown plants. In Japan, infected seed, straw, stubble, and natural weed hosts may all contribute to the initial inoculum. However, in monocropped areas, all the infected tissues including stubble, straw, and other plant material perish during land preparation for the subsequent crop, particularly for wheat in northern and northwestern India. BB has appeared as an epidemic in such situations despite the absence of naturally infected wild rices. In such situations, the "jumping theory" of the inoculum from the wild rices to the cultivated rices may not form an important source of inoculum. The only plant part in which the inoculum may be in a protected condition is the seed, which is regularly used for raising subsequent crops. In China and India, transport of infected seed within the country has spread the disease. Hence, disease incidence could be reduced in the field by using seed collected from disease-free crops or by subjecting the seed to chemical or hot-water treatments. That will reduce or eliminate the number of focal centers of initial infection, thus minimizing the ultimate spread and severity of the disease.

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Bacterial blight: crop loss assessment and disease management

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Disease management strategies need to consider the impact of disease severity on crop yield. The available information on crop losses associated with bacterial blight (BB) of rice caused by *Xanthomonas campestris* pv. *oryzae* and its management is reviewed. Although substantial data have been generated during the past two decades on grain yield losses due to BB at experimental stations, very little information has become available about crop losses in tropical Asia on a regional or national basis. There are no standard methods for BB management in tropical Asia; the rice culture systems are so diverse that no single method is likely to apply to all. Therefore. the development of methodologies, technical capabilities, and workable systems to monitor crop losses is a research priority. Among the available technologies for BB management. the use of resistant varieties has been most successful. The roles of other methods in disease management are discussed.

Because agriculture faces increasing resource constraints, in actual production or in research, we need to justify and rationalize inputs. Crop loss information provides a means for evaluating the potential benefits of implementing any management decision. Crop loss assessment may also be viewed as problem definition, providing objective criteria for determining priorities. Crop loss data collected over many seasons enable the evaluation of changing agricultural technologies concerning disease control and their economic effects on crops (James 1974, Teng 1985).

Many techniques, concepts, and innovative ideas on crop loss assessment have been generated in recent years in developed countries. Some of them are relevant to the less developed, rice-growing, tropical Asian countries; few are graftable onto existing plant protection technologies for rice; and many are difficult for the rice researchers in tropical Asia to implement. This paper takes stock of the situation in crop loss assessment and disease management with particular reference to bacterial blight (BB) of rice.

CROP LOSS ASSESSMENT

Several production constraints to rice productivity exist in tropical Asia, of which BB of rice caused by *Xanthomonas campestris* pv. *oryzae* (Uyeda and lshiyama, Dowson) Dye has been considered by many rice scientists as significant (Mackill 1986, Mew et a1 1986). This disease occurs as vascular wilt at the early stages of crop growth (nursery stage to tillering) and as leaf blight at later stages (panicle initiation [PI] to flowering). It reduces grain yield to varying levels depending on the stage of the crop and the degree of cultivar susceptibility. Losses due to BB are thought to be higher in tropical Asia than in temperate regions (Mizukami and Wakimoto 1969, Ou 1985) because of the prevalence of more virulent populations of the pathogen (Buddenhagen and Reddy 1972, Wakimoto 1967).

Disease effects

The pathogen affects the stem (tiller), leaf sheath, leaf, and panicle of the rice plant. When kresek precedes leaf blight, the damage is extensive. All yield components—panicle number per unit area, maturity percentage, and 1,000-grain weight—are adversely affected (Ahmad and Singh 1975, Rao and Kauffman 1977, Reddy et al 1978). If the disease occurs from PI to flowering, grain development is impaired. Consequently, sterility increases, and maturity percentage and 1,000-grain weight are reduced (Mohiuddin et al 1977; Reddy et al 1978, 1979b; Tagami and Mizukami 1962). However, the postflowering infections commonly observed in marginal BB-prone areas have no measurable effect on grain yield or yield components (Reddy et al 1978, 1979b). In short, the earlier BB appears on the rice plant, the more the plant suffers.

Quantitative loss estimates

Although crop losses due to BB had been extensively studied and well documented in Japan (Mizukami and Wakimoto 1969, Tagami and Mizukami 1962), precise estimates of such crop losses were not available for tropical Asia as of the early 1970s (Ou 1972), and the situation has not changed much. However, a large volume of data has been generated on yield losses associated with BB in India and a few other Asian countries.

The estimated yield losses due to BB in tropical Asia vary from 2 to 74%, depending on location, season, weather, crop growth stage, and cultivar (Ahmad and Singh 1975; Exconde et al 1973; IRRI 1967, 1972; Jain 1968; Mohiuddin et al 1977; Muralidharan and Rao 1979; Rao and Kauffman 1977; Ray and Sengupta 1970; Singh and Ahmad 1975; Singh et al 1977; Srinivasan 1982; Srivastava and Kapoor 1982). Most of these estimates are based on experimental plot observations, and little information is available on crop losses on a regional or national basis. The few available reports on crop loss estimates in India (AICRIP 1984; ICAR 1964; Raina et al 1981; Reddy 1980; Srivastava 1967, 1972; Srivastava and Rao 1963, 1966) were collected by the Departments of Agriculture of various states or were educated estimates, and they cover not only BB but other constraints as well.

Nevertheless, the information has attracted the attention of policymakers and researchers, and has helped to promote rice pathology research in India.

Disease severity vs yield loss

Quantification of the functional relationship between disease severity and yield is a basic necessity for crop loss assessment. Many scales and formulae have been developed in Japan for measuring disease severity and for estimating losses (Mizukami and Wakimoto 1969, Tagami and Mizukami 1962), but they have limited applicability in tropical Asia. In recent years, attempts were made by the All-India Coordinated Rice Improvement Project, the International Rice Research Institute (IRRI), and others to generate basic information on the functional relationship between yield loss and BB. Some useful information has been generated on methodology, experimental designs, disease enumeration sampling techniques, and data processing (Reddy et al 1979b,c).

Disease progress curves of BB epidemics were generated by manipulating the initial inoculation date, using bactericides, and selecting rice cultivars with differing susceptibility to the pathogen. A significant linear relationship between grain yield and BB severity at the soft dough stage (SDS) of plant growth allowed the construction of a critical point model to predict BB-associated rice crop losses. Regression analysis of BB severity at SDS and grain yield produced the linear function $\overline{Y} = a - bx$ ($\overline{Y} = 100\text{-}0.49 \pm 0.021\ X$), where $\overline{Y}$ is the predicted rice yield, a is the expected rice yield in the absence of the disease, x is the percentage of diseased leaf area at SDS, and -b is the linear yield loss (%) associated with specific BB levels. The potential yield of a variety (without disease) in a given season determines the functional relationship between disease and yield loss (Reddy et al 1979b). This kind of single host-pathogen empirical model has limited relevance for existing rice management systems in tropical Asia, and, as suggested by Teng (1985), efforts should be made to develop synoptic models for rice crop loss assessment.

DISEASE MANAGEMENT

In national rice crop improvement programs and at IRRI, breeding for BB resistance is given top priority (Mackill 1986). During the past two decades, there has been a surge of publications on various aspects leading to disease management, and several researchers have reviewed the available information on disease management (Devadath 1985; Mizukami and Wakimoto 1969; Ou 1972, 1985; Padmanabhan 1974; Srivastava 1967, 1972; Tagami and Mizukami 1962).

BB disease management has centered around methods that reduce the initial inoculum and subsequent development of the pathogen on host plants. Since some of the earlier reviews (Mizukami and Wakimoto 1969, Srivastava 1972, Tagami and Mizukami 1962) give an excellent account of the former, I will dwell on the latter aspect in this discussion.

Rate-limiting BB management methods include chemical protection, host plant resistance, and N management.

Chemical control

Despite extensive efforts of researchers and extension agencies in tropical Asia directed toward chemical control of BB, no effective chemical can yet be recommended for general practical use, albeit valuable information has been revealed through these studies (Ou 1985, Reddy et a1 1985).

Host plant resistance

During the past two decades, extensive research at IRRI and in India, Bangladesh, Thailand, Indonesia, and elsewhere has focused on the identification of BB germplasm and the incorporation of resistance genes in otherwise productive genotypes.

In IRRI's breeding program, diverse gene sources have been used and resistant varieties developed (Khush 1977, Mew and Khush 1981). BB-resistant or -tolerant IR20, IR26, IR36, IR54, and IR64 are now grown extensively in Asia and have also served as parents in numerous crosses at IRRI and in national programs. Many IRRI breeding lines released in other countries for commercial cultivation—Pant Dhan 4 (IR262/ Remadja), Prasad (IR747B26-3/IR57948), Pant Dhan 6 (IR8608-298-3-1/IR10179-23), and PR109 (IR19660/IR2415//IR5883) in India; Chandina (IR532-1-1-76) and Mala (IR272-4-1) in Bangladesh; and TN73-2 (IR1561-228) in Vietnam—are also resistant to BB (Khush 1977, Reddy and Reddy 1987, Reddy et a1 1986).

In Bangladesh several BB-resistant improved varieties like BR4, IR20, Mala, BR6, BR8, and BR14 are cultivated commercially (Miah et al 1986, Reddy et al 1986). In other countries as well, several IRRI breeding lines have been used to incorporate resistance and for commercial cultivation (Khush 1977, Mackill 1986)

In India, diverse gene sources for BB resistance were used (Kauffman and Rac 1972; Reddy et a1 1980, 1986) and several varieties resistant to BB were released (Reddy et a1 1980, 1986). Of the 286 varieties released in India (up to 1987), 30 possess moderate resistance or resistance to BB (Table 1). However, only a few are widely grown (ca. ≥P50,000 'ha): IR20, IR36, IR64, IET2815, Saket 4, Prasad, Swarna, Mahsuri and its derivatives, Biraj, Radha, Sujata, and a few others.

Field resistance and disease management

A high degree of tissue resistance to BB is desired and has been achieved through systematic breeding programs in tropical Asia. Artificial inoculation methods such as the clip inoculation technique (Kauffman et al 1973) used in the breeding programs have precluded the possibility of exploiting other forms of resistance. This and similar techniques do not measure how a rice variety would react in the field to the step-by-step epidemiological requirements of a pathogen population (Budden-hagen and Reddy 1972, Ou 1985). Some of the popular commercial cultivars in India, such as IR20, Saket 4, and Swarna—rated as moderately susceptible to susceptible in disease nurseries (Reddy et a1 1980, 1985; also Table 2)—do not suffer much when planted in large areas. Disease progress in this kind of variety is comparatively slower than in susceptible varieties, resulting in lower terminal disease severities and yield losses (Table 3). In preventing disease outbreaks, field-resistant varieties seem to be highly beneficial in BB-prone irrigated areas (Table 4).

Table 1. Bacterial blight-resistant varieties released in India (Reddy and Reddy 1987; Reddy et al 1980, 1985, 1986).

Releasing body	BB-resistant varieties released	Cross	Year released
Central Variety	IR20	IR262/TKM6	1970
Release Committee	Sasyasree	TKM6/IR8	1979
	IR36	IR1561//IR244/*O. nivara*///CR94-13	1981
Andhra Pradesh	Sona Mahsuri	Sona/Mahsuri	1982
	Vijaya Mahsuri	Mahsuri/Vijaya	1982
	Samba Mahsuri	GE824/TN1//Mahsuri	1986
	Swarna	Vasista/Mahsuri	1985
Bihar	Janaki	Sel. from Chenab of North Bihar	1978
	Radha	IR20/IR5-114-3	1984
	Sujata	Peta/TN1//Remadja	1984
Karnataka	Sona Mahsuri	Sona/Mahsuri	1986
Madhya Pradesh	Asha	IR22/W1283	1980
	Deepti	IR22/NP130	1980
	Usha	IR22/W1263	1980
Maharashtra	Ratnagiri 68-1-1	IR8/Sigadis	1976
Orissa	Rama Krishna	TKM6/IR8	1980
	Udaya	CR129-118/CR57-49-2	1985
Pondicherry	Bharathidasan	IR3403-267/Ptb 33//IR36	1984
Punjab	PR4141	IR8/BJ1//IR22	1982
	PR109	IR19660/IR2415//IR5883	1986
Tamil Nadu	CO 43	Dasal/IR20	1982
	CO 44	ASD5/IR20	1982
	IR50	IR2153-14-18-8-2/IR28//2070	1982
Uttar Pradesh	Saket 4	TKM6/IR8	1971
	Prasad	IR747826-3/IR57948	1978
	Govind	IR20/IR24	1982
	Pant Dhan 4	IR262/Remadja	1983
	Pant Dhan 6	IR8608-298-3-1/IR10179-23	1986
	Biraj	OC1393 mutant	1982
West Bengal	Suresh	IR262/Kheo Nahng Muey 11	1982

Table 2. Reaction of resistant, moderately resistant, and susceptible rice varieties when clip-inoculated and under natural disease development. All-India Coordinated Rice Improvement Project, 1978 WS.

Variety or line	Disease score (0-9 scale)[a]		Disease severity at the soft dough stage (%)
	Clip-inoculated	Natural	
IET1444	8	9	75
TN1	8	9	80
Java	7	6	53
IR8	8	6	50
Pankaj	7	8	48
IR20	6	4	18
IR26	5	3	9
IET2815	6	5	25
IET1798	6	5	19
IET4140	4	2	7
IET4141	3	2	4

[a] By *Standard evaluation system for rice* (IRRI 1980).

Table 3. Effect of BB on yield of susceptible, moderately resistant, and resistant rice varieties (Reddy et al 1979b. 1980).[a]

Treatment[b]	IET2895		IR20		IET4141	
	Disease severity	Yield (t/ha)	Disease severity	Yield (t/ha)	Disease severity	Yield (t/ha)
Inoculation at 60 DAS	66 a	3.9 c	12 a	5.0 a	5a	5.3 a
Inoculation at 70 DAS	38 b	4.3 b	7 a	5.2 a	3a	5.4 a
Inoculation at 70 DAS + 1 spray of TF-130**	30 c	4.8 a	2 a	5.3 a	2a	5.5 a
No inoculation + 5 sprays of TF-130	10 d	5.2 a	1a	5.3 a	0 a	5.5 a

[a] AV Of 4 replications; values followed by a common letter do not significantly differ at the 5% level by analysis of variance. [b]DAS = days after sowing. TF-130 = Celdion S 10% wettable powder at 1 g/liter.

Table 4. Comparison of BB incidence in East and West Godavary Districts of coastal Andhra Pradesh (1976, 1977, 1985, 1987 WS).

Year	Cultivars	Fields or villages surveyed (no.)	Fields (no.) with			
			Bacterial blight at severity of		Sheath blight at severity of	
			I-10%	>11%	1-10%	>11%
1976[a]	RP6-17, RP193-1, Jagannath, Mahsuri	52	5	47	10	0
1977[a]	RP6-17, RP193-1, Java, MTU8002, MTU8089	45	18	27	13	0
1985[b]	Swarna, Vajram (MTU5249), MTU2067	48	5	1[c]	0	31
1987[b]	Swarna, Vajram, MTU2067, MTU2077	49	2	3[c]	3	25

[a] Source: Reddy et al 1979c. Sample size 0.75 ha. Covered area about 200,000 ha. [b]Source: Directorate Of Rice Research (1985, 1987). Surveyed area about 300,000 ha. [c]Recorded exclusively on MTU2067, a BPH-resistant, BB-susceptible commercial cultivar.

Currently, little is known about field resistance and its mode of inheritance. Existing screening techniques are inadequate to identify and exploit field resistance.

In contrast to field resistance, some cultivars and lines can endure the disease and still give satisfactory returns to the farmers, despite having no inherent resistance. The agronomic performance of one such selection, IET2937, in the BB-endemic region of Andhra Pradesh is shown in Table 5. It showed a significantly different quantitative response to infection. This kind of field tolerance is well documented in other crop-pathogen systems (Caldwell et al 1958, Hooker 1967, Simons 1966). However, in disease-favorable years, the so-called field-tolerant varieties cause

Table 5. Yield of selected medium-duration entries under BB pressure in Maruteru, Nitrogen Variety Trial II, 1974 WS (Reddy et al 1980).[a]

Selection	Disease severity	Yield (t/ha)
IR26	6.0 a	5.1 a
IET2815	33.5 b	3.7 b
IET2254	50.0 c	1.3 d
IET2295	52.0 c	2.2 c
IET2937	60.0 cd	3.9 b
IET2895	68.3 d	1.8 cd

[a]Av of 4 replications. Values followed by the same letter are not significantly different at the 5% level.

havoc for farmers when planted over large areas. Unfortunately, many such selections (because of the law of averages) are used by farmers in tropical Asia.

Crop management

Increased productivity brought about by modern production practices (modern varieties, high levels of applied N, etc.) tends to create ideal conditions for the development of diseases and insects. The problem is particularly acute in monoculture areas where two or more rice crops are planted in succession (Barker et al 1985). Among the factors favoring BB development, application of high N levels is important in increased disease incidence and severity (Mizukami and Wakimoto 1969, Ray et al 1967, Tagami and Mizukami 1962, ten Have and Kauffman 1972). High N levels either favor pathogen multiplication and lesion enlargement (Kim and Cho 1970) or, through promoting increased vegetative growth of the plant, influence the microclimate in favor of the pathogen (Ou 1972).

Reddy et al (1979a) attempted to quantify the relationship between yield reduction and increased BB severity associated with high N application to rice: The relationships between yield and N level, and between BB severity and N level, were best described by quadratic and linear functions, respectively. Increased N levels increased BB and reduced yield. High disease severities negated N response, and beyond a critical N level the law of diminishing returns operated in susceptible cultivars. The optimum level of N application to derive maximum yield (with minimum disease effects) was 76 kg/ha for susceptible cultivars grown in wet season rice. To cut costs and increase productivity in BB-prone areas, a positive prognosis was suggested involving omission of topdressing of rice with N when BB is severe at PI to minimize disease effects and optimize N response.

To manage BB, various researchers have suggested integrated control measures (Devadath 1985, Padmanabhan 1983, Srivastava 1972), including using resistant cultivars, removing inoculum sources, using healthy seed, avoiding flooding in nurseries and main fields, avoiding excess N, and applying N in splits.

Recommendations of this type and man; others that involve nonmonetary crop management inputs to control BB are not readily accepted in rice-farming communities, because the farmers do not see immediate benefits. Psychologically,

farmers expect from scientists and extension specialists ready-made solutions in the form of agrochemicals to combat the disease when they see it.

CONCLUSIONS

Disease management strategies need to consider the impact of disease severity on crop yield. To develop rational and economical control measures, the extent of crop losses must be evaluated and related to the potential gain obtained from control practices. Therefore, development of methodologies, technical capabilities, and workable systems to monitor crop losses from year to year is a research priority deserving support from policymakers, administrators, and extension experts.

There are no standard methods for BB management in tropical Asia. The rice culture systems are so diverse that no single method is likely to apply to all. In many rice pathology, programs, little time is devoted to research on disease management, and more knowledge needs to be generated on different aspects of disease management suited to diverse rice ecosystems.

The technology available today to farmers for managing BB is limited to disease-resistant varieties. Other feasible technologies deserve more attention. Furthermore, strategies and methods of disease management used at experimental stations need to be validated in farmers' fields before recommendations are made for large-scale adoption.

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Chemical control of bacterial blight of rice

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Bacterial blight (BB) is one of the most devastating diseases of rice. A reliable forecasting system is necessary to determine the proper time to apply chemicals for effective control. Forecasting methods currently used are discussed, as are the effects of chemicals on eradicating seedborne inoculum and on field control of BB. Effective chemical control that can be used in different rice ecosystems is still not available against this intractable disease.

There is a pressing need to double or even triple rice production in Asia to keep pace with the increasing population. To do so, we must concentrate our efforts on increasing yields and eliminating the yield losses inflicted year after year by pests.

Intensive cultivation of high-yielding susceptible cultivars with reduced genetic variability, higher plant population per unit area, high doses of fertilizer. and staggered sowing and planting have intensified the severity of bacterial blight (BB) of rice in most Asian countries. BB is widely distributed and is more devastating than any other bacterial disease of rice, because we lack truly effective control measures and full knowledge of the biology and ecology of the pathogen. An integrated approach involving cultural, biological, genetic, and chemical control measures is necessary. In this paper, we discuss the use of chemicals in controlling BB of rice.

DISEASE FORECASTING

Forecasting is essential to determine the proper time to apply chemicals for effective control. Several methods for forecasting BB have been reported, but the validity of some is questionable.

Forecasting from natural infection

Fairly severe infection results when infected seedlings are transplanted. Therefore, inspecting the seedlings toward the end of the nursery stage at the entrance, middle, and near the outlet of the irrigation water in the nursery is suggested to detect primary infection and to assess subsequent disease development. Resistant and susceptible rice cultivars can be grown under heavy N fertilization in a forecasting

field, and the incidence and severity of BB assessed periodically and extrapolated to surrounding areas based on knowledge of the distribution of cultivars (Tagami and Mizukami 1962). Test cultivars should have different degrees of resistance and differing maturity durations (Aoyagi et al 1960).

In another method, rice leaves are periodically wounded by needles, and disease development of the wounded leaves is estimated and used to forecast the disease.

The occurrence of the disease on collateral hosts like *Leersia sayanuka* and susceptible wild rices can also be examined to forecast occurrence on rice, since the collateral hosts show the disease earlier than transplanted rice (Devadath et al 1974, Tagami and Mizukami 1962, Thri Murty et al 1982).

Forecasting from climatic conditions

Floods, rainfall, number of rainy days, sunshine, temperature, and typhoons influence disease occurrence and severity. The correlation between BB incidence and climatic conditions has been worked out in Japan (Tagami and Mizukami 1962) (Table 1).

In India, with more than 27 rainy days during August, September, and October, and with a total rainfall of at least 20 mm, kresek occurs, while leaf blight occurs if the number of rainy days and the amount of rainfall are lower (Mohiuddin et al 1977). Mean minimum and mean maximum temperatures of 24.3 and 34.0 °C, respectively, favor disease spread, while temperatures of 18.2 and 37.2 °C, respectively, adversely affect the spread (Premalatha Dath et al 1978). More kresek develops at a high temperature range of 28-34 °C than at low temperatures, and no kresek occurs below 16 °C (Hsieh and Chang 1977, Ou et al 1971). In general, with an increase in temperature, irrespective of the susceptibility of the rice cultivar and of the bacterial isolate used for inoculation, lesion development increases significantly (Horino et al 1982, Premalatha Dath et al 1979), which partly explains why BB is more severe in tropical than in temperate countries. Of course, other factors such as inoculum availability, level of N applied, planting density, and crop growth stage also play a role in determining the severity of disease.

Table 1. Correlation between bacterial blight incidence and climatic conditions (Saga Agric. Exp. Stn. 1960, cited by Tagami and Mizukami 1962).

x-value	Forecasting equation	Correlation
Rainfall, end of July	y=1.507+0.043x	0.902
Rainy days, end of July	y=0.214+0.793x	0.605
Rainfall, middle of August	y=2.035+0.0097x	0.915
Rainy days, middle of August	y=1.809+0.596x	0.794
Rainfall, beginning and middle of August	y=1.621+0.009x	0.829
Rainy days, beginning and middle of August	y=0.610x-1.427	0.752
Rainy days, end of August	y=0.689x-0.309	0.842
Rainfall, middle and end of August	y=1.259x-0.0098x	0.924
Rainy days, middle and end of August	y=0.455x-0.519	0.958
Rainfall, August	y=0.489+0.011x	0.961
Rainy days, August	y=0.481x-2.963	0.976

Forecasting from bacterial population

In endemic areas of Japan, small amounts of *Xanthomonas campestris* pv. *oryzae* (Xco) are found on weeds in early spring, but Xco is abundant on *Leersia* spp., and in June many weeds are infested. In field water, the pathogen population increases as the population on rice plants increases, Thus, by estimating the bacterial population, it is possible to forecast the disease. Ten to 20 leaves from the top, middle, and base of resistant, moderately resistant, and susceptible rice cultivars are collected twice from tillering to the young ear formation stage and washed in water. The resulting bacterial suspension is concentrated by centrifugation and inoculated into a susceptible cultivar. The bacterial population is estimated from disease development (Mizukarni 1961). But disease development is influenced by a number of factors, not by the inoculum potential alone. Saprophytes present in the inoculum can also play an important role in disease development, depending on their number (Hsieh and Buddenhagen 1974).

Forecasting from bacteriophage population

The phage population increases much in advance of disease occurrence. Hence, this has been utilized to forecast BB occurrence in Japan (Tagami et al 1958), Korea (Lee 1975), Thailand (Tabei and Eamchit 1974), and Sri Lanka (Watanabe 1975).

Water from nurseries or ricefields from many sites is pooled. Serial dilutions are prepared, and 1 ml of each dilution is shaken separately with 2 ml of Xco having broad phage susceptibility. This is added to 5-6 ml of potato-semisynthetic agar and poured onto a petri plate. The number of plaques is counted after 15 h of incubation at 20-25 °C (Tagami and Mizukami 1962, Wakimoto 1967).

In nurseries, the number of phages detected is generally less than 30/ ml. When the phage population exceeds 100/ml, seedling infection begins. If many more phages are detected at a later stage of the nursery, a severe disease outbreak is forecast at early tillering.

Immediately after transplanting, the phage population in the fields is low. But if more than 200 phages/ ml are observed, diseased plants must exist in the field. At the time of transplanting, if 50 phages/ml are detected in the irrigation canals, the first disease occurrence is expected on *L. oryzoides*. If more than 100 phages: ml are detected, the first disease outbreak on rice is expected within 10-14 d. At midtillering, if the phage population is <50/ml in the ricefield water, the disease is forecast to be slight; if >l00/ ml, moderate; and if >1,000/ ml, severe. If the phages detected in the irrigation canals number >100/ml, a disease outbreak is expected shortly. The disease already prevails if the phage population is >1,000/ ml. At maximum tillering, if the phage population in the ricefield water is <l00/ ml, the disease occurrence is going to be slight; if >500/ ml, moderate; and if >5,000/ ml, severe. When the phage population is regularly >1,000/ml, the disease is widely established in the area. When 1,000-2,000 phages/ml are detected frequently in a wider area, many secondary infection sources already exist and a high possibility of disease is forecast (Mizukami 1966). Kresek incidence is expected after transplanting if the phage population in the field water of nurseries exceeds 500/ml (Watanabe 1975).

The phage population is influenced by irrigation water depth, climate, rain, humidity, and the time of collecting water samples for phage estimation (Thri Murty and Devadath 1982, Watanabe 1975, Yoshimura et al 1960). The phage population is larger at the outlet of the irrigation channel than at the entrance, and hence the water sample has to be collected at the outlet (Tagami and Mizukami 1962). The estimate of the phage population from a given nursery or field will forecast the occurrence of the disease only in that particular nursery or field. To forecast disease occurrence in large areas, phage populations in many fields and irrigation canals need to be studied (Tagami et al 1961, Yoshimura et al 1960).

Although there is some relationship between phage population and disease development at early crop growth stages in India, it becomes indistinct as the crop develops. Moreover, no particular relationship is apparent between phage population and disease development when a single cultivar is grown at different locations (Thri Murty and Devadath 1982).

Although phage counts provide valuable information for disease forecasting, the theoretical grounds for the use of phage have not been satisfactorily explained (Buddenhagen 1969). Some questions one may ask in relation to forecasting are

- What are the minimum levels of Xco and phage required for interaction in field water?
- How frequently do phage-resistant mutants arise in nature under different rice ecosystems and cropping patterns?
- What role do resistant mutants play in epidemiology?
- As some Xco phages are able to lyse Xco and other xanthomonads infecting some rotation crops and vice versa, how reliably can we use phages under different rice ecosystems and under different rotation crop cycle systems in Asian countries?

Therefore, we need to learn more about the interactions of Xco, rice, rotation crops, phage, *Bdellovibrio,* saprophytes, and the environment to develop a fully dependable forecasting method (Devadath 1985).

CONTROL WITH CHEMICALS

Seed treatment

Immersing the seed in hot water at 57 °C for 10 min or disinfecting with Hg compounds was suggested in earlier days to eradicate seedborne inoculum (Tagami and Mizukami 1962). Other methods reported to be effective in eradicating seedborne inoculum are

- steeping seed for 12 h in a mixed solution of 0.05% wettable ethylmercury chloride and 0.02% Agrimycin 100, followed by hot-water treatment at 52-54 °C for 30 min (Srivastava and Rao 1964);
- soaking the seed for 8 h in 0.1% wettable ethylmercury chloride and 0.6 g streptocycline in 20 liters of water (Jain et al 1966);
- soaking infected seed in 100 ppm of streptacycline solution overnight (Devadath and Padmanabhan 1970);
- steeping the seed in water for 12 h followed by hot-water treatment (Sinha and Nene 1967);

- soaking the seed for 16-24 h in decinormal nitric acid followed by washing in tap water and finally drying well under the sun (Subramoney and Abraham 1969);
- steeping the seed in triphenyl tin chloride and 2-hydroxy propyl-methane-thiosulphonate (Singh and Rao 1982); and
- dressing with duter and captan dissolved in dichloromethane and copper oxychloride, oxytetracycline, and benomyl dissolved in acetone (Singh and Monga 1985).

Sundrying the seed for 5 d, and hot-water treatment alone cannot eliminate the pathogen (Natural 1975). Seed treatment (Srivastava and Rao 1964) and fumigation of the seedbed by drenching with 1 liter of commercial formalin in 50 liters of water 1 mo before sowing is suggested to provide a pathogen-free environment for at least 20-25% of the total growth period (Srivastava 1966). Seed treatment with still higher concentrations (300-500 ppm) of streptocycline cannot prevent secondary invasion of the pathogen even within 5 d of seed treatment (Devadath 1973).

Chemical application

Attempts to control BB through chemicals began in Japan over a decade before its bacterial nature was established. For about four decades, Bordeaux mixture with or without sugar, Bordeaux mixture and copper soap mixture, and Cu compounds were tried without much success; furthermore, these chemicals are phytotoxic. After World War II, Cu-Hg fungicides were used. Although these were less phytotoxic than Bordeaux mixture, they were less effective in controlling the disease (Tagami and Mizukami 1962). Hg fungicides considerably inhibited lesion development when sprayed, but with the lapse of time after infection, their inhibitory effect was reduced (Mizukami and Seki 1954). Ethyl derivatives or ethyl radical-containing Hg fungicides were more effective than phenyl derivatives (Inoue and Tsuda 1957).

Spraying copper oxychloride (Sulaiman and Ahmed 1965) or rabbing followed by copper oxychloride (Jain et al 1965) was reported to control the disease. Because streptomycin was more effective than Cu-Hg fungicides, spraying streptomycin solution frequently at short intervals was recommended (Seki and Mizukami 1956). But high concentrations of streptomycin induced etiolation (Devadath and Padmanabhan 1970), thereby reducing yield (Yoshimura et al 1961). Penicillin was found effective in reducing disease intensity (Mary and Mathew 1983). Chloramphenicol was as effective as streptomycin, and less phytotoxic; PCP-salts, 1-naphthalene acetic acid, potassium permanganate, and some respiratory inhibitors inhibited lesion development (Tagami and Mizukami 1962).

Spraying the nursery with Bordeaux mixture, Cu-Hg fungicides, or streptomycin effectively reduces the disease after transplanting. Streptomycin-containing antibiotics must be sprayed at least 3-4 times at 4-5 d intervals to minimize subsequent disease severity, since the persistence of these antibiotics is no more than 5 d in rice plants (Devadath 1973, Reddy and Reddy 1971, Tagami and Mizukami 1962). Achromycin, cellocidin, streptomycin, and streptocycline are readily translocated upward in rice seedlings. Chloromycetin at 400 ppm and streptocycline at 500 ppm totally arrested lesion development when they were root-fed (Devadath 1973). Streptomycin translocates more readily upward than downward (Reddy and

Reddy 1971). Spraying chemicals or antibiotics at the nursery stage is helpful when the nurseries are severely infected or when the secondary spread of the pathogen in the field is mild (Tagami and Mizukami 1962).

Dipping seedling roots or whole seedlings in antibiotics or Hg compounds was also somewhat effective (Tagami and Mizukami 1962). Submerging infected or contaminated seedlings for 24 h in 500 ppm of Agrimycin 100, Dicrystin-s, or streptocycline before transplanting was recommended by Durgapal(1983). Dipping seedlings in Cu (IRRI 1979, Schure 1953) and Zn (IRRI 1979) compounds effectively controls kresek.

The spread of secondary infection in a tolerant cultivar was arrested to a large extent by seven sprays of streptocycline, while it was somewhat checked in a highly susceptible cultivar. More frequent sprays during the initial infection period may be more effective in checking the secondary spread of the disease in a highly susceptible cultivar (Jain et al 1966).

Chlorinating field water decreased the disease as effectively as streptocycline and Cu. Seed treatment followed by streptocycline, Cu spray, and one or two applications of chlorine—cutting the number of antibiotic sprays to the minimum—has been suggested (Padmanabhan and Jain 1966). Chlorinating irrigation water with stable bleaching powder was also reported to be effective in minimizing the disease (Chand et al 1979). Adding stable bleaching powder reduced the survival and population of the pathogen in soil (Sivaswamy and Mahadevan 1986). But Palaniswami and Ahmed (1979) found that bleaching powder did not effectively control the disease, and high concentrations of 30-50 ppm were phytotoxic and reduced yield.

Phenazine or its 5-oxide at 150-200 ppm effectively controlled the disease (Devadath and Premalatha Dath 1970, Lee 1975, Oda et a1 1966, Sekizawa et al 1965), but phenazine sprayed after the entry of the pathogen did not give satisfactory control (Devadath and Prerhalatha Dath 1970), and it cannot translocate in rice plants (Devadath 1973). F-48 gave better control at 750 ppm than phenazine at 500 ppm (Lu et a1 1983), and the best time for applying F-48 was at heading to flowering. Cellocidin, chloramphenicol, and phenylmercury acetate were found effective in reducing the disease. Synthetic organic bactericides such as nickel dimethyl dithiocarbamate, dithianone, phenazine, and phenazine N-oxide were also recommended (Fukunaga 1966). Spraying Agrimycin 100 (Mary and Mathew 1983, Singh et al 1980), Agrimycin 500 (Krishnappa and Singh 1977), sankel and new sankel (Mukherjee et a1 1976), and streptocycline along with Cu-containing compounds (Chauhan and Vaishnav 1980, Jain et al 1966) was reported to be effective, but the efficacy of some of these chemicals has not been confirmed (Devadath and Premalatha Dath 1970). Ammonium sulfate (Hoa et a1 1984) and cowdung extract (Mary et a1 1986) sprays were also reported to be effective; cowdung extract was as good as penicillin, paushamycin, and streptomycin.

Several dyes seemed to control the disease in the greenhouse (Ishii et al 1966). A very low dosage of root-fed 1-methyl thiosemicarbazide showed preventive as well as curative effects, but it was neither effective when sprayed nor inhibitive in vitro (Ohmori et a1 1976a). When applied to irrigation water, the compound is converted

into three substances including 2-amino-l,3,4-thadiazole, an antibacterial substance that translocates readily in rice plants (Ohmori et al 1976b). A substance extracted from *Streptomyces zeomyceticus* str. SF-1836 was also effective (Iwata et al 1979).

Spraying techlofthalam was more useful than soil application, and it translocated readily and inhibited bacterial multiplication in rice plants (Nakagami et al 1980a, b; Takahi 1985). Formycin, produced by a strain of *Nocardia* spp., showed curative effects (Chen et al 1980). Although probenazole (Oryzemate) was not inhibitory to the pathogen, it induced resistance in rice plants through host mediation and was easily translocated in rice plants when applied to soil (Ohashi 1980, Sekizawa and Mase 1980). Nursery spraying once or twice with phenazine, ambam, or *Oittea camellia (Camellia oleosa)* cake and 2-3 sprays in the field were recommended. Use of Xco-contaminated field water for chemical spraying is discouraged (Lin and Yu 1981).

Extracts of *Artabotrys uncinatus* and *Allium sativum* (Grainge et al 1985) and a number of organic and inorganic chemicals at very low concentrations were inhibitory to the pathogen (Gossele et al 1984) and worth further evaluating.

Because the invasion of the pathogen occurs almost throughout the rice-growing season, bactericides must be applied at the most appropriate time. Chemical sprays at the later nursery stage, at the initiation of the disease at maximum tillering, and during the early stages of secondary spread of the disease at booting were suggested (Yoshimura and Tagami 1967). Spraying chemicals at high concentration at the critical time, just before the manifestation of the disease, is more successful than spraying frequently with dilute formulations (Tagami and Mizukami 1962).

Variation in the sensitivity to antibiotics of the causal bacterial isolates, and the existence or development of drug-resistant strains in nature pose serious problems in formulating foolproof chemical control (Cho and Shim 1977, Devadath 1971, Shekhawat and Srivastava 1968, Wakimoto and Muko 1963, Yamamoto and Jusaka 1965). Applying chemicals for BB control in deepwater and floating rice has handling limitations. Effective and economical chemical control has yet to be developed against this disease.

The best chemical would be

- effective at very low concentrations either in killing or inhibiting the multiplication of the pathogen by blocking some of the pathways essential for multiplication;
- able to translocate readily both downward and upward, and stable in rice plants for a considerable time; and
- selective and environmentally acceptable; it must be minimally harmful to fish, snails, eels, crabs, etc., because ricefields are also used for producing these in some countries.

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Methods for epidemiological research on bacterial blight of rice

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Modern epidemiology relies heavily on quantifying biological phenomena and their relationships with the environment and other factors. Most research to date on rice bacterial blight (BB) caused by *Xanthomonas campestris* pv. *oryzae* has been descriptive and ecological. Quantitative analyses of BB epidemics may be done using a "bottom-up" approach through studies on components of infection cycles, or by using a "top-down" approach with analysis of disease progress curves (DPCs). The components of the BB infection cycle are primary inoculum source, colonization on host surface, entry into host, symptom development, dissemination, survival in air, deposition, and survival on plant surface, weeds, and nonliving media. BB pathogen populations are measured either as number of cells per unit area or volume, or as number of groups of cells that function collectively to cause infection. Bacterial populations may be characterized by standard bacteriological tests, phage and serological reactivity patterns, fatty acid composition, and isoenzyme analysis. The effects of specific environmental variables on components of the infection cycle are quantified into functional relationships, which collectively constitute a system model and which may be used to explain epidemics in different environments. Many mathematical models are available for quantifying the DPCs of BB. However, fundamental knowledge of how to assess blight symptoms and of the spatial distribution of the pathogen and diseased rice plants is required for accurately interpreting DPCs. Much of this knowledge does not currently exist. Neither does knowledge of the quantitative effects of BB on rice yield. In this paper, we discuss these and other knowledge gaps, and make suggestions for filling them.

Modern epidemiology is a quantitative science (Zadoks 1972) in which pathosystem analysis is used to explain and predict biological phenomena. It is the study of the dynamic interactions between pathogen and host that result in disease, as influenced by man and the environment (Kranz 1974). To fully understand the epidemiology of a specific disease, therefore, requires information on the components of the pathosystem (pathogen and host), their resultant interaction (the disease), and how the physical and human environments affect them. The time and space scales and the

level of biological organization are important considerations in epidemiology. Epidemiology research may, for example, be concerned only with what happens in a single field during one cropping season. Or, it may deal with epidemics occurring over a large area and over several seasons. Disease can develop on single plants within a crop population; not all plants within the same population need be infected, and even if they are, they need not have the same disease severity. There is thus much spatial, temporal, and biological heterogeneity in the epidemics of any pathosystem.

Although bacterial blight (BB) caused by *Xanthomonas campestris* pv. *oryzae* (Xco) is a potentially yield-limiting disease of rice (Mew 1987), surprisingly little is known of its epidemiology. The quantitative relationships among environmental and host factors, and the population dynamics of the pathogen are poorly understood. Ou (1985), for example, showed that most research on epidemiology has concerned the descriptive, ecological aspects of the disease cycle. Based on experience with other pathosystems, we believe it is essential that quantitative knowledge be accumulated to improve our understanding of the epidemiology of BB. Without this quantitative knowledge, it is difficult, if not impossible, to develop sound disease management tactics.

Many workers have used the disease cycle, commonly called the infection cycle, as a starting point to understand epidemiology (Teng and Bowen 1985). With polycyclic, "compound interest" type diseases, the field epidemic is an integration of many overlapping infection cycles. The dynamics of a field epidemic, studied by assessing the disease in a field through repeated visits, is commonly represented in the form of a disease progress curve (DPC). Quantitative studies on the DPC done by Van der Plank (1963) and others have resulted in many mathematical models. Other workers have studied the component processes of the infection cycle, i.e., the "building blocks" of epidemics such as infection, germination, latent period, sporulation, and dissemination. Some workers have then attempted to reconcile the "top-down" (starting with the DPC) and the "bottom-up"(starting with component processes) approaches to epidemiology through the use of systems models (Teng and Bowen 1985). Because the micro- and macroenvironments are important influences on pathogens and disease, many epidemiologists have utilized environmental monitoring equipment of varying sophistication. As has happened with fungal diseases, the effect of bacterial epidemics on crop yield is receiving increased attention.

In this paper we will take a systems analysis view of BB to suggest questions that still need to be addressed, and then recommend methods to answer those questions by studying the BB infection cycle, the DPC, the effect of BB on yield losses, and BB management using epidemiological knowledge.

METHODS FOR STUDYING THE RICE BACTERIAL BLIGHT INFECTION CYCLE

The BB infection cycle serves as a convenient level of biological organization on which to base either more reductionistic or more expansionistic studies. A conceptual model of the infection cycle is first needed, and this is done using a systems analysis approach. Methods are then needed to provide information on

various aspects of the infection cycle. However, to fully understand field epidemiology requires that all available knowledge be synthesized into a model, and that the synthetic form with its underlying hypotheses be tested under natural conditions.

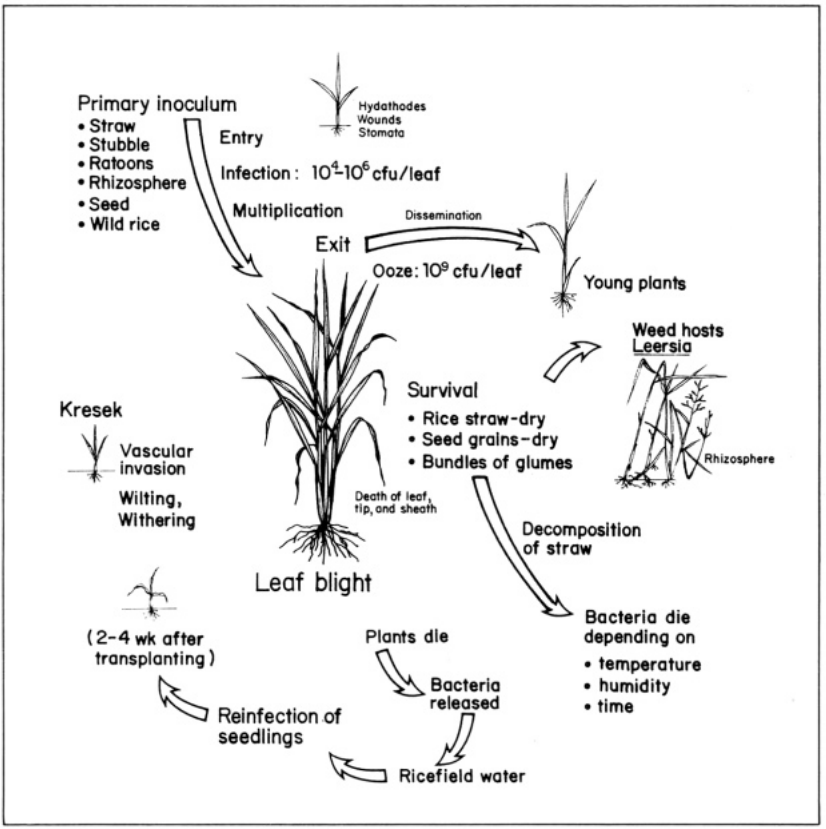
Pathosystems analysis

Systems analysis is a useful technique for analyzing the components of infection cycles, for identifying the main factors that drive these components, and for revealing weaknesses in present knowledge of any relationships, thereby defining areas that require further research (Zadoks 1971). Systems analysis commonly starts with specifying the problem and objectives of the analysis, followed by activities to understand the system better through the literature and through interviews, and then proceeds to the formulation of a conceptual model of the system showing its main components (Teng and Zadoks 1980). With fungal pathogens, on which much epidemiological theory has been developed, the major components are infection, latency, sporulation, and dissemination. There are obvious differences among bacterial pathogens. For BB (Fig. 1), we consider the components and their major influencing variables to-be

- *Primary inoculum source.* The primary inoculum source may be seed. wild rice, weeds, infected straw, stubble, or ratoons. Influencing variables are relative humidity, temperature, sunlight, soil moisture, and microbial interactions.
- *Colonization on plant surface.* Bacteria may exist as an epiphytic population. Influencing variables are leaf wetness, leachates, nutrients, and microbial competition.
- *Entry into host.* Entry is achieved principally via wounds and hydathodes (water pores). Ingress is influenced by time on plant surface, cut surfaces, plant age, exudates from hydathodes (Mew et al 1984), pathogen virulence, and host resistance factors.
- *Multiplication within host.* Theoretically, a single bacterial cell may multiply to reach a population of several million within 3-4 d. Parry and Callow (1986) found that an initial inoculum of 10^5 cells/leaf increased to 10^7 cells/leaf during the first 70 h and then increased gradually to 10^8 cells/leaf. Nevertheless, little is known about the influencing variables. Whether or not the pathogen multiplies in the host depends on the cellular environment. With other bacterial pathogen-host interactions, the bacteria may elicit a host defense reaction that prevents further multiplication. If the host fails to respond with an effective defense mechanism in time, the pathogen multiplies unchecked, and symptoms occur. With BB on rice, differences in the rates of host colonization have been observed for both compatible and incompatible host-pathogen combinations (Leach et al 1989, this volume), but such differences are not universal (Parry and Callow 1986).
- *Symptom development.* When the bacteria reach a sufficiently large population to disrupt the normal physiology of the rice plant, symptoms occur. The number of bacteria within the tissue required to produce symptoms

is not known, although a minimum population of 10^4-10^6 colony-forming units (cfu)/ml is needed in the inoculum for eventual symptom expression (Mew 1987). Increased temperatures up to 35 °C were associated with longer lesions that developed as a result of needle prick inoculation (Horino et al 1982). Increased N fertilizer applications favored disease development in susceptible cultivars (Reddy et al 1979). Little other information has been found on the rate of bacterial growth and possible driving variables. However, much insight into the host response to pathogen invasion has been provided by the scanning electron microscopic studies of Mew et al (1984).

- *Dissemination.* Bacteria exit from the plant in ooze and become available for dissemination. A number of questions need to be answered: How many bacteria are released per leaf? When are they released? It is known that windstorms and rainstorms increase dissemination in the Philippines and many other areas of the humid tropics; but how far are the bacteria spread, and what is the mode of spread? Such data on airborne inoculum are required for a quantitative approach to epidemiology.



1. Epidemiological cycle of bacterial blight of rice.

- *Survival in air.* It is not known how long the bacteria remain viable in air: nor do we know which influencing variables, such as temperature or sunlight, reduce viability.
- *Deposition.* Several questions may be asked about the deposition of bacterial cells on plant surfaces. What is the "catching" ability of rice plants? What is the role of wind speed on impaction? What is the role of aerosols? What is the number of hydathodes per leaf and the relationship between that number and successful infections per leaf?
- *Survival on plant surface, weeds, and nonliving media.* Survival time is affected by temperature, moisture, relative humidity, sunlight, and other factors; but, again, little quantitative information is available.

The equivalence theorem of Van der Plank (1963) suggests that certain components of epidemics have equivalent effects in terms of their influence on the rate of epidemic development. This has not been tested empirically or through modeling. The latter would require 1) that a computer systems model be developed linking all the above components into a complete system, and 2) that the effect of specific influencing variables on each component be quantified and used to explain observed phenomena under natural conditions. The above summary points out that there is much we still do not know about the BB infection cycle.

Measuring bacterial blight pathogen populations

Measurement of a bacterial population is a fairly simple and relatively accurate procedure as long as the population is homogeneous and the measurements are made in the laboratory. This is done by relating the number of cfu that grow on a culture medium to its optical density in pure culture; growth of the population is then a function of the increase in optical density of the pure culture.

In the plant, bacterial infections usually arise not from a single cell but rather from a group of cells. For example, it takes about 10^5 ml in the inoculum to obtain symptoms on rice by pin prick methods, and about 10^9 cfu/ ml are required if rice is inoculated by spraying (Mew 1987). Growth of a bacterial population in the plant can be measured as a function of cfu recovered from a sample of plant tissue. However, this measurement tells nothing about how a population moves from plant to plant, nor about how the spatial distribution of one bacterial population relates to another in a field.

Types of population measurements must be clearly distinguished. One is a measure of the number of bacterial propagules (cells) that make up the population within a given space (e.g., area of leaf tissue or volume of water); another is a measure of the population that functions collectively to establish infection. Successively higher levels of integration may also be considered, e.g., populations within a leaf, a farm, or a wider geographical region.

In epidemiology, it is not particularly useful to measure the population of bacterial cells within a small space (a lesion, for example); rather, one needs to measure the number of separate populations that function as infection units. In this paper, "population" is used in the latter sense. We have also compared populations of organisms isolated from many different rice-growing regions.

Bacterial populations in a field can currently be measured only if they produce visible and discrete symptoms per leaf area, per plant, or per field. Movement of the population thus becomes a function of the number of new infections over time. This simplified measurement may be erroneous if the population loses virulence over time or if resistant plants are present. The question then becomes, how can the real population be measured, and how can such populations be distinguished from each other in space and in time?

Bacterial populations can be qualitatively characterized by standard bacteriological tests, phage and serological reactivity patterns. fatty acid composition, and isoenzyme analysis. With the exception of serological analysis, each of these methods requires that a representative strain (progeny of one bacterial cell) be isolated from the population and characterized by various laboratory assays. In addition, all of the methods are rather cumbersome, making it virtually impossible to analyze large populations efficiently. Serological identification has the advantage that, once a serological marker has been found that identifies a strain or population, bacteria with that marker (antigenic determinant) can be readily identified by a highly specific antibody, even in a mixed population. Genetic probes that recognize only a predefined piece of DNA have also been developed that will identify specific bacterial strains within a mixed population. However, such probes have not been found for Xco.

Serological identification of Xco is performed primarily with polyclonal antisera. Problems have thus arisen with respect to reproducibility of the reaction and to loss of reactivity of a particular Xco strain that has been successively cultured. In addition, colony-type variants may have different serological properties (Chio et al 1981). Such problems are not unique to Xco; rather, variable reactions should be expected because of the nature of polyclonal antisera, which are actually heterogeneous mixtures of antibodies produced by a mixed assembly of antibody-secreting lymphocytes.

More recently, hybridoma technology has produced highly specific monoclonal antibodies (MAbs), which are homogeneous products of a single antibody-secreting lymphocyte. The hybrid lymphocyte (hybridoma) is isolated, cloned, and expanded in such a way that all the antibodies resulting from this clone are identical. For practical purposes, the hybridomas subsequently can be reproduced indefinitely, making their antibody products very useful to independent researchers for obtaining reproducible results in the field. Several MAbs have been produced for identifying Xco strains that appear to be useful for epidemiologic purposes (Table 1). One MAb, designated Xco-1, reacts with 142 Xco strains tested and does not react with any other of the tested xanthomonads or other bacterial genera of plant or animal origin. The strains were isolated from many geographical locations; thus, the epitope detected by this antibody appears to be a unique marker characteristic of all Xco strains. Other MAbs appear to be useful for subgrouping Xco strains and perhaps for identifying Xco races. For example, one MAb (Xco-2) reacts with most but not all Xco strains. The significance of this subgrouping is not known. The potency of the hybridoma technique is illustrated by the generation of a MAb that reacts only with strains from a recent outbreak of BB in Texas. The “Texas antibody”(Xco-5)

Table 1. Reactivity of *Xanthomonas campestris* pv. *oryzae* strains with monoclonal antibodies.

Strain designation	Monoclonal antibodies			
	No.	Xco-1	Xco-2	Xco-5
PXO (original)[a]	14	+	+	-
PXO (new)[b]	59	+	+	-
Lyo-A[c]	26	+	+	-
XO20	1	+	-	-
PXO 35	1	+	-	-
LA-X81A[d]	1	+	-	-
Texas (new)[e]	6	+	-	-
Lyo-B[c]	4	+	-	-
Laxia	1	-	-	+
Texas, 5, 7[e]	2	+	+	+
Texas (new)[e]	16	+	+	+
Texas 6, 8, 10[e]	3	+	-	+
Texas (new)[e]	9	+	-	+
Total	142			

[a] Two strains from each of 6 races and 2 avirulent strains received from T. W. Mew, International Rice Research Institute, 1985. [b] Strains from 6 races received from T. W. Mew, 1987. [c] "Lyo-A" and "Lyo-B" represent 30 cultures collected from Australia, Bangladesh, Burma, India, Indonesia, Japan, Sri Lanka. Thailand, and Taiwan, China, by I. W. Buddenhagen. [d] Strain from the outbreak in Louisiana received from J. E. Leach, Kansas State University. [e] Strains recovered from the outbreak in Texas: Texas strains 5, 6, 7, 8, and 10 were early isolations from leaf blight received from R. Jones, Texas A&M University; "new" Texas strains were more recent isolations received from C. Gonzalez, Texas A&M University.

does not react with any of the Xco strains from other geographical locations. Although the Texas strains have a unique antigen, they can be separated into 2 groups based on the reactivity of Xco-2; only 50% of the 36 strains isolated from the Texas outbreak react with this antibody. Further studies on the application of MAbs for epidemiologic, diagnostic, and taxonomic purposes are desirable. The methods by which bacterial populations are monitored using specific monoclonal antibodies will be described later.

Effect of environment on component processes

Epidemiology is concerned with understanding how different environments affect disease development. With BB, the environment affects not just the dynamics of the bacterial population, but also how it causes injury to the plant (measured as disease symptoms) and the consequent damage. The type of quantitative relationship required for modeling epidemics may be represented as

$$\text{Component process} = f(\text{influencing variables})$$

For example,

$$\text{No. of viable bacteria} = f(\text{days after exit from plant, ambient temperature})$$

in which f means a "mathematical function of." Each of the components would then have at least one equation associated with it, and the entire infection cycle would be represented by a series of linked equations (Teng 1985). Each equation may also be

viewed as a stimulus-response equation in which the component process is the response and the stimulus or stimuli are the various influencing variables such as temperature and relative humidity (Zadoks and Schein 1979).

Controlled-environment studies in greenhouses or phytotrons provide data for quantifying stimulus-response relationships (Zadoks 1972). The design is commonly a complete factorial with replication. For example, to determine the survival of Xco over time under the influence of various temperature regimes would require an experiment in which phytotrons are adjusted to the experimental temperature regimes. The percentage of viable cells would then be sampled at known intervals from the onset of the experiment. The aim in data collection is to generate a response to as wide a range of stimuli as feasible, i.e., to have many points on a curve so that its full shape can be explored. This is important if a reliable prediction of system behavior is to be achieved.

Pathosystem modeling

Ad hoc knowledge of the components of the infection cycle has a heuristic value for understanding field epidemiology. The ad hoc knowledge, when assembled into a single entity (a system model), would lead to a better explanation of why a specific location favors BB epidemics. Different approaches are available for pathosystem modeling (Teng 1985), a common one being to develop a computer simulation model from the functional relationships previously discussed. The computer programming aspects of pathosystem modeling are facilitated by user-friendly software such as STELLA (Structured Thinking and Experiential Learning Laboratory) by High Performance Systems Inc. (Richmond 1985) for the Apple Macintosh microcomputer, and P-CSMP (P-Continuous System Modeling Program) developed by modelers from the Agricultural University, Wageningen, Netherlands, for MS-DOS microcomputers. Computer simulation models have also been written in general-purpose languages such as PASCAL and FORTRAN.

A recent review did not list any simulation model for bacterial epidemics (Teng 1985).

METHODS FOR STUDYING THE RICE BACTERIAL BLIGHT DISEASE PROGRESS CURVE

That disease increases in both space and time (Van der Plank 1963) suggests that not all plants in a population are infected or show symptoms at the same time. To study the dynamics of field epidemics, therefore, requires a methodology for assessing the amount of disease and for collecting samples of diseased material. The latter is best done with knowledge of the spatial distribution of the diseased units; thus, sampling method and spatial distribution studies are often interlinked. The assessment and sampling provide data for plotting DPCs, data that are commonly analyzed using one of several statistical models.

Blight assessment

Two parameters of disease are commonly assessed in epidemiological studies (James and Teng 1979): Disease incidence is the proportion of infected plant units,

commonly expressed as a percentage of the total population, while disease severity is the proportion of tissue infected by disease and showing symptoms, commonly expressed as a percentage of total tissue area. Assessment methods enable quantification of either incidence or severity, and may sometimes result in the determination of disease indices that incorporate both incidence and severity. Disease assessment methods consist of descriptive keys as well as standard area diagrams (James and Teng 1979). With either, use is made of the abilities of the human eye to discriminate between grades of disease symptoms and to estimate the area occupied by disease.

Several BB assessment methods have been reported. One method widely used by plant breeders and pathologists in tropical Asia is the scale in the *Standard evaluation system for rice* of the International Rice Testing Program (IRRI 1980). In this scale, 6 grades of disease incidence or severity are recognized: 0 = no BB, 1 = <1%, 3=1-5%,5 = 6-25%, 7=26-50%, and 9=51-100% incidence. A problem with this method is that much useful information is lost because of the wide range of disease associated with each grade. A standard area diagram was used for BB severity by Teng (1975) in surveys of farmers' fields, in which grades showing different percentages of whole-plant severity were specified.

For epidemiological studies, we suggest that attention be paid to the warning by James and Teng (1979) that assessment methods be used that take into account the inherent abilities of the human eye. For example, the Weber Fechner Law outlined by Horsfall and Barratt (1945) specifies that the distinguishing ability of the human eye follows a logarithmic relationship with respect to stimulus intensity. This would suggest that assessment grades follow a geometric division, and that the eye can equally distinguish between 25 and 50%, as between 12.5 and 25%. This would also suggest that the eye cannot distinguish, with the same accuracy, between 5 and 6% disease, as between 5 and 10% disease. Assessment keys should therefore contain the primary grades of 0, 12.5, 25, 50, 75, and l00%, and their interpolation points (James and Teng 1979).

Sampling and spatial distribution

Techniques that allow accurate identification and monitoring of a bacterial population are needed for epidemiological studies. Techniques used for this purpose involve phage typing, serotyping, and detection of mutant strains with antibiotic resistance. Serotyping has three main advantages over the other two methods: 1) specific antibodies can identify a pathogen in mixed culture, whereas for phage typing, the target cells must be in pure culture and in the logarithmic growth phase for accurate detection; 2) use of antibiotic resistance markers to detect movement of different strains may distort the results, since mutants may not have the same capacity as wild-type strains to infect and spread in the field; and 3) specific antibodies may be used to track a number of wild-type strains simultaneously.

In studies with *X. campestris* pv. *campestris,* a related pathovar that causes black rot of crucifers, strains that in greenhouse tests showed no differences in capacity to infect cabbage plants moved at very different rates in the field (Yuen et al 1987). Disease progress was measured, and the strains were tracked by using a panel of MAbs that differentiated them according to their surface antigens. It was also

observed that the disease spread in the direction of groundwater flow and not in the direction of prevailing winds as was expected. These spatial differences among strains have epidemiological significance and need to be quantified so that patterns of diseased plants may be understood. Statistical methods and software for quantifying spatial attributes are commonly available (Ferrer and Teng 1987).

Spread of BB in the field can be measured using techniques similar to those described for black rot of crucifers. Even with the limited number of MAbs available that detect and differentiate strains of Xco, it is possible to design field experiments to measure disease progress resulting from two separate inoculum sources. For example, using two MAbs (Xco-1 and Xco-2), one Philippine strain (PXO 35) can be differentiated from most of the Philippine Xco strains tested so far. By using these two MAbs, the movement of strains having different reactivity patterns can be traced in field plots. However, before such studies are undertaken, we need to continue the search for more useful MAbs, namely those that distinguish Xco races.

Disease progress curve models

When sequential measurements are made of BB symptoms or of BB pathogen populations and are plotted against time, a sigmoid DPC is commonly obtained. The DPC represents population growth, and many mathematical models have been suggested for describing it (Jowett et al 1974). When a DPC is related to weather data, it is sometimes possible to discern the influence of weather variables on overall disease development.

Among the models used to describe the DPC are the exponential, the logistic, the Gompertz, and the Richards. Van der Plank (1963) pioneered the use of the exponential and logistic models for describing DPCs. These models are represented in differential form as

$$\text{Exponential } dy/dt = r_1 \cdot y, \text{ and}$$

$$\text{Logistic } dy/dt = ry\ (1.0 - y),$$

where dy/dt is growth rate of the DPC, r_1 is logarithmic growth rate of the population, r is apparent infection rate, and y is amount of disease. These models may be fit to data by solving the equations and then using linear regression to quantify the parameters (Van der Plank 1963). Little information is available on which models are best for BB epidemics, suggesting that much field work remains to be done. It is also important to have mathematical models of the DPC so that disease variables that are suited for estimating the relationship between BB severity and crop yield can be identified.

CONCLUSION

Two other research areas are commonly associated with epidemiology — crop loss assessment and disease management. The former is concerned with understanding and quantifying the effects of BB on the crop, while the latter is an application of epidemiological knowledge. Yield loss experiments can be greatly improved when

methods for rapid diagnosis are available. Because of confounding factors and complex symptomatology in the field, yield losses specifically due to BB can be estimated with greater accuracy if the pathogen identity is confirmed by serological tests when symptoms are obscured by other stress factors.

The work of Reddy et al (1979) illustrates the difficulties of quantifying the effect of BB symptoms on yield. The researchers could not derive any predictive equations for estimating loss independent of potential yield, and the pathogen may have had greater effects than the symptoms showed (MacKenzie and King 1980). Experiments at the International Rice Research Institute involving field studies and a computer simulation project will provide further data for modeling BB effects on yield.

It is hoped that these approaches will strengthen and refine the epidemiological basis for disease management. Generation of pertinent information depends, in part, on developing recognition techniques for individual populations of the BB pathogen, and on utilizing these techniques in field studies. By identifying serological markers for strains that represent Xco races it should be possible to study the stability of such strains in the field, as well as their capacity to survive and compete with indigenous bacterial populations. Information regarding the diversity of BB races in the various rice-growing countries will help breeders to evaluate the genetic background of resistant cultivars. In addition, a more detailed analysis of the components of the pathosystem should lead to predictive models that can be used as decision aids in disease management.

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Notes

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Methods for assessing resistance to bacterial blight

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Infection by *Xanthomonas campestris* pv. *oryzae* can lead to three forms of disease symptoms, of which bacterial blight lesions are the most common. Three types of resistance can be discerned: 1) seedling resistance is expressed at all growth stages of the plant; 2) adult plant resistance is expressed only in adult plants; and 3) quantitative resistance is expressed as reduced susceptibility. The degree of symptom expression is assumed to reflect the degree of resistance. Symptom expression is assessed by estimating the percentage of diseased leaf area (%DLA), sometimes expressed as a class within a discrete scale. Two scales are often used—a 0-7 disease index scale and the 0-9 Standard Evaluation System scale. It is also possible to measure the length or area of the bacterial lesions. In the field, the frequency of hills, plants, or tillers with disease symptoms can be assessed. Proper assessment requires uniform exposure to the pathogen. If the whole plant or plot is exposed to the inoculum, as in the field or with spray inoculation, the %DLA assessment procedures are adequate. For more accurate comparisons, inoculation of specified leaves at specified plant stages is carried out. In such cases, %DLA assessment is inadequate, because it measures the ratio between diseased leaf area and total leaf area. Entries with large leaves would give a lower %DLA than entries with small leaves and so may be erroneously classified as more resistant. In this case (pin prick and clip inoculation methods), the lesion length or lesion area is a better assessment parameter.

Resistance is defined as the ability of a host to reduce the growth and development of a pathogen. Measuring this reduction in the pathogen population is most often difficult, if not impossible. In many host-pathogen relationships, including the rice-bacterial blight (BB) relationship, the areas invaded by the pathogen become visibly affected, and one can use this "area affected" as an indirect measure of the size of the pathogen population. One assumes that the smaller this affected area is, the smaller the pathogen population and the greater the resistance.

Assessment of resistance is needed for 1) screening a large collection of cultivars, 2) characterization and genetic study of the resistance, and 3) utilization of the resistance in a breeding program. The choice of assessment method for each stage

can determine the accuracy of the conclusions reached, but it can also determine the size limits imposed on a program.

This paper discusses the types of disease expression occurring in the rice-BB system, the forms of resistance that can be expected, and the suitability of the inoculation methods and assessment methods in use.

DISEASE SYMPTOMS

Three sets of symptoms can be distinguished following infection of rice by *Xanthomonas campestris* pv. *oryzae* (Xco).

- Infection through the leaves results in watersoaking and necrosis typical of leaf blight; leaf blight can occur at all growth stages and usually develops following leaf damage, such as after a storm.
- Systemic infections in seedlings infected through wounds of roots or leaves during transplanting lead to kresek wilt symptoms; young plants often die 24 wk after infection.
- Newly developing leaves of systemically infected plants may be pale yellow, although bacteria are not present in the leaves (Ou 1985).

Leaf blight is the most widespread symptom of Xco infection and is therefore the symptom for which resistance is most often sought. Kresek wilt symptoms are locally of great importance. Differences between genotypes have been shown for kresek infection (Watanabe 1976). The relation of resistance to the two forms is not clearly documented (Horino and Yamada 1982).

TYPES OF RESISTANCE

Mew (1987) refers to seedling, adult plant, and quantitative resistance to BB. Seedling resistance is conferred by any one of a number of known major genes and is characterized by a high level of resistance throughout crop growth (Ezuka and Horino 1976). Adult plant resistance is characterized by a high level of resistance in adult plants, but seedlings are highly susceptible (Zhang and Mew 1985). Quantitative resistance is characterized by reduced symptom expression in relation to a highly susceptible check cultivar (Wasano and Dhanapala 1982, Yamada 1986).

INOCULATION METHODS

A reliable assessment of the differences in resistance between genotypes requires a method by which the inoculum is uniformly introduced into the population of entries to be tested. The lesions initiated by this inoculation should be representative, and the differences between different genotypes should represent actual differences in resistance as observed in the field.

Most assessment procedures depend on artificial inoculation of a leaf, a whole plant, or a field. Spray inoculation, which most resembles field infection, results in long, narrow lesions along the leaf margins. The bacteria enter through the

hydathodes or through wounds and therefore move down the vascular system against the water gradient.

Because spray inoculation often gives a low percentage of infection, prick or clip inoculation may be preferred (Kauffman et al 1973, Morinaka et al 1978). Both methods deposit the inoculum directly in the vascular system, bypassing the natural points of entry (Preece 1982). Prick inoculation deposits the inoculum in a small point at one or both sides of the midrib, halfway between the leaf tip and base. Clip inoculation deposits the inoculum across the whole leaf width near its tip. Both methods are usually carried out on plants at maturity or at maximum tillering. When the inoculum contains 10^8 or more colony-forming units (cfu)/ml, nearly 100% chance of infection is assured. Lesion size in compatible reactions may increase with dosage. Lesions develop earlier following clip than prick inoculation, probably due to the larger inoculum dosage actually delivered into the leaf tissue. Lesions following clip inoculation move down the leaf against the xylem water flow, while lesions following prick inoculation develop in both directions, but more toward the leaf tip than the leaf base. Lesions are well defined and measurable.

Despite these differences, Morinaka et al (1978) reported correlations of 0.81-0.92 between clip and prick inoculation and of 0.74-0.83 between spray and clip inoculation, depending on the isolate used. The results of clip or prick inoculation therefore represent the more natural infection process, as shown by the significant correlation between spray and clip inoculation. Spray inoculation has a larger experimental error due to escapes, the greater variation in inoculum deposited onto the leaves, and the irregular form and placement of the lesions. It is therefore unsuitable for studies where small differences between lines or between individual plants are important. For screening lines for quantitative resistance, or for selection among segregating plants, the more efficient clip or prick methods are preferable. These methods allow standardization of inoculum concentration, plant age, and leaf position to increase accuracy. It is also possible to use more than one isolate per plant.

ASSESSMENT OF SYMPTOM DEVELOPMENT

The diseased leaf area is assessed by various methods. One can measure the length or area of the lesion, or the percentage of the leaf area affected by the lesions (percentage of diseased leaf area = %DLA). Disease incidence, which is the percentage of leaves, plants, or hills showing disease, can also be assessed.

The choice of method depends on the intended use of the assessment data. It is clear that if a relationship with crop damage is studied, the %DLA procedures are preferable, because there should be a relation between damage and the leaf area affected by the pathogen. In assessing resistance—the aim of this paper—vit is important to distinguish various aims. Screening large numbers of entries for their level of resistance asks for an assessment that is rapid, easy, and fairly reproducible and that classifies the entries into resistance classes. On the other hand, if research into the genetics of resistance is the aim, one needs a method that estimates the

resistance of individual plants, rather than of groups of plants in the field (plots). It is therefore imperative first to describe the assessment procedures in use and then to discuss which procedures should be used.

Percentage of diseased leaf area

Estimating %DLA allows rapid scoring of large numbers of plants, although estimates can vary significantly among scorers when more than one person does this work (Baw 1985). In seedlings, lesions can vary from nearly 100% to less than 1% DLA, whereas on plants at maximum tillering or older, the maximum expected %DLA is about 50. Highly resistant reactions will result in a %DLA of not more than 5 and often less than I (Baw 1985).

Estimates of %DLA, even when aided by field keys, are more precise at the extremes than in the midrange, because visual accuracy is proportional. to the logarithm of the intensity of the impulse (Horsfall and Barrett 1945). This will result in large differences in error variance between highly resistant and moderately susceptible cultivars so that when this type of screening is done using %DLA, a transformation may be required before an analysis of variance can be carried out (Finney 1973).

For speed of scoring, many prefer to use a disease scale based on %DLA (Horsfall and Cowling 1978). Such scales divide the reactions into a number of defined classes. The well-known Horsfall-Barrett (HB) scale was designed on a logarithmic scale for estimates of %DLA up to 50% and for estimates of the remaining green leaf area from 50 to 100% (Horsfall and Cowling 1978). This division of classes largely replaces the need to transform the data. When %DLA estimates are averaged, the arithmetic mean is found. When the equally spaced codes of this score are averaged, the log of the geometric mean of the %DLA is calculated, which is less distorted by extreme individual scores (Large 1966), again reducing error variance.

The HB scale itself has not often been used to assess BB; two other scales have been developed for this purpose:

- Ezuka and Horino (1974) developed a scale to score prick-inoculated leaves. This scale has 8 classes (coded 0-7) ranging from symptomless to 100% coverage of the leaf area above the inoculation point (Table 1). Tests are most often done on flag leaves on the assumption that this reaction is the most stable (Mew 1987). The scale deviates from the HB scale in that it puts greater emphasis on resistant reactions. Distinctions are made between symptomless plants, plants showing a slight discoloration around the point of inoculation, and plants with lesions less than 15 mm long. These extra classes allow greater discrimination when selecting for high resistance.
- The Standard Evaluation System (SES) was developed at the International Rice Research Institute (IRRI) (IRRI 1980) to score clip-inoculated leaves on booting or flowering plants. The scale has 6 classes (coded 0, 1, 3, 5, 7, 9) ranging from symptomless to 100% DLA (Table 2). The last class contains all severities greater than 50% DLA, an area not usually found in plants at maximum tillering or later, but often found in seedlings. Highly resistant cultivars score 1 or 3, whereas highly susceptible cultivars score 7 or 9.

Table 1. Scale developed by Ezuka and Horino (1974) for assessing lesions resulting from inoculation by the double-needle prick method on either side of the midrib at the center of the leaf blade. The ratio of the lesion length to the leaf length from the inoculation point to the tip is assessed.

Code	Description
0	No symptoms
1	Slight discoloration at the inoculation point
2	Lesions 15 mm long
3	Lesion 1/4 of the length from the inoculation point to the leaf tip
4	Lesion between 1/4 and 1/2 of the length
5	Lesion between 1/2 and the whole length
6	Lesion covering the whole length, but some green area remaining
7	Lesion covering the whole area above the inoculation point

Table 2. Standard Evaluation System (IRRI 1980), developed for assessing diseased leaf area. The mean percentage of diseased leaf area (%DLA) on the upper 3 leaves of plants at booting or later is assessed.

Code	Description
0	No lesion
1	1% DLA
3	1-5%DLA
5	6-25% DLA
7	26-50% DLA
9	51-100% DLA

Horino et al (1981) found a significant correlation between Ezuka and Horino's scale (1974) and the SES scale (r = 0.753), and between Ezuka and Horino's scale and absolute lesion length (r = 0.728).

Baw (1985) compared %DLA, SES score, the HB scale, and a modified HB scale for assessing 39 cultivars ranging from highly resistant to highly susceptible at the seedling and maximum tillering stages. The %DLA estimates distinguished more statistically different groups of cultivars than the three scales, and also more than parameters based on multiple scorings of %DLA on single leaves.

An assessment based on %DLA is probably the most suitable procedure if the total leaf area of the plant or plot is exposed to the inoculum. If individual leaves are inoculated, however, such as with the pin prick and the clip inoculation methods, estimates based on %DLA may not be the best. With these latter inoculation methods, the size or length of lesions that develop is determined by the resistance of the inoculated leaf. The %DLA does not measure the size of the lesion but rather the ratio between diseased leaf area and total leaf area. If two genotypes carry the same resistance level but vary largely in leaf size, the genotype with the larger leaves will give a lower %DLA than the genotype with the smaller leaves. This will be interpreted as a higher level of resistance for the large-leaved genotypes. Also, if one compares resistance at different plant stages, %DLA in young plants will be much higher than in older plants even if the actual lesions are of the same size, because the

leaves are smaller in young plants. This reasoning assumes that lesion size and leaf size are not correlated. Comparing lesion length and leaf length of a large number of cultivars for two isolates showed no association of any significance, the correlation coefficient *r* between lesion length and leaf length being less than 0.1.

Lesion length or lesion size

Several workers have preferred to use absolute lesion length (Yoshimura et a1 1984) or lesion area (Yamamoto et al 1977) for studies of BB. While %DLA measures the relative damage to the leaf caused by Xco, lesion length is a direct measure of the extent of the rice-Xco interaction, and so of the level of resistance. Unlike the scattered symptoms caused by spraying or natural infection, the single lesion resulting from clip or prick inoculation is well defined and large enough to measure, although agreement must be reached on whether to include or exclude chlorotic tissue in the measured length. Measuring requires more time than estimates of %DLA, although electronic equipment may make rapid measurements possible. A scale based on lesion length should be considered; it would speed scoring while eliminating the confounding effects of %DLA.

Because lesion length measurements are continuous and not subject to the discrete grouping of %DLA or scales, the power of discrimination of small differences should be greater. This may be important in distinguishing among highly resistant individuals that would all fall into a single class of a scale, but is especially important when selecting for moderate resistance, because it is in this range that the eye can less accurately detect small differences. This can also mean that fewer leaves need to be scored to achieve a desired level of variance.

Appearance of symptoms

The large differences in the reported time of appearance of BB symptoms (Horino and Hifni 1978, Kaku and Kimura 1983, Rao et al 1979, Yoshimura et al 1984) may be due to differences in plant age, inoculation method, or concentration of isolates. Major differences between cultivars following inoculation with a single isolate have not been reported. Differences in time of appearance of bacterial ooze have not been reported and would be difficult to measure. This indicates that assessments based on differences in time of symptom appearance are not likely to be useful.

Rate of lesion extension

Differences among cultivars in rate of lesion extension have been reported (Baw 1985, Yoshimura et al 1984) using either lesion length or %DLA as a measure of lesion development. Logit and Gompertz transformations of the original data have been used (Baw 1985, Yoshimura et al 1984) to aid in comparison. The integral of the lesion extension curve was also used as a measure of comparison by Baw (1985). Whereas it could be expected that information from repeated scorings would improve the power to distinguish between cultivars, Baw (1985) concluded that a single assessment better distinguished 39 cultivars ranging from highly resistant to highly susceptible than the parameters derived from repeated scores. For general

screening, the rate of lesion extension seems to be excessively time-consuming for the added information gained, and is therefore not a suitable procedure.

ALTERNATIVE ASSESSMENT PARAMETERS

Although visual symptom assessment will remain the major parameter used in BB research, other parameters have been used to help characterize resistance. Such measurements are generally more tedious, but the added information may help distinguish among otherwise similar reactions and may offer potential insights into the host-parasite interaction. It is especially important to determine whether fundamental differences exist in the working of the various known major genes for resistance to Xco. The near-isogenic series of lines now in advanced stages of development (Ogawa and Yamamoto 1987) can be expected to aid detailed comparisons between known resistances.

Bacterial counts from infected leaves

Sap extracted from leaves infected with an incompatible isolate reaches a maximum bacterial count 100- to 1000-fold lower than sap from the same cultivar infected with a compatible isolate (Barton-Willis et al 1986, Kaku and Kimura 1983). There is some evidence (Kaku and Kimura 1983) that a relationship between lesion length and bacterial concentration can be found for susceptible reactions where the cultivars vary in quantitative resistance. Kaku and Kimura (1983) noted a 10- to 100-fold difference in bacterial counts between highly susceptible cultivars and a more moderately susceptible cultivar 12 d after inoculation.

Horino and Yamada (1975) tried to quantify the bacteria indirectly by measuring the exudation from leaf segments immediately after cutting. Their exudation index combined observations on the size of the bacterial cloud from each vascular bundle with a count of the number of bundles exuding bacteria, The index was used to rate 20 cultivars 1 d before symptoms appeared and was found to better distinguish between cultivars than a 0-5 disease scale based on an estimate of absolute lesion area (Horino and Hifni 1978). It is unclear whether the advantage of the exudation index would remain if lesion length or %DLA were used instead of this disease scale. The exudation index requires microscopic analysis, which must be made immediately after cutting the leaf pieces.

Bacterial presence does not always lead to symptom development (Goto 1965). The relation between lesion size and bacterial concentration can be confounded by factors related to the sensitivity of the tissue to destruction by bacteria. For this reason, a number of tests have been used to follow latent bacterial presence in apparently healthy tissue. Parry (1985) followed the spread of bacteria during the first 172 h of infection in compatible and incompatible interactions of IR20 and Cas 209. He cut inoculated leaves into 2-cm segments and scored for the presence of Xco colonies after incubation on peptone sucrose agar for 4 d. Large differences in movement were found between the two cultivars, but little difference could be found between the compatible and incompatible interactions for each cultivar. Goto (1965)

developed a technique for colored dye uptake in detached leaves. Using a basic fuschin solution, he showed that the area of vascular blockage was considerably larger than the area of visible lesion. Reddy and Kauffman (1973) used this technique to show differences between a resistant and a susceptible cultivar.

The conductivity of leaked electrolytes has been used to compare cultivars for damage caused by bacteria to leaf tissue. Kohno et al (1981) reported that, 3 d after infection, a compatible reaction showed greater conductivity than did an incompatible reaction. These differences remained detectable up to 18 d after infection (Rao et al 1979).

Infectivity titration

Infectivity titrations can be used to derive a number of parameters useful in varietal screening (Ercolani 1984). The dose-response curve can be used to compare test cultivars for the dosage needed to elicit a certain response, usually the effective dose at which 50% of the inoculated units show symptoms (ED_{50}), or to compare the response at 1 or more dosages. The slope of the line at the ED_{50} point can also be used to compare reactions. Increasing levels of heterozygosity tend to reduce both the slope of this curve and differences between test lines (Ercolani 1984). Two cultivars with equal ED_{50} but different dose-response curves will show differences at higher and lower dosages. The researcher must therefore realize that, when using a single value for comparison, caution should be taken that this value is related to concentrations expected in field infections (Ercolani 1984).

IRRI (1981) reported differences in the ED_{50} of Cas 209 for compatible and incompatible reactions at different growth stages. Cho (1975) found differences in the dose-response curves of 7 cultivars using 4 concentrations varying from 10^5 to $2x10^9$ cfu/ml. Because infectivity titrations require many homogeneous plants and much work to carry out, they are not suitable for screening or selection. Their use for characterization needs to be investigated further.

Epiphytic growth, surface reactions, and chemotactic responses

Phytopathogenic bacteria have been found to survive and multiply on leaf surfaces, providing inoculum for disease. Studies with *Pseudomonas* spp. and *Xanthomonas campestris* pv. *phaseoli* have shown that epiphytic populations of pathogenic bacteria are generally lower on resistant than on susceptible cultivars of a given host species (Hirano and Upper 1983). Because methods to quantify epiphytic populations are tedious and the results variable, direct measurement of this value does not appear a useful parameter for cultivar screening, although it might explain unexpectedly low or high field resistance of a cultivar as compared with levels following clip or prick inoculation.

One aspect of this epiphytic bacterial interaction is the attractive quality of host exudates. The ability of rice water pore exudate to attract Xco was studied by Feng and Kuo (1975). The numbers of bacterial cells attracted from a central source into a capillary tube filled with exudate were compared. Exudates from highly susceptible cultivars and synthetic medium attracted up to eight times as many bacteria as

exudates from nonhosts, several resistant cultivars, and distilled water. Different isolates were not tried.

Histological evidence

Histological studies of compatible and incompatible interactions have shown differences in bacterial spread (Kaku and Kimura 1983). In addition, there is evidence of cellular disintegration, both in the vascular bundle and in the adjacent parenchymatous tissue, as the bacteria translocate from the point of inoculation, except in symptomless reactions (Kaku and Kimura 1978).

Electron micrographs show dramatic changes in the integrity of the host vessel wall 3 d after prick inoculation. The inner vessel wall is loosened and released into the vessel. Bacteria within the vessel are misshapened and immobilized in a mass of fibrillar material (FM). When the cytoplasm of vascular parenchyma cells is disrupted, the bacteria appear to be dead (Horino 1978). Some FM was found in compatible interactions 20 d after inoculation, but the bacteria were not as misshapened as in the incompatible reaction (Horino and Yamada 1982). The time taken until FM appears may be a parameter for varietal comparisons.

Mew et a1 (1984) reported differences in the reaction of water pores following contact with compatible or incompatible bacteria. This reaction should be further studied on more cultivars.

Most histological studies have compared highly compatible with highly incompatible reactions (Huang and De Cleene, 1989, this volume). Detailed histological studies of moderately resistant reactions might reveal essential differences, if they exist, between major gene resistance and quantitative resistance.

ASSESSMENT FOR SCREENING PURPOSES

For screening purposes, such as at the earlier stages of a breeding program, it is important to be able to classify the entries according to their level of BB resistance; but this classification does not need to be accurate. Far more important is a representational assessment, i.e., whether the resistance observed in the screening trials represents the resistance in the farmer's field. For this purpose, a procedure such as the SES scale on clip-inoculated leaves (Table 2) seems adequate. An exception can be made in screening for moderate resistance when there are large differences among leaf sizes of the cultivars, as when both traditional and short-statured rice cultivars are tested. A seemingly acceptable resistance on a long-leaved cultivar can be less acceptable when later transferred into an improved short-statured cultivar. In such cases, lesion length or a scale based on lesion length is advisable.

For screening purposes, one could also assess differences in resistance by scoring the incidence of disease. This is the percentage of hills; plants, or tillers that show symptoms. Assessment of the incidence of kresek symptoms is the most accepted way to screen for resistance to this form of the disease. Spray-inoculated plots or

plots that are infected from spreader rows can also be assessed for BB in this manner. Although this is a relatively fast method, it requires more plants per cultivar than the previously mentioned methods to get a good estimate of the relative levels of resistance of the cultivars, because spraying is the least uniform inoculation method. Incidence should be used only to assess resistance in the earlier phases of the epidemic, before the majority of the plants show symptoms. When most plants show symptoms, one must shift to assessing severity, such as by estimating %DLA. Because the bacteria spread via the ricefield water, a high level of interplot interference can be expected, and differences among cultivars may be hard to demonstrate later in an epidemic.

The relationship between incidence, which measures the number of successful infections, and severity, which measures the size of an infected area, is not well documented. Resistance factors relating to entry may be independent of factors relating to spread within a leaf following establishment (Buddenhagen 1983). Mew et a1 (1984) showed that the exclusion of bacteria from the water pores in an incompatible reaction was an active process of resistance by the plant. Until further evidence is available, the two parameters should be viewed separately and, where possible, both assessment techniques should be used during screening and characterization.

ASSESSING TYPES OF RESISTANCE

Seedling resistance

Seedling resistance is usually identified following clip or prick inoculation both at the seedling stage and at maximum tillering or booting. A single isolate is usually sufficient to indicate the presence or absence of resistance. Because the most highly resistant plants are usually sought, attention should be paid to detecting fine distinctions between plants showing resistant reactions. Attention to lesion type and speed of appearance may aid in developing the resistance to its fullest expression.

Adult plant resistance

Identification and characterization of adult plant resistance will necessarily involve tests at various stages to determine at what plant stage resistance begins to be expressed and when it reaches its maximum expression, as well as its maximum level. These tests should be done as staggered plantings to avoid the confounding influence of environment.

Quantitative resistance

Quantitative resistance can be identified after prick or clip inoculation. Yamada (1986) recommended the use of a highly aggressive isolate. Interest in this form of resistance arises from the assumption that it is a form of durable resistance (Parlevliet 1979). Quantitative resistance in BB is expected to be expressed as reduced lesion size, best assessed by measuring lesion length or area, compared with a highly susceptible cultivar. Unfortunately, major genes do not usually show complete expression, and there may be considerable lesion growth, as in *Xa-4* when challenged with Philippine race 4 isolates, for example. This makes it very difficult to

discriminate true quantitative resistance from resistance genes with incomplete expression. Progeny tests and tests with more than one isolate may help to distinguish between these two forms of incomplete resistance.

ASSESSMENT FOR RESEARCH PURPOSES

To characterize different resistances or to determine the genetic background of resistance requires accurate assessments. The resistance of a leaf can vary greatly with its age, the position of the leaf, and the age of the plant. Inoculation methods that expose the whole plant, such as spraying, are thus not exact enough. If one exposes the whole plant to the pathogen, the researcher should assess only specified leaves to avoid confounding resistance with other factors such as plant or leaf age. Prick or clip inoculation is much more suitable. To avoid such confounding factors, only specified leaves should be inoculated; and these should later be assessed in the most accurate way, by measuring lesion length.

Genetic studies to characterize resistance require information on the distribution of the reaction in the population. Confirmation of monogenic resistance requires the division of a population into resistant and susceptible types. Because there are no qualitative differences between resistant and susceptible lesion types, the limits of the two groups need not necessarily be determined beforehand (Lin 1987, Saha 1984). A study of the histogram of the F_2 should show whether there is a natural division of the population into groups, In this way, shifts in the level of the entire population due to variation in inoculum, environment, or genetic background are not confounded with the resistance in question.

Use of a scale, such as that described in Table 1, to assess resistance for genetic studies not only carries the danger of confounding leaf size with resistance; it also suffers from the facts that the classes are not of equal width, and that the number of effective classes (6) is small. These two aspects make such a scale less suitable for genetic analysis, especially genetic analysis of quantitative resistance. The best assessment parameter for a polygenically inherited trait is a completely continuous one such as lesion length or lesion size, or the transformed data (if necessary) of these measures.

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Types of resistance in rice to bacterial blight

Zhang Qi and T. W. Mew

Resistance to bacterial blight has been identified in many rice cultivars. Several types of resistance comparable to those against fungal pathogens have been recognized. We studied seedling and adult plant resistance to clarify the types of resistance. IR944 and IR1695 resistance to 4 races of *Xanthomonas campestris* pv. *oryzae* appeared at the 6th to 10th leaf position. Zenith and Malagkit Sungsong showed resistance at one or two leaf positions earlier than their progenies. IR1545 was differentially resistant to the four races at all growth stages, whereas TN1 was susceptible. Expressed as the plants matured, the resistance of Zenith and Malagkit Sungsong was not affected by temperature change, but the lesions of IR944 and IR1695 were longer at higher temperature. A strong differential interaction was noted on selected japonica cultivars and lines from China. The differential resistance appears moderate at maximum tillering and increases at maturity; it may not be fully expressed at booting. However, seedling resistance to incompatible races was stable.

Bacterial blight (BB) of rice caused by *Xanthomonas campestris* pv. *oryzae* (Xco) has been recognized as an important rice disease since the introduction of improved but susceptible cultivars. These modern cultivars respond to fertilizer with luxurious growth and thus have modified the rice microenvironment to make it highly conducive to disease development. Until cultivars with genetic resistance to the disease were bred and introduced, BB posed a serious threat to rice production in the major rice-growing countries of Asia.

Many attempts have been made to control the disease, including chemical treatment and modified cultural practices, chiefly reducing the rate of N fertilizer; but the impact of such management tactics has been limited in scale and locality. Host plant resistance has proven more effective and economic. Since the release of IR20, the first modern rice cultivar in tropical Asia with genetic resistance to BB, breeding for BB resistance has become an important component of varietal improvement programs. Many varieties and breeding lines have been developed with certain levels of resistance, but have later been discovered to be resistant only to specific isolates in one locality. Variety Asakaze in Japan is a good example (Ezuka and Sakaguchi 1978). IR20, after its release in the Philippines in early 1970, was

found susceptible in Isabela Province (Ou 1985). These setbacks suggest not only the existence of different pathogen races in different regions, but also that the types of resistance to BB in rice must be assessed.

The subject has been reviewed recently (Mew 1987); in this paper we provide additional information on the topic.

GENERAL CONCEPT OF HOST RESISTANCE

According to Van der Plank (1968), host plant resistance can be classified into vertical resistance (VR) and horizontal resistance (HR), considering both genetic and epidemiological concepts. VR is monogenic and is effective against specific races. It delays the disease by reducing the amount of effective inoculum. HR is polygenic and is effective against all pathogen races. When a disease has started, HR lowers its severity by reducing the apparent infection rate. HR is characterized by the absence of interaction between host cultivars and pathogen isolates. The absence or presence of interaction can be studied either by an analysis of variance or by ranking the isolates in order of virulence. It is believed that a reduction of the apparent infection rate is associated with the lasting resistance of a cultivar, and that it can be measured quantitatively.

Parlevliet (1979) assessed the resistance of barley to leaf rust caused by *Puccinia hordei* Orth. at different crop growth stages. He noted several types of resistance:

- Seedling resistance is against low-infection types (0-2) at all growth stages.
- Intermediate resistance is against infection types 2-3 at all growth stages.
- Adult plant resistance is characterized by susceptible infection types (4^{-}-4^{+}) at the seedling stage and a resistant reaction at the adult plant stage (0-2).
- Partial resistance shows a susceptible reaction at all growth stages (4 in seedlings, 34 in adult plants).

On analyzing the resistance of cabbage to the yellows caused by *Fusarium oxysporum* f.sp. *conglutinans* and of potato to late blight caused by *Phytophthora infestans,* Fry (1982) established that different forms of resistance have different characteristics. The magnitude of the effect may be very high, moderate, or relatively low. Its inheritance may be conditioned by one or several genes. It may be effective against some but not all pathogen isolates; that is, it may be differential. If there are at least two possibilities for each of the three characteristics, there are eight categories of resistance.

To assess host plant resistance, the growth and development of the pathogen should be measured. Equally important is the stage of host plant development. Response to infection varies with the age of the host. In fungal diseases, we can measure the growth and development of the pathogen, e.g., its sporulation, latent period, and infection frequency.

RESISTANCE TO BACTERIAL BLIGHT

Resistance of plants to bacteria shares some similar characteristics with resistance to fungal pathogens. Ercolani (1972) proposed to measure the generation time in host tissues as an effective means of measuring resistance to bacterial diseases; this may be

accurate, but it is tedious. Mew et al (1981) used incubation period as a component for varietal resistance to BB in rice; this appears feasible, but it is highly influenced by the inoculum dose deposited on the infection court. The initial inoculum dose is difficult to control, and it affects the incubation period, especially for moderate resistance levels. In dicots, lesion type has been used to indicate resistance type. On BB of cotton caused by *X. campestris* pv. *malvacearum,* two distinct types of lesion are observed: watersoaked and dry (Brinkerhoff 1963).

BB resistance has been detected in many rice cultivars. In some cases, it is conditioned by a single gene, in other cases by many genes (Mew 1987; Ogawa and Khush 1989, this volume). In an inventory of rice germplasm for BB resistance, Mew et al (1981) recognized several types of resistance comparable to some of those reported by Parlevliet (1976) for barley leaf rust; this has been reviewed recently (Mew 1987). To further clarify the types of resistance relative to adult plant resistance and seedling resistance, the following experiments were done.

When 6 rices were inoculated on the 6th leaf to the flag leaf of the same plant, TN1 was susceptible to the 4 Philippine pathogen races at all leaf positions, and IR1545 was differentially resistant to races 1-4 at all growth stages. IR944 and IR1695 were susceptible to the 4 races at the 6th to the 10th leaf positions and became resistant from either the 1lth or 12th leaf to the flag leaf (Table 1). Zenith and Malagkit Sungsong reacted similarly, but resistance appeared one or two leaf

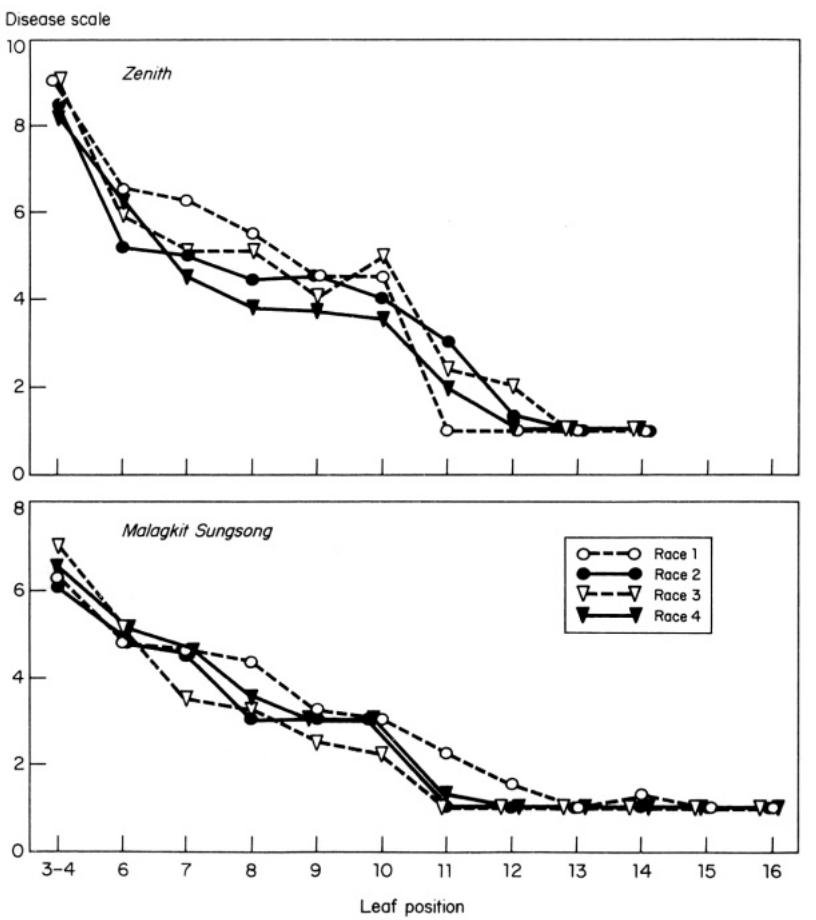
Table 1. Disease reactions of rice leaves at different leaf positions in the main culm to *Xanthomonas campestris* pv. *oryzae* races.

Leaf position	Disease score (1-9)[a] against race							
	TN1	IR1545	IR1695	IR944	TN1	IR1545	IR1695	IR944
	PXO 61				*PXO 71*			
6	8.5 a	3.0 a	7.0 a	5.7 ab	8.5 a	7.0 a	7.0 a	6.0 b
7	7.0 b	3.0 a	6.0 a	6.0 a	7.5 b	6.5 b	7.0 a	6.5 a
8	7.0 b	3.0 a	7.0 a	5.0 b	7.5 b	6.5 b	7.0 a	5.0 c
9	7.0 b	2.5 b	7.0 a	5.5 ab	7.0 b	5.0 d	5.0 b	5.0 c
10	7.0 b	3.0 a	5.0 b	5.0 b	5.0 c	7.0 a	5.0 b	5.0 c
11	7.0 b	1.5 c	5.0 b	5.0 b	7.0 b	5.0 d	5.5 b	4.5 d
12	7.0 b	1.0 d	3.0 c	1.5 f	6.5 b	5.0 d	1.0 d	1.0 f
13	6.5 c	1.0 d	1.5 d	2.0 e	6.0 b	5.0 d	1.0 d	1.0 f
14		1.0 d	1.0 d	2.5 d		6.0 c	1.0 d	1.0 f
15			1.5 d	4.0 c			2.0 c	2.0 e
	PXO 79				*PXO 86*			
6	7.0 b	2.0 b	7.0 a	7.0 b	7.0 a	3.0 a	6.5 a	6.5 a
7	8.5 a	2.5 a	5.0 c	8.0 a	6.0 b	3.0 a	5.5 b	6.0 b
8	7.5 b	2.0 b	5.7 b	7.0 b	5.0 c	3.0 a	6.5 a	5.0 c
9	7.0 b	1.5 c	5.7 b	5.8 c	5.0 c	2.5 b	5.5 b	5.0 c
10	7.0 b	2.0 b	5.0 c	5.0 d	5.0 c	3.0 a	5.0 b	5.0 c
11	7.0 b	1.8 bc	5.0 c	5.0 d	5.0 c	1.8 c	5.0 b	5.0 c
12	5.0 c	1.0 d	1.0 d	1.5 f	5.0 c	1.0 d	1.0 c	1.0 e
13	6.5 c	1.0 d	1.0 d	1.0 g	5.0 c	1.0 d	1.0 c	1.0 e
14		1.0 d	1.0 d	1.0 g		1.0 d	1.0 c	1.0 e
15			1.0 d	3.0 e			1.0 c	2.0 d

[a]Disease scale: 1 = <1% leaf area infected, 9 = >75%. in a column, figures followed by a common letter are not significantly different at the 5% level by Duncan's multiple range test.

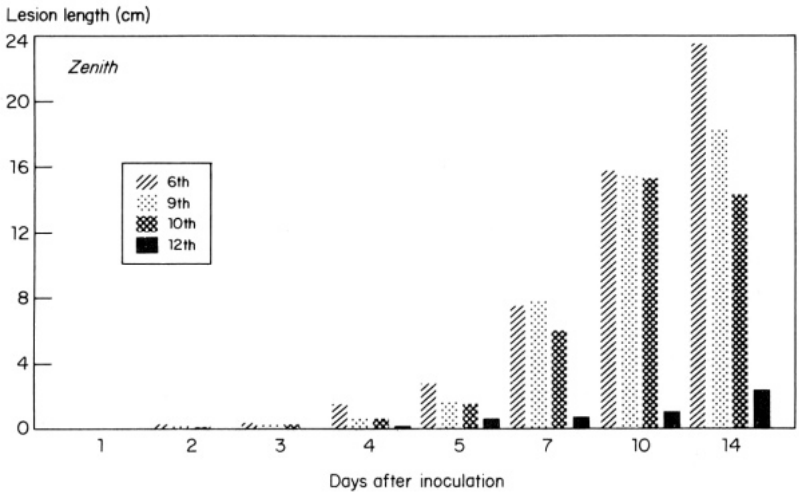
positions earlier than in the two progeny lines (Fig. 1). The lesions on leaf positions other than the flag leaf expanded with time (Fig. 2). Both experiments showed the same results.

When the resistance of Malagkit Sungsong, Zenith, IR944, and IR1695 was evaluated against the 4 pathogen races at 3 temperature ranges (33/25,29/21, and 25/20 °C) in a growth chamber, the disease reaction of Malagkit Sungsong and Zenith was not affected by temperature change (Fig. 3). The adult plant resistance of these cultivars was expressed as the plants matured. The response to infection when the susceptible reaction was expressed according to leaf position, however, was not consistent in different environments, i.e., greenhouse vs growth chamber. The resistance of Malagkit Sungsong and Zenith was demonstrated well at booting with fully expanded flag leaf. BB lesions on the two cultivars developed very slowly,

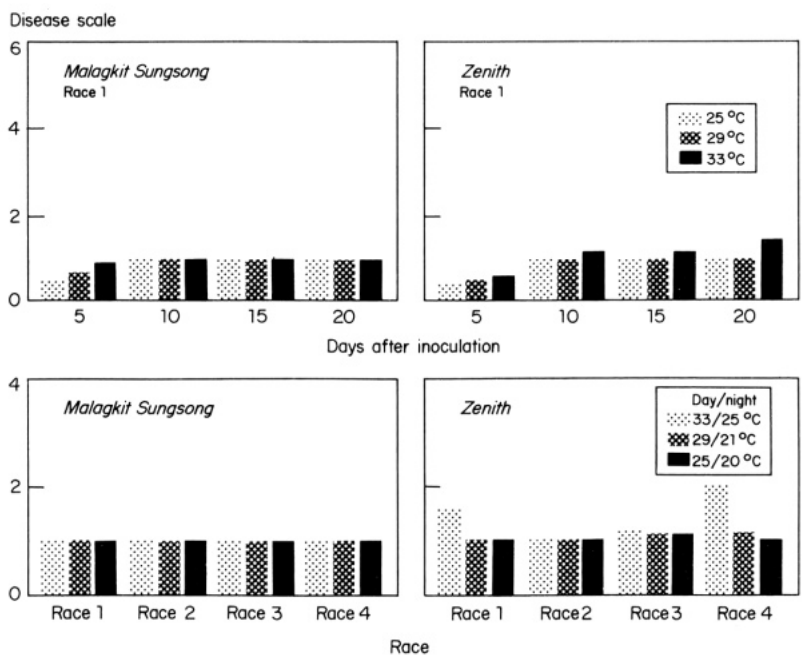


1. Reaction of 2 varieties inoculated at the same time to 4 races of *Xanthomonas campestris* pv. *oryzae* from 3d leaf to flag leaf.

gradually became yellowish brown, and then ceased to progress. At low temperatures (25/20 °C), these varieties showed resistance several days earlier than that at 29/21 °C.



2. Lesion development of bacterial blight at dfifferent leaf positions in cultivar Zenith in the greenhouse



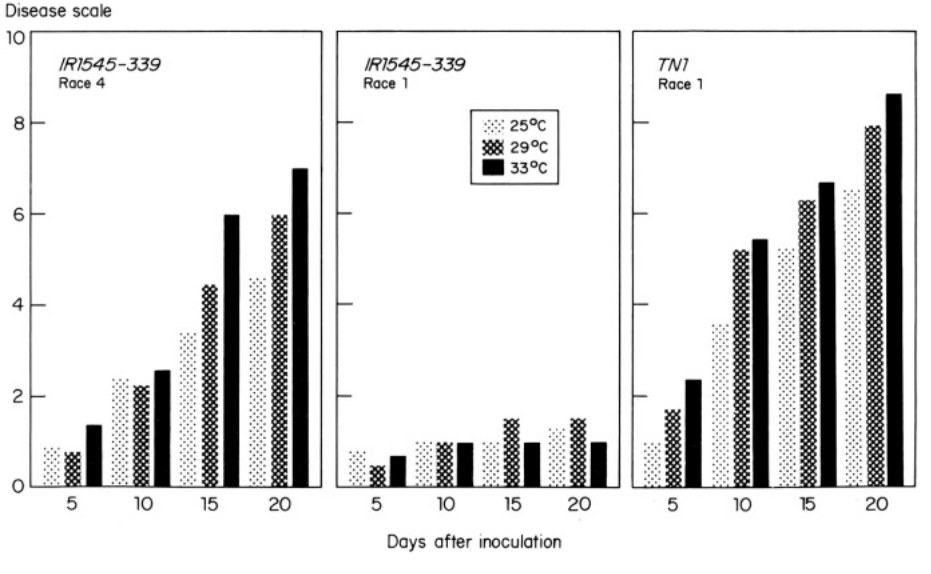
3. Effect of temperature on disease reaction caused by 4 races of Xco on Malagkit Sungsong and Zenith.

The adult plant resistance of Malagkit Sungsong and Zenith was also fully expressed on lines derived from them—IR944 and IR1695, respectively. The lesions, however, appeared to be longer than those on their parents at higher temperature. At lower leaf positions at booting, the difference was not obvious.

Adult plant resistance can be differential (Zhang and Mew 1985), but differential resistance at maximum tillering may not be fully expressed at booting if seedling resistance is moderate. Such resistance may be differential, yet it may not be expressed in later plant development.

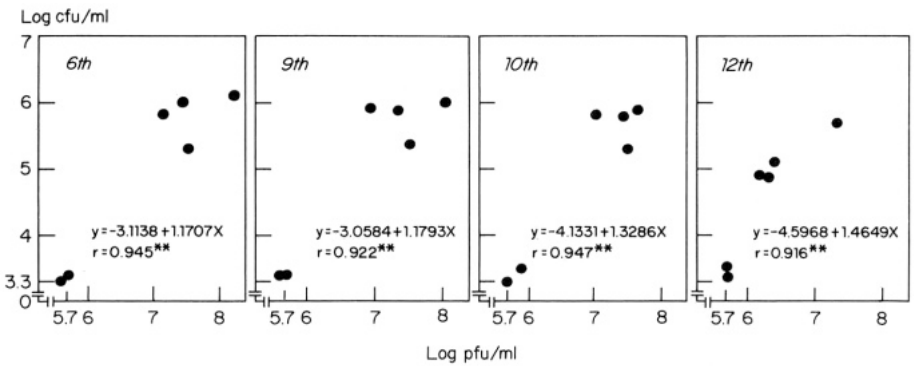
Under the same temperature range in response to incompatible races, seedling resistance was stable. Lesion development was not altered by any temperature tested, but in compatible races, lesion development was faster at higher (33/25 °C) than at lower (25/20 °C) temperatures (Fig. 4).

The growth of Xco in leaf tissues was also assessed. Bacterial number was estimated by direct plating of leaf extracts on peptone sucrose agar (PSA) plates and by bacteriophage method. An aliquot (0.1 ml) of the leaf extract was uniformly spread on the surface of a petri dish containing 20 ml of PSA medium. The rest was mixed with a standard phage suspension obtained by culturing P80 in PXO 61 suspension for 20-24 h. Ten minutes after incubation at 28 °C in aeration, the mixture was centrifuged to remove excess phage. The procedure was repeated once. The precipitate was then resuspended and incubated for 20 min to estimate phage adsorption by the bacterial cells. Final phage plating was 10 h later by serial dilution with PXO 61 as the indicator strain. The phage count, as an indication of bacterial number, was expressed as plaque-forming units (pfu)/cm^2 of leaf area. There was a good correlation (Fig. 5) between bacterial count by dilution plating and phage

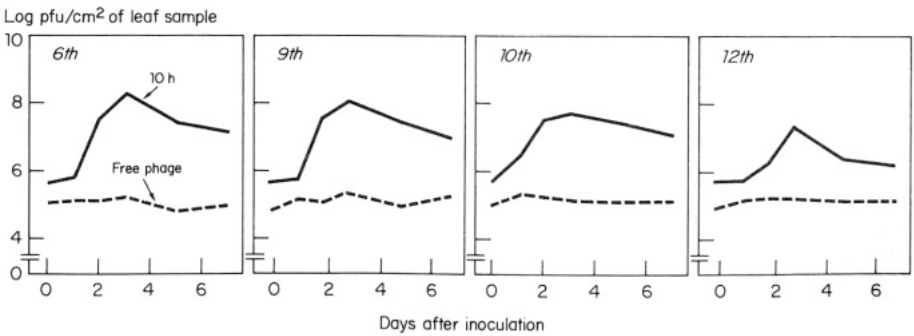


4. Effect of 3 temperatures on lesion development induced by races 1 and 4 of Xco on 2 varieties at booting.

count for the bacteria in the leaf extract. Based on these results, multiplication of Xco in leaves 6, 9, 10, and 12 of Zenith was established (Fig. 6). When phage was added to the leaf maceration, the phage counts were all increased at 10 h compared to 20 min after incubation, which indicated the presence of numerous bacterial cells in the leaf extracts. Similarly, the increase in plaque counts from leaf tissues 24 h after inoculation indicated rapid bacterial multiplication at all 4 leaf positions, regardless of their BB resistance. Based on this indirect method of bacterial enumeration, the bacteria seem to have increased rapidly, achieved a peak population at 72 h, and then slightly declined in all 4 leaves. At 6 and 7 d (120 and 158 h), the sample leaf specimens were totally infected, yet the bacterial numbers in the leaf tissues were even lower than those at 2 or 3 d (24-72 h) after inoculation. Lesion development or expansion was significantly different on leaves 6, 9, and 10 from that on leaf 12 (Fig. 2), yet bacterial number and rate of multiplication followed the same trend; but the lag phase of bacterial multiplication in leaf 12 was longer, and the number was less and declined faster than in the other 3 leaves. The resistance of



5. Relationship between plaque-forming units (pfu) cm^2 and colony-forming units (cfu) cm^2 in leaf samples with bacterial blight lesions at different positions in cultivar Zenith.



6. Population trends of *X. campestris* pv. *oryzae* in the 6th, 9th, 10th, and 12th leaves of Zenith at different days after inoculation,

leaf 12 is likely controlled by mechanisms that have no effect on bacterial multiplication, e.g., inhibition of pathogen growth.

Although the phage method provides an indirect method for counting bacteria, the number of bacterial cells can be estimated with reasonable confidence from the 1-step growth curve of the phage, with 4-6 phages per bacterial cell. The advantage of the phage method in such a study with Xco is its rapid assay and minimal contamination. Xco is a slow-growing bacterium, and no selective medium is available to reduce contamination, especially to assay bacterial multiplication in a host plant that is grown in humid environments.

Finally, 20 selected japonica cultivars from China were tested at both maximum tillering and booting against Chinese isolates differing in virulence. Planting was staggered to synchronize growth stage and inoculation. Differential responses of the varieties were recorded (Table 2), indicating strong interaction at maximum tillering. The difference in lesion length was rather small. The differential interaction was not shown at booting (Table 3). It appears that the differential response is no longer significant in some varieties that show strong interactions at maximum tillering with some isolates, and the resistance increases as the plants approach maturity. In this group of cultivars, resistance appears moderate at maximum tillering and increases to a high level of resistance at maturity.

DISCUSSION

Rice pathologists have just begun to understand the types of resistance in rice to Xco.

The issues of quantitative resistance and HR in relation to host plant development and to disease measurement scales and indices have been reviewed recently (Mew 1987); in response to infection, the resistance of rice plants may be expressed as different levels of resistance, or the resistance may be expressed at different stages of host plant development. Analyzing some of the data presented by Yamamoto et al (1977) and Horino (1985), both Parlevliet (1981) and Mew (1987) recognized that differential resistance is likely at the moderate level of resistance.

Table 2. Strong variety × isolate interaction of selected Chinese cultivars at maximum tillering and booting in provinces of China, Chinese Academy of Agricultural Sciences, 1986.

Variety	Lesion area (%)[a] with isolate				
	Zhe 173	HN84-31	KS6-6	HB82-21	HB84-21
Yangeng 2	30	13	10	-	-
Yunyu 1	12	42	-	-	-
Chaitang	12	-	55	5	-
Aoyu 24	9	40	-	-	-
Wei 84-894	-	-	5	28	
H84-213	8[b]	-	-	-	50[b]
Zhong Xi 8409	35[b]	-	-	-	5d[b]

[a]Mean of 3 replications. (-) = no interaction with any of the 5 isolates. [b]Interaction at booting.

Table 3. Differential resistance of selected cultivars to some Xco isolates in China, Chinese Academy of Agricultural Sciences, 1986.

Cultivar	Lesion area (%) at								
	Maximum tillering			Booting					
	LN84-44	LN85-49	KS6-6	NH85-37	NX85-40	JL85-65	HL85-69	BJ84-3	Heilang Jiang
Tetep	-	-	-	31	14	24	10	-	-
Jingang 30	-	-	-	16	25	-	-	-	-
Chugoku 45	-	-	-	-	-	15	41	-	-
Cas 209	-	-	-	-	-	-	-	22	15
Jae Ye Qinq	-	-	-	-	-	-	-	2	26
Cheongcheongbyeo	60	1	-	-	-	-	-	-	-
Pungsenbyeo	-	1	35	-	-	-	-	-	-
Milyang	3	55	1	-	-	-	-	-	-

Baw and Mew (1988) measured lesion development on rice cultivars at different growth stages with a leaf area meter. Neglect of either category will not facilitate a good understanding of host-pathogen interactions. This applies to genetic analysis of resistance, and to the use of host plant resistance in disease management.

In response to infection by a single pathogen race, rice cultivars were classified into three or four groups. In general, susceptible cultivars such as TN1 and IR8 showed very severe symptoms at 20 d after sowing (DAS), and kresek often developed. But from 30 to 50 DAS, the lesion areas gradually decreased and became constant from 50 to 80 DAS. In cultivars with adult plant resistance, lesion areas decreased from 20 to 40 DAS, but the reaction remained susceptible. Lesions decreased abruptly at 50 DAS, and became less than 5% of the leaf area at 80 DAS. In plants with seedling resistance, the lesion area never exceeded 10% of the leaf area even at 20 DAS; differential resistance was also evident (Chu Juzheng and T.W. Mew, International Rice Research Institute, unpubl. data). However, seedling resistance varied from the 3- to the 6-leaf stage. Moderate resistance may increase as the plants mature. Hence, even though a differential response is present, the reaction may change when plants mature.

Resistance of rice to BB has many characteristics in common with resistance to other diseases. Types of resistance can be distinguished, but whether the resistance is stable or not depends very much on the host-pathogen interaction, which warrants further study.

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Defense mechanisms of rice against bacterial blight caused by *Xanthomonas campestris* pv. *oryzae*

O. Horino and H. Kaku

Induced resistance was recognized when the rice plant was challenged with an incompatible isolate prior to a compatible one. The primary inoculum with stronger aggressiveness inhibited symptom development in the secondary inoculum more effectively than one with normal aggressiveness. In the vascular tissues of the incompatible cultivar, the bacteria were enveloped by fibrillar material (FM), which depressed bacterial multiplication and finally killed the bacteria in the vessels. In the incompatible combination, the greatest amount of ^{14}C was found in the FM and on vessel walls, and less on the bacteria, suggesting association of the FM with the vessel wall. Water pores on rice leaves are involved in the specificity of the rice-*Xanthomonas campestris* pv. *oryzae* interaction. The resistance of *Leersia japonica* to hydathodal invasion by the pathogen is attributed to the well developed outer ledges on the upper sides of the guard cells. Eight antibacterial components from the leaves of susceptible and resistant cultivars were purified and identified. Differences in the antibacterial activity of these components extracted from rice of different resistance levels were closely related to the genes conveying resistance.

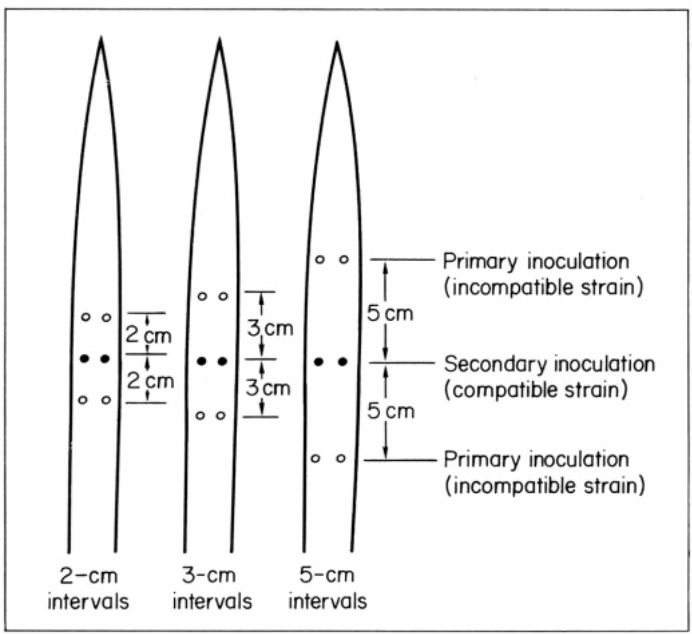
Bacterial blight (BB) of rice caused by *Xanthomonas campestris* pv. *oryzae* (Xco) is one of the most serious diseases of the crop in Asia. Chemical control of BB has not yet been completely successful, and efforts have concentrated on breeding for disease resistance. Systematic attempts to develop BB-resistant rice cultivars in Japan did not begin until after the highly resistant cultivar Asakaze was severely attacked in 1957—the first time a resistant cultivar had become susceptible to a BB pathotype. The epidemic also made Japanese plant pathologists and breeders aware of the importance of resistance mechanisms in rice.

This review discusses the progress of research on the resistance mechanisms of rice cultivars in response to Xco, especially induced resistance, protective reaction, antibacterial components, and infection types in cultivar-bacteria interactions.

INDUCED RESISTANCE

To investigate induced resistance, leaves of a resistant cultivar were always inoculated first with an incompatible pathotype, and subsequently with a compatible pathotype as the secondary inoculum (Horino 1976). As shown in Figure 1, the secondary inoculation was done between the two primary inoculation points. The interval between the primary and secondary points was 2, 3, or 5 cm.

Symptom development on cultivars of the Kogyoku group challenged by compatible pathotypes was greatly inhibited by pre-inoculation with an incompatible pathotype of the pathogen (Table 1). Without pre-inoculation, the average indices for the symptoms induced by compatible pathotypes were 6.3 on cultivar Kogyoku and 5.7 on Hagareshirazu, a nonsignificant difference. On the other hand, if the incompatible pathotype was pre-inoculated on both cultivars, symptom development induced by the compatible pathotype was apparently inhibited (Table 1). The inhibitory effect was greater if the compatible pathotype was inoculated closer to the site of the pre-inoculation with the incompatible pathotype. Table 1 lists the bacterial pathotypes in decreasing order of aggressiveness to the compatible cultivar Kinmaze. The results suggest that a primary inoculum with stronger aggressiveness inhibits symptom development by a secondary compatible pathotype. The effect of pre-inoculation with incompatible pathotypes was also observed on cultivar Tetep. Pre-inoculation with *Erwinia aroideae* and *X. citri,* both nonpathogens of rice, inhibited the development of the challenger, Xco.



1. The double-needle prick method of inoculation.

Double inoculations were next conducted to determine the pattern of resistance induction. The secondary inoculation was carried out 0, 2, 4, 6, and 8 d after primary inoculation. Kogyoku served as the host plant, and the distance between the inoculation points was 3 cm. The inhibitory effect was dependent on the time interval between the two double inoculations. A 2-d interval gave the most inhibitory effect, followed by 0-d and 4d intervals (Table 2). These results indicate that pre-inoculation with either incompatible pathotypes of Xco or nonpathogens can induce resistance in rice against primary compatible challengers.

Table 1. inhibitory effect of pre-inoculation with incompatible pathotypes on disease symptom development induced by compatible pathotype.[a]

Bacterial pathotype used for primary inoculation (incompatible pathotype)	Disease rating index by secondary inoculation (compatible pathotype T7147)					
	Kogyoku			Hagareshirazu		
	2 cm	3cm	5cm	2 cm	3cm	5 cm
T7123	1.7	3.6	3.0	2.0	2.8	2.8
T7177	1.4	1.8	2.3	1.8	1.8	2.6
T7107	1.6	3.6	3.1	2.2	2.5	3.0
Q6808	1.8	3.4	5.8	2.5	2.3	2.6
Giken 44	1.7	4.2	5.2	2.6	3.2	4.2
X-18	2.0	3.8	4.7	2.8	2.1	3.6
Control (needle prick only)	6.3	6.6	6.1	6.0	5.4	5.7

[a] Secondary inoculation was done 3 d after primary inoculation.

Table 2. Symptom development as affected by the time interval between primary incompatible and secondary compatible inoculation.[a]

Time interval (d)	Disease rating index by secondary inoculation
0	3.4
2	2.2
4	3.8
6	4.9
8	4.9
Control	
T7123 (incompatible pathotype only)	0.0
T7147 (compatible pathotype only)	5.8
Needle prick only	5.9

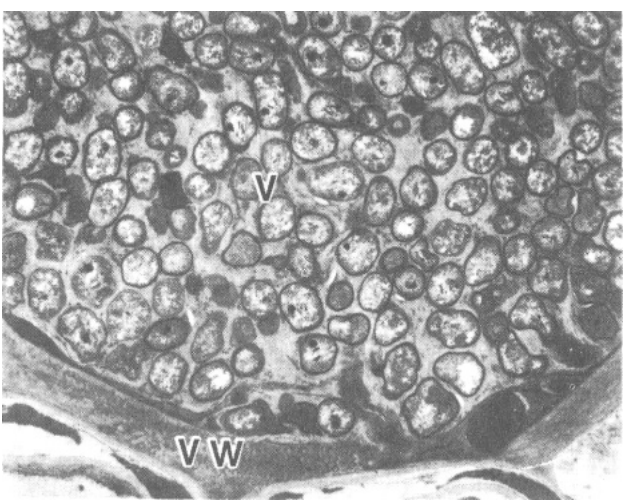
[a] Rice cultivar Kogyoku; distance between points of primary and secondary inoculations = 3 cm.

PROTECTIVE REACTION

Ultrastructural changes of cells in the leaf tissues were studied in rice plants challenged by a compatible pathotype after primary infection with an incompatible pathotype (Horino 1976). Flag leaves of Kogyoku and Kinmaze were inoculated with pathotypes I, II, and III. More than 10 leaves of each combination were inoculated in the center by the double-needle prick method. Specimens were obtained at 3 and 20 d after inoculation (DAI). Leaf pieces, 0.5 cm long, were excised from the inoculation site and prepared for microscopic examination. Comparable tissues from uninoculated but wounded leaves served as controls. The excised tissues were fixed in Millonig's glutaraldehyde (Millonig 1963) and postfixed in 1% OsO_4 in phosphate buffer (pH 7.2). The tissue samples were embedded in epoxy resin after dehydration in a graded series of ethyl alcohol. Ultrathin sections were stained with uranyl acetate and lead citrate.

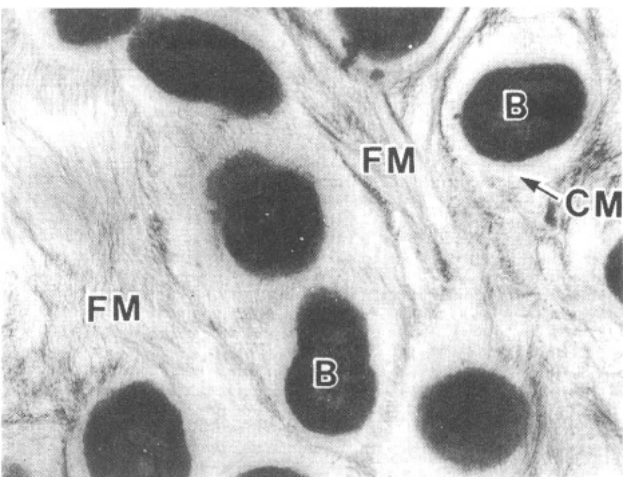
The bacteria in the host tissue had spread both upward and downward through the xylem vessels from the inoculation point. The pathogen had reproduced only in the vessels of the vascular bundles. Figures 2 and 3 show thin sections of vessels at 20 DAI with a compatible pathotype. Bacterial cells are surrounded by abundant fibrillar material (FM) in the vessels. With the compatible combination, the bacterium is surrounded by an electron-transparent layer (Fig. 3) that appears to be identical with the capsular material composed of polysaccharides. Such capsular material is very similar in appearance to that formed in cabbage tissues infected with *X. campestris* (Wallis et al 1973). FM is also observed around the bacteria.

Figure 4 shows the incompatible (T7174) and Figure 5 shows the compatible (T7147) bacterial strains in the xylem vessels of Kogyoku at 3 DAI. In the

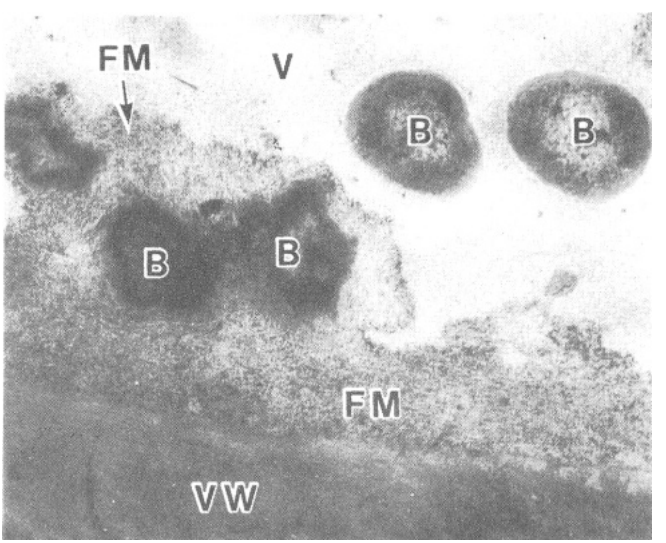


2. Cross-section of a vessel (V) in aleaf inoculated with a compatible bacterial strain. Abundant bacteria are surrounded by fibrillar material, which formed in the vessel wall (VW) (20 d after inoculation, 5000X).

incompatible combination, most of the bacteria were enveloped within 3 DAI by FM near the vessel walls (Fig. 4). The normal bacterium is rod-shaped, but the one enveloped by FM has become irregular, and at the same time its cytoplasm has become electron dense. The bacteria enveloped by FM seem to have been suppressed



3. Fine structure of the fibrillar material (FM) and bacteria in a vessel. The bacteria (B) are surrounded by an electron-transparent layer. This layer appears to be identical with the capsular material (CM) composed of polysaccharides (20 d after inoculation of the compatible cultivar. 25200X).



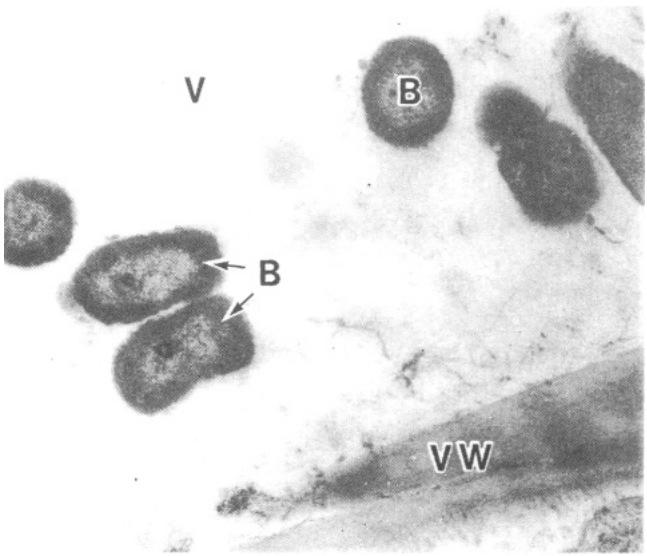
4. Electron micrograph of bacterial infection in the incompatible cultivar. Bacteria (B) enveloped by fibrillar material (FM) became irregular in shape, and their cytoplasm became electron dense (3 d after Inoculation. 6500X). V = vessel. VW = vessel wall.

or killed in the leaf vessels of the incompatible cultivar, based on their electron microscopic appearance (Fig. 4). Thus, FM may inhibit bacterial reproduction. Bacteria enveloped by FM seem to have degenerated, but the structure of the non-enveloped ones appears normal. Nearly 65% of the bacteria have been enveloped by FM in the incompatible combination. The FM induced in the incompatible cultivar is not always present together with the bacteria, and is sometimes present in vessels where the bacteria are not found (Fig. 6). FM is not observed in vessels of the compatible tissues, and the bacteria appear to have multiplied actively there (Fig. 5).

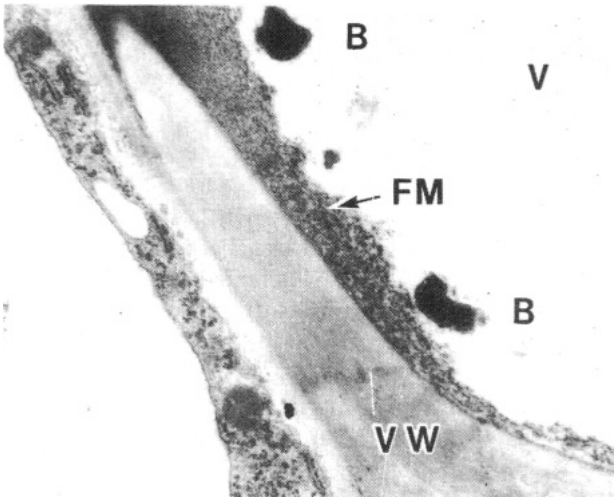
Thus, at 3 DAI, FM has been produced in the xylem vessels, but only when the leaves were inoculated with the incompatible strain. Such FM is also found in cultivars of the Rantai Emas and Wase Aikoku groups inoculated with incompatible strains..

At 20 DAI, however, FM also forms in vascular tissue infected with compatible strains, although alteration of the bacterial fine structure is not as extensive as that observed in the compatible combination (Fig. 3).

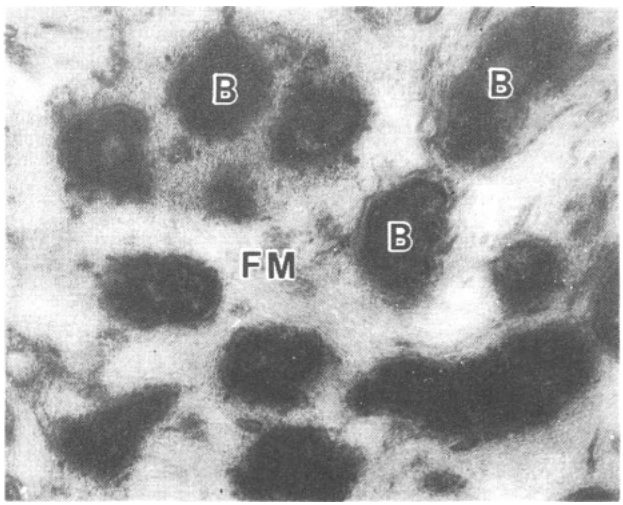
To examine the origin of this FM, sections of bacteria grown on peptone sucrose agar (PSA) medium were observed under an electron microscope. No FM was seen around the cultured bacteria, but large amounts were present around bacteria in the vessels of the incompatible cultivar (Fig. 7). This FM could hardly have been of bacterial origin, since it was ultrastructurally different from the bacterial capsule and was not observed around bacteria treated with a bactericidal chemical, 2-amino-l,3, 4-thiadiazole. When cultured bacteria were treated with the chemical, the bacterial



5. Electron micrograph of bacterial infection in the compatible cultivar. No fibrillar material can be observed around the bacteria (B) in the vessel (V), and the bacteria appear to reproduce actively (3 d after inoculation, 13600X). VW = vessel wall.



6. Cross-section of a vessel (V) in a leaf inoculated with an incompatible strain. Fibrillar material (FM) is present near the vessel wall (VW), where bacteria (B) are not found (3 d after inoculation, 8700X).



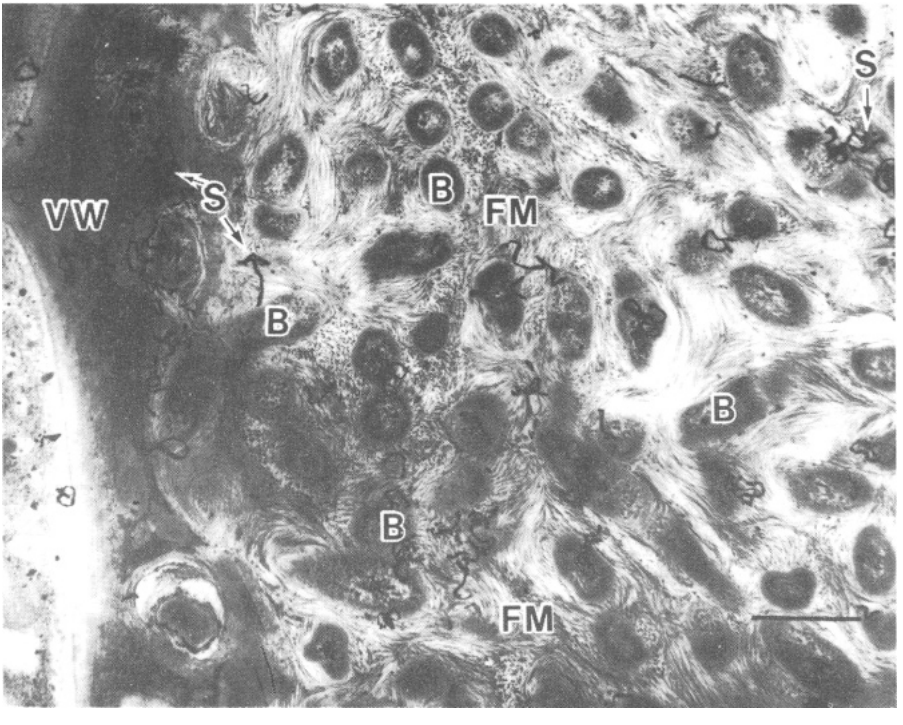
7. Fine structure of bacteria in the incompatible cultivar. The bacteria (B) have become weak or almost dead based on their appearance. Note the fibrillar material (FM) filling in between the bacteria (20 d after inoculation, 11800X).

wall remained intact, but the cytoplasm disappeared (Horino 1972). The absence of FM around bacteria killed by this chemical treatment suggests that the material was not produced as a result of bacterial death. It is conceivable, therefore, that the FM is of host origin and may be involved in the protective reaction of the host. These results thus substantiate the conclusion of Wallis et al (1973) for cabbage black rot caused by *X. campestris.*

FM thus develops within 3 DAI as the result of an incompatible interaction in the leaf vessels. It appears to depress bacterial reproduction, and finally to kill bacteria in the vessels. Because in the compatible cultivar, FM appeared at a later stage after inoculation, at around 20 DAI, it seems to have little ability to kill the bacteria. Thus, rapid FM appearance might be associated with BB resistance.

Kelman and Sequeira (1972) classified mechanisms of induced resistance into two types: hypersensitive reactions and protective reactions. The resistant reaction to BB could hardly be a hypersensitive reaction, because no visible necrosis of host cells is observed after inoculation with incompatible Xco strains. Rather, the resistance mechanism may be attributable to FM formed in the vascular vessels, which probably inhibits the growth or movement of the bacteria.

To obtain information on precursors of FM, rice leaves infected with Xco were treated with $[^{14}C]O_2$, then investigated by autoradiography (Horino and Inaba 1979). In the incompatible host-bacterium combination, the vessel became filled with abundant FM. More ^{14}C was incorporated into the vessels than other leaf tissues. In the vessels, more silver grains could be counted on the FM than on the bacteria (Fig. 8). On the other hand, in the compatible host-bacterium combination,



8. Electron microscope autoradiograph of the incompatible combination. Four days after inoculation, rice plants were exposed to $^{14}CO_2$. Leaf pieces were taken from the inoculation site immediately after exposure to $^{14}CO_2$. In the vessel, more silver grains (S) could be counted on the fibrillar material (FM) than on the bacteria (B) (12000X). VW = vessel wall.

FM was not seen in the vessels. The distribution of ^{14}C in the compatible and incompatible host-bacterium combinations immediately after ^{14}C assimilation is summarized in Table 3. In the incompatible combination, the average number of silver grains was greatest on the FM, followed by the chloroplasts, the vessel walls, and the bacteria. By contrast, in the compatible combination, the number of silver grains was greatest on the chloroplasts, then on the vessel walls, on the bacteria, and in the vessels. Thus, FM that is specifically related to the incompatible combination is at least partly composed of ^{14}C assimilated by photosynthesis. The fact that in the incompatible combination the greatest number of silver grains was found on the FM and vessel walls, and fewer on the bacteria, suggests an association of the FM with the vessel walls.

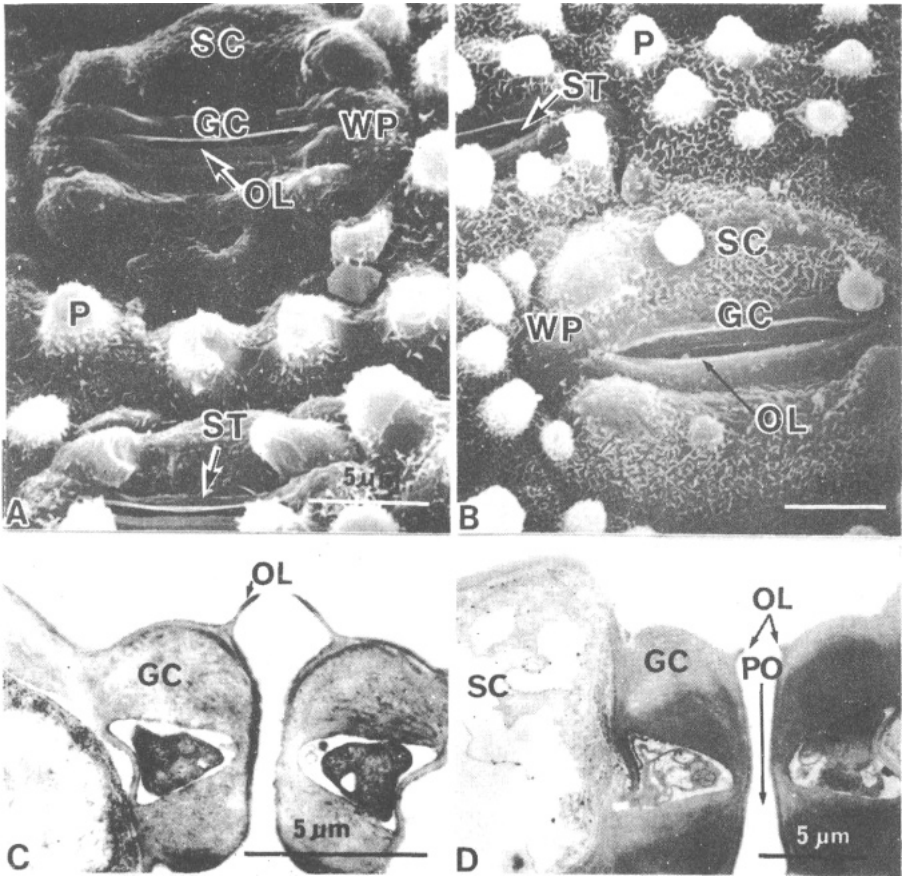
Leersia sayanuka is a major host of overwintering Xco in Japan. *L. japonica* grows under the same environmental conditions as *L. sayanuka* and shows clear BB symptoms only by the wound inoculation method. *L. japonica,* however, is not infected with BB naturally and plays no role in its hibernation (Tabei 1977). Horino (1984) reported the results of transmission and scanning electron microscopic examination of water pores in *L. japonica* and *Oryza sativa* for Xco penetration (Fig. 9). He attributed *L. japonica* resistance against hydathodal invasion by the pathogen to the well-developed outer ledges on the upper sides of the guard cells (Fig. 10).

Mew et a1 (1984) investigated the establishment of virulent and avirulent Xco strains on the leaf surfaces of resistant and susceptible cultivars. They showed that water pores are not only portals of entry for the causal bacterium but are also involved in the specificity of the rice-Xco interaction. Twenty-four hours after inoculation, bacteria of strain PXO 61, which is virulent to cultivars TN1 and Cas 209, multiplied immediately outside the water pores, and some bacteria had gained entrance through these pores. Cells of PXO 101, a strain that has lost its virulence, did not multiply significantly on the leaf surfaces and appeared to be embedded in a thin layer of exudate secreted by the water pores. The exudate eventually sealed the openings of these pores. Bacterial cells of PXO 86, which is virulent to TN1 and

Table 3. Comparison of the number of reduced silver grains (RSG) on the autoradiograph at 4 d after inoculation, immediately after exposure to $(^{14}C)O_2$.

Part	Incompatible combination (T7174 - Tetep)			Compatible combination (T7133 - Tetep)		
	Total RSG[a] (no.)	Total area observed (μm^2)	RSG (no./μm^2)	Total RSG[a] (no.)	Total area observed (μm^2)	RSG (no./μm^2)
Bacteria	284	617	0.46	818	880	0.93
FM structure	3197	1872	1.75	—	—	—
Vessel	—	—	—	1629	1917	0.85
Vessel wall	562	410	1.37	243	238	1.02
Chloroplast	1931	116	1.73	21 62	1150	1.88

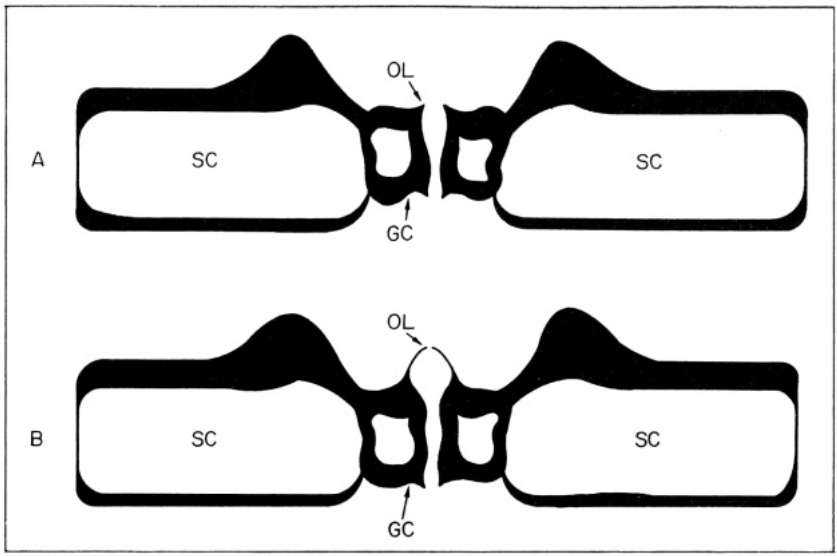
[a] Total number of RSG in 25 fields, each field 16.2 X 12.3 μm.



9. Scanning (A, B) and transmission (C, D) electron micrographs of water aperture in *Leersia japonica* and *Oryza sativa.* A and B are *L.japonica,* C and D are *O. sativa.* The water pore aperture (WP) consists of guard cells (GC), subsidiary cells (SC), and outer ledges (OL). P = papilla, PO = pore opening, ST = stomate.

avirulent to Cas 209, multiplied on the water pores of TN1 but were trapped in the exudate of Cas 209 at 48 h after inoculation. Bacterial cells of all three strains were not observed to multiply on stomata of either cultivar. These results suggest that bacteria are immobilized and inhibited from dividing by excretions from water pores in incompatible host-bacteria combinations.

A postinfection defense mechanism with tissue specificity was also demonstrated (Tsuno and Wakimoto 1983). When Xco was infiltrated into the intercellular spaces in parenchymatous tissue, the host plasmalemma adjacent to the bacterial cells receded from the host cell wall, showed vesiculation, and became indistinct. Seven days after infiltration, the bacterial cells had increased to some extent in the intercellular spaces and inside the cells of the host tissue. Most of them, however, were morphologically abnormal and were surrounded by FM originating from host cell walls and cytoplasm, and they had become immobilized.



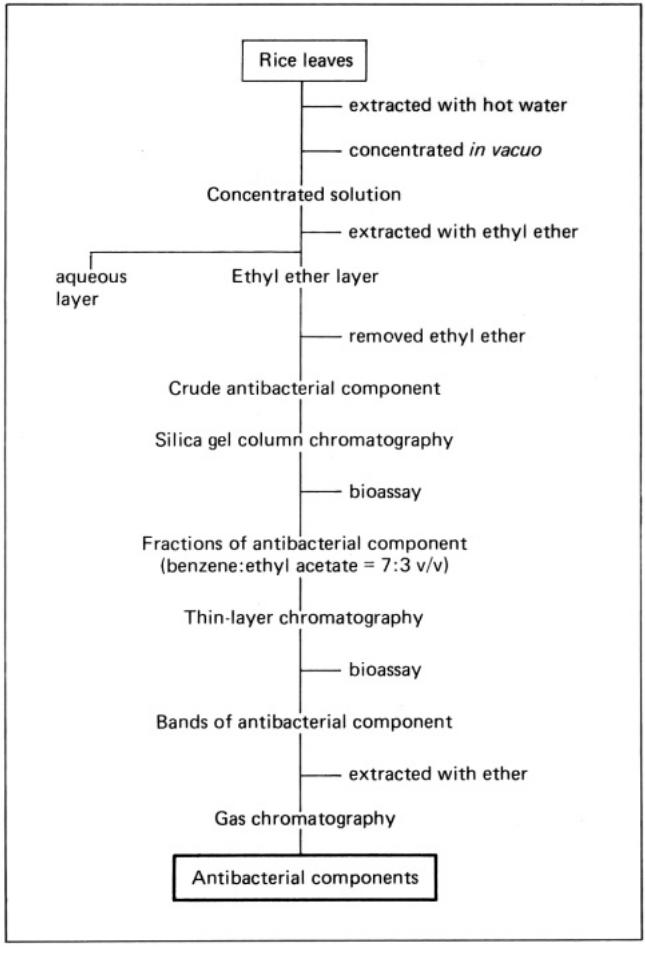
10. Cross-sections of water pores of *O. sativa* (A) and *L. japonica* (B). *L. japonica* has welldeveloped outer ledges (OL) on the upper sides of the guard cells (GC). SC = subsidiary cells.

ANTIBACTERIAL COMPONENTS

To elucidate the defense mechanism of rice, Horino (unpubl. data) and Sato (1979) isolated antibacterial components of rice leaves from susceptible and resistant cultivars following the steps shown in Figure 11. Dried powder of healthy rice leaves was treated with hot water. The solution was concentrated, then treated with ethyl ether. The resulting crude antibacterial components were subsequently fractionated by column and gas chromatography. The final products showed antibacterial activity. For the bioassay, 2 ml of antibacterial component solution, 1 ml of bacterial suspension, and 2 ml of liquid PSA medium were mixed in a test tube and inoculated on a rotary shaker at 27 °C for 12 h. The antibacterial components obtained from 10 g of dried leaf powder were dissolved in 10 ml of water.

When solutions diluted four times were tested, the components of the susceptible cultivar Kinmaze permitted slight bacterial multiplication, but the components of three resistant cultivars completely inhibited multiplication. A similar bioassay was conducted using test solutions of an eightfold dilution. The antibacterial components of Kinmaze and Kogyoku did not suppress bacterial multiplication, while those of Tetep and Chugoku 45 showed 100% inhibition. There were considerable differences in the activities of antibacterial components extracted from healthy rice cultivars having different levels of resistance. Antibacterial activity was higher in Chugoku 45, Tetep, and Kogyoku, in that order, and lower in Kinmaze. Based on these results, Chugoku 45, a representative resistant cultivar, and Kinmaze, a representative susceptible cultivar, were used as test plants for subsequent experiments.

The antibacterial activities of the fractions obtained from susceptible and resistant cultivars are compared in Table 4. In both cultivars, the fourth fraction



11. Method for isolating antibacterial components from healthy rice leaves.

Table 4. Antibacterial activity of leaf components fractionated from susceptible and resistant cultivars by column chromatography.

Cultivar	Inhibition rate[a]							
	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8
Kinrnaze (susceptible)	–	–	–	+++	–	–	–	–
Chugoku 45 (resistant)	–	–	++	+++	–	–	–	–

[a]Inhibition rate of bacterial multiplication <20% = –, 20-39% = ±, 40-69% = +, 70-89% = ++, >89% = +++.

showed high antibacterial activity. The 3rd fraction of Chugoku 45 also showed high activity (70% inhibitory effect). When the antibacterial components were fractionated by column chromatography, Chugoku 45 tended to show higher activity than Kinmaze.

The column chromatography fraction that had antibacterial activity was concentrated and analyzed by thin-layer chromatography, Among eight bands, obtained from both cultivars, the sixth and seventh had high antibacterial activity (Table 5), suggesting that such activity depends on at least two components that are identical in susceptible and resistant cultivars, and that the amount of the components is greater in resistant cultivars.

To identify the antibacterial components, they were isolated and purified from 10 kg of dried leaf powder of Kinmaze and Chugoku 45 by column, thin-layer, and gas chromatography, successively. Components were further analyzed and identified, by ultraviolet absorption spectroscopy, infrared spectroscopy, mass spectroscopy, gas chromatography-mass spectroscopy, and nuclear magnetic resonance (Fig. 12). *Trans*-2-hexenal was obtained by steam distillation of fresh leaves.

The antibacterial activities of the eight components were compared. Syringaldehyde at 200 µg/ml, p-hydroxybenzaldehyde at 400 µg/ ml, vanillin at 400 µg/ ml, and coniferylaldehyde at 200 µg/ ml completely inhibited bacterial multiplication (Fig. 13). By contrast, the activity of acetosyringone was relatively lower: 400 µg/ ml inhibited only 60% of multiplication (Fig. 13). The activity of trans-2-hexenal was greatest: 50 µg/ ml showed a 100% inhibitory effect, and 25 µg/ ml was 50% effective. The activities of *trans*-2-hexenoic acid and *cis*-3-hexenoic acid were also great: 100 µg/ml showed 100% inhibition (Fig. 13).

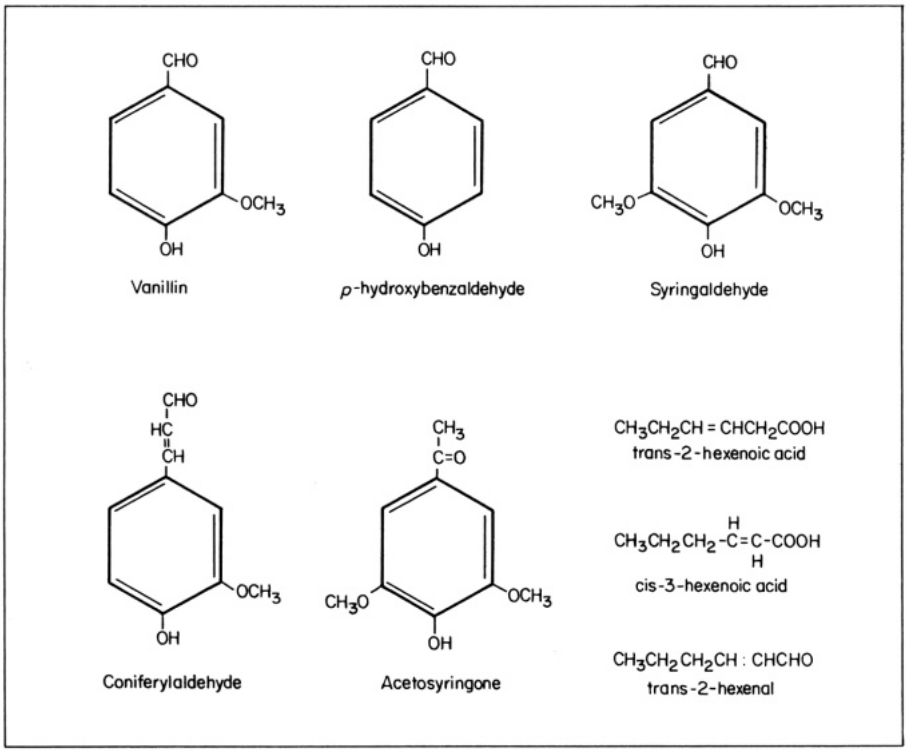
The effect of these antibacterial components in controlling BB in vivo was examined. Hexenoic acid was administered to rice roots, which were then inoculated with Xco by the spray method. Infection in treated plants was effectively controlled, compared with that in untreated plants.

BB of rice is a typical vascular disease, and thus bacterial multiplication is limited to the vessels. Of the eight antibacterial components found, all except the hexenoic acids are oxidized lignin products with aldehyde and phenol groups. The relation of

Table 5. Antibacterial activity of leaf components fractionated from susceptible and resistant cultivars by thin-layer chromatography.

Cultivar	Inhibition rate[a]							
	Fraction[b] 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6	Fraction 7	Fraction 8[c]
Kinmaze (susceptible)	–	–	–	–	–	+	++	–
Chugoku 45 (resistant)	–	–	–	–	–	++	+++	–

[a]Inhlbition rate of bacterial multiplication <20% = –, 20-39% = ±, 40-69% = +, 70-89% = ++, >89% = +++. [b]Original point. [c]Front.

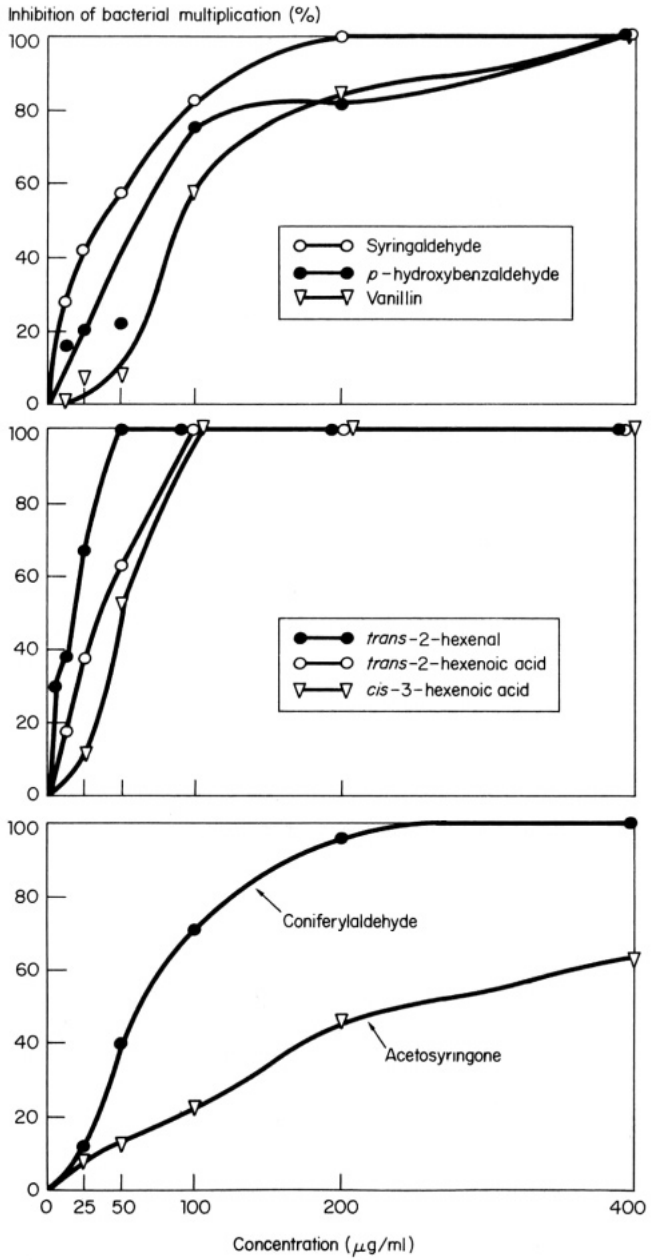


12. Chemical structures of antibacterial components of rice effective against *Xanthomonas campestris* pv. *oryzae*.

these components to vessel lignin, and their association with resistance mechanisms, are interesting problems. To establish the amounts of antibacterial components in the leaves and to examine whether or not the various levels of resistance are explained by varied amounts of the components would be important research subjects.

INFECTION TYPES

Kaku and Kimura (1978) grouped the resistance reactions of rice cultivars to Xco strains into three categories: symptomless, small yellow lesions, and brown necrosis (Table 6). They also found that the types of resistance reaction are closely related to resistance genes (Table 7). Kogyoku group cultivars carrying gene *Xa-1* showed symptomless resistance reactions to strains of bacterial group I. The reactions of Rantai Emas group cultivars to bacterial groups I and II were symptomless and small yellow lesions, respectively. The former was shown to be controlled by gene *Xa-1* and the latter by *Xa-2* (Sakaguchi 1967). All Wase Aikoku group cultivars with gene *Xa-w (Xa-3)* showed browning reactions to bacterial groups I, II, and III. The



13. Inhibitory effects of antibacterial components of rice on the multiplication of *Xanthomonas campestris* pv. *oryzae.*

reaction of Kogyoku and Java group cultivars (having gene *Xa-kg*) to bacterial group V was symptomless to small yellow lesions. The reaction of Java 14 to bacterial group I, whose resistance is controlled by both *Xa-1* and *Xa-3,* was minute brown necrosis.

Ogawa et a1 (1986) indicated that gene *Xa-6* could be identical to *Xa-3.* The line IR1695 with Xu-6 showed browning reaction to incompatible pathotypes 1,2,3, and 4 in the Philippines.

Thus, the different types of resistance reaction have been shown to be gene-controlled. In addition, they also appear to be very similar to the "infection types" reported for some fungal diseases. Infection types in the host-parasite interaction

Table 6. Resistance reaction[a] in differential cultivars to 5 races of *Xanthomonas campestris* pv. *oryzae.*

Cultivar	I	II	III	IV	V
Kinmaze	TS	TS	TS	TS	TS
Kogyoku	NS (*Xa-1*)	SS (*Xa-2*)	TS	TS	SS (*Xa-kg*)
Rantai Emas 2	NS (*Xa-1*)	SS (*Xa-2*)	TS	TS	NS (?)
Wase Aikoku 3	BR (*Xa-w*)	BR (*Xa-w*)	ER (*Xa-w*)	TS	TS
Java 14	BR (*Xa-1, Xa-w*)	BR (*Xa-w*)	ER (*Xa-w*)	TS	SS (*Xa-kg*)

[a]*NS* = symptomless, SS = small yellow lesions, BR = brown necrosis, TS = typical symptoms. Genes for resistance are given in parentheses.

Table 7. Known resistance genes and their relation to reaction against incompatible strains of *Xanthomonas campestris* pv. *oryzae.*

Cultivar group	Cultivar	Resistance gene	Reaction to incompatible isolate
Kogyoku	Kogyoku	*Xa-1*	Symptomless
	Norin 27	*Xa-1*	Symptomless
	Zensho 26	*Xa-1*	Symptomless
Rantai Emas	Rantai Emas 2	*Xa-1, Xa-2*	Symptomless, limited lesion
	Chinsurah Boro II	*Xa- 1, Xa-2*	Symptomless. limited lesion
	Kele	*Xa-1, Xa-2*	Symptomless, limited lesion
	Tetep	*Xa- 1, Xa-2*	Symptomless, limited lesion
	Tokushu-Daisui-To	*Xa-1, Xa-2*	Symptomless, limited lesion
Wase Aikoku	Wase Aikoku 3	*Xa-w*	Browning
	Nagomasari	*Xa-w*	Browning
	Java 14	*Xa-w*	Browning
	Koentoelan	*Xa-w*	Browning
	TKM6	*Xa-4*	Symptomless, limited lesion

seem to be caused by two factors: the degree of bacterial multiplication in host tissue, and the ability of the host tissue to undergo necrosis.

Through population studies (Kaku 1980, Kaku and Kimura 1978), the threshold point of typical symptom expression appears to be around 10^6 colony-forming units (cfu)/inoculated tissue. In the resistance reaction conditioned by *Xa-1,* the maximum level of bacterial growth was less than 10^6 cfu/ inoculated tissue, while it was slightly higher than 10^6 cfu/inoculated tissue in reactions conditioned by Xu-2 or *Xa-kg.* As mentioned above, plants remained symptomless in the former, and small yellow lesions appeared in the latter. In compatible cultivar-strain combinations, bacteria could multiply above the level of 10^8 cfu/inoculated tissue, and large typical symptoms developed.

The atypical symptom—browning—controlled by *Xa-w* was induced below the level of 10^5 cfu/inoculated tissue, and the population of incompatible strains reached its peak before browning ceased to spread. Histological studies showed that incompatible strains could multiply and move to some extent from the inoculation point without inducing browning. Once browning was induced, however, it spread in advance of the bacterial movement. The incompatible strains were finally confined to the necrotic area. The cytological change in tissue browning was very similar to that in typical hypersensitive reactions in fungal diseases. Thus, the browning reaction conditioned by *Xa-w* appears to be involved in the resistance mechanisms.

BB of rice is a typical vascular disease. Vascular browning has been described as a typical symptom in many vascular diseases (Beckman 1964). In contrast, browning in rice-Xco relationships is characterized as an atypical symptom. That vascular browning is a result of the oxidation and polymerization of phenolics through the action of polyphenol oxidase was established by Dimond (1970). In rice leaves, however, such an enzyme has not been detected. Comparative analysis of polyphenol compounds and oxidation enzymes among cultivars with each resistance gene is necessary to elucidate resistance mechanisms.

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Notes

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How variable is *Xanthomonas campestris pv. oryzae?*

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Xanthomonas campestris pv. *oryzae* (Xco), the pathogen of bacterial blight in rice, varies in virulence to rice varieties with inherent resistance. Six races of the bacterium have been identified on a set of differential varieties. Each race has specific virulence to rices with different resistance genes following a gene-for-gene concept in the host-parasite interaction. Xco also exhibits variation in phenotypic characters, but not enough to indicate the existence of biotypes. The relationship between these characters and virulence was studied. No distinct cluster among 52 Xco isolates on 103 phenotypic features was detected by numerical analysis. They were homogeneous regardless of their cultivar specificity. However, the ability to grow on a medium with trehalose and with sodium aconitate as the C source was associated with certain races. More than 90% of all races except race 1 utilized trehalose, whereas more than 90% of races 2, 3, and 5 utilized sodium aconitate as the C source. Using 5 phage groups to type 225 Xco isolates in relation to virulence, 8 lysotypes were identified. Race 4 was sensitive to group D phages, whereas race 6 was sensitive to none of the phage groups. Most of race 1 (56.1%) fell into cluster 1. whereas most of race 2 (50.9%) and race 3 (72.1%) fell into cluster 3. Overlapping reactions of the races were noted.

Plant pathogens are variable. Although plants and pathogens have co-evolved over long periods, human interference through breeding and management practices has disturbed this balanced condition. When pressure is exerted on the pathogens to change, they may do so rapidly and dramatically, either by mutation or by genetic recombination. The subject of variability is of vital importance for a comprehensive understanding of the problems involved in control through host resistance.

Variation, in terms of the host-pathogen interaction, means a potential shift of the pathogen to match the resistance of a currently grown variety. The dynamic nature of pathogenicity is demonstrated by the continuous appearance of new pathogenic races under natural conditions. In cotton, new virulent races of the bacterium *Xanthomonas campestris* pv. *malvacearurn* became a problem almost immediately after resistant varieties were developed and cultivated (Brinkerhoff

1970). In rice, the variability of *X. campestris* pv. *oryzae* (Xco) was realized when the resistant variety Asakaze succumbed to bacterial blight (BB). In the Philippines, BB-resistant varieties were also infected with the disease.

Bacterial races have been identified in various host-pathogen systems such as those involving cotton (*X. campestris* pv. *malvacearum* [Brinkerhoff 1970]), pepper (*X. campestris* pv. *vesicatoria* [Cook and Stall 1969]), soybean (*Pseudomonas glycinea* [Cross et al 1966]), and several other hosts (*P. solanacearum* [Buddenhagen et al 1962]). To determine whether differences in pathogenicity on different varieties of the host are correlated with characters of the pathogen determinable in the laboratory, various properties including physiological and biochemical characters and sensitivity to phages were studied by many workers before the advent of molecular genetic techniques.

Since a comprehensive review of current research on BB has recently been published (Mew 1987), this paper presents our work on and a review of variation in the rice BB pathogen, with emphasis on phenotypic variation and virulence to rice varieties and the relationship between these properties.

PHENOTYPIC VARIATION

Physiological and biochemical characteristics

The identity of Xco, the causal organism of BB of rice, has been known since Ishiyama (1922) described it. However, some of the physiological and biochemical descriptions in the literature were conflicting (Chakravarti and Rangarajan 1967, Mukoo and Isaka 1964, Shekhawat and Srivastava 1968). Reddy and Ou (1976) compared some physiological and biochemical characteristics of Xco reported by various researchers. Conflicting results were found for gelatin liquefaction, H_2S production, NH_3 production, starch hydrolysis, litmus milk reaction, lipase activity, pectolytic reaction, and acid production from lactose, maltose, and dextrin. Reddy and Ou (1976) confirmed the results of earlier workers regarding gelatin liquefaction (+), production of NH_3 and H_2S (+), starch hydrolysis (-), litmus milk reaction (alkaline), and acid production from certain sugars. From a comparison of 40 Xco isolates from different Asian countries, they concluded that no distinct biochemical types existed in Xco, in contrast to those in *Pseudomonas solanacearum* (Hayward 1964b) and *X. campestris* pv. *malvacearum* (Hayward 1964a). However, they noted minor quantitative variations as expected with other microorganisms. Within *P. solanacearum,* Hayward (1964b) classified four biochemical types by conventional bacteriological methods, namely their capacity to oxidize three disaccharides (maltose, lactose, and cellobiose) and three hexose alcohols (mannitol, sorbitol, and dulcitol). In *X. campestris* pv. *malvacearum,* Hayward (1964a) classified the cultures into two groups on the basis of their utilization of casein, gelatin, and lactose and on differences in colony form and texture when first isolated. However, Shekhawat and Srivastava (1968) found two distinct biochemical groups in Xco; group 1 was similar to the normal Xco, while group 2 hydrolyzed starch completely, produced an acidic reaction to litmus milk, and was insensitive to penicillin. Reddy and Ou (1976) presumed group 2 to be contaminants.

Recently, Tsuchiya et al (1982) compared the physiological and morphological characteristics of wild virulent Xco isolates and induced mutants. All the strains of pathogenic groups I, II, and III showed similar characters in most of the 32 bacteriological tests examined. The researchers concluded that most of the virulent strains. of Xco from the Philippines have similar bacteriological characteristics, as reported by Reddy and Ou (1976).

Based on a more recent comparison of 133 bacteriological characters and protein electrophoregrams of 35 Xco isolates from Japan (11), India (7), Bolivia (l), Nepal (l), Indonesia (4), China (1), Philippines (7), Mali (1), and Colombia(2), the isolates were fairly homogeneous (Vera Cruz et al 1984), confirming the results of Reddy and Ou (1976), Hifni et al (1975), and Tsuchiya et al (1982). No phenotypic features were found that allowed a subdivision of Xco. Neither the geographical distribution nor the virulence of the, strain was reflected in the dendrogram of similarities. Strains from Japan and the Philippines representing the various pathogenic groups or virulence groups did not show clear correlation with the biochemical or physiological features tested. However, among the Xco strains from the Philippines, PXO 61 and PXO 10 (representing race 1) did not grow on a medium with trehalose, while PXO 82 and PXO 86 (race 2) and PXO 79 (race 3) grew on a medium containing sodium aconitate.

Sensitivity to bacteriophages

Xco was one of the first bacterial pathogens reported to be infected by bacteriophages. If obtained from an adequate number of samples, phages can be used to distinguish bacterial species or strains. For complete accuracy, Okabe and Goto (1963) suggested the possibility of distinguishing bacterial species or strains by phages when the pathogen under consideration is specifically lysed by given phages, and when no mutants are present either in the phages or their host bacteria. This is the main reason why the application of phages to the problem of species identification or strain differentiation is difficult. However, in *X. citri, X. pruni,* and *X. translucens,* the presence of several strains differing in sensitivity to each of their specific phages has been clearly detected (Eisenstark and Bernstein 1955, Goto and Okabe 1962, Katznelson and Sutton 1953).

The bacteriophage of Xco was first isolated from diseased ricefield soil in Fukuoka Prefecture, Japan. Since then, Xco phages in Japan have been grouped and characterized. All studies, however, have shown that the sensitivity of a bacterial strain to phages has no relation to its virulence (Ou 1985).

At the International Rice Research Institute (IRRI), several attempts were made to classify Xco strains from different geographical areas in the Philippines by their reactions to phages as an aid to epidemiological studies of BB. The phages were isolated from ricefield water from the IRRI farm and from the same provinces where the bacterial isolates were collected. Two hundred seven Xco isolates maintained either in skim milk or lyophilized culture were typed against 67 phage isolates maintained in CaVf-medium at 4 °C. The bacterial isolates were revived in peptone sucrose agar, while a single plaque from each phage stock culture was multiplied in the homologous host Xco isolate determined by previous tests. The phage isolates

were individually inoculated by multipoint inoculator to duplicate plates seeded with 1 ml each of bacterial isolate at 10^9 colony-forming units/ml. After a 24-h incubation at 28 °C, plaque formation based on 4 types of reaction was observed. The results were analyzed by Ward's Minimum Variance Cluster Analysis.

The results indicated two major groups of lysotypes, groups A and B, consisting of two clusters each. Lysotypes are bacterial strains differentiated only by their response to phages. Clusters 1 and 2 of group A consisted of 82 and 53 isolates, respectively, collected from different geographic areas in the Philippines. The majority of cluster 3, consisting of 21 isolates from Laguna and Palawan, and cluster 4, consisting of 45 Xco isolates, was a mixture of isolates originating from Palawan, Banaue, the IRRI farm, and a few other major rice-growing areas. Clusters 1 and 2 appeared to be distributed in most of the major rice-growing areas in the Philippines (T. W. Mew and M. Baraoidan, IRRI, unpubl. data).

VARIATION IN VIRULENCE

The basic unit of variation in classifying physiological specialization is the physiologic race, which is a combination of virulent and nonvirulent reactions evoked on a standard set of differential host varieties (Wolfe and Schwarzbach 1975). A differential set of host varieties that can distinguish the responses evoked by isolates of the pathogen is an important prerequisite for understanding pathogen variation.

In *Pseudomonas glycinea,* seven separate pathogenic races were distinguished when seven differential soybean varieties were identified (Cross et al 1966). Regarded as the universally susceptible soybean variety, Acme was later found resistant to races 5 and 6, while variety Flambeau, described as resistant, was susceptible to 4 of 7 races. The race that can be identified is a function of the differential host cultivars.

Variation in Xco virulence was recognized in Japan and in tropical Asia. Races of Xco were distinguished, and the research has been reviewed recently (Mew 1987). Currently, there are six races in the Philippines identified on the basis of differential cultivars (Table 1) (Mew 1987; Mew and Vera Cruz 1979. 1985; Mew et al 1982).

RELATIONSHIP BETWEEN VIRULENCE AND BACTERIOLOGICAL FEATURES

Using *X. campestris* pv. *malvacearum,* Hayward (1964a) studied biochemical groups and phage sensitivity in relation to pathogenicity for different cotton varieties. He found some correlation between the presence of group 1 or 2 xanthomonads and the group specificity of the phage isolated from the same material.

In rice, Xco has been much studied from the pathological aspect, and attempts have also been made to correlate its pathological characteristics, mainly virulence, with others determinable in the laboratory such as bacteriological characters (Choi et al 1981, Hifni et al 1975, Mukoo and Isaka 1964, Reddy and Ou 1976, Tsuchiya et al 1982) and sensitivity to phages (Goto 1965, Wakimoto 1960).

Table 1. Interaction between rice and Xco in the Philippines.

Differential variety[a] (resistance gene)	Reaction to races[b]					
	Race 1 (PXO 61)	Race 2 (PXO 86)	Race 3 (PXO 79)	Race 4 (PXO 71)	Race 5 (PXO 112)	Race 6 (PXO 99)
IR24 (0)	S (40-45)	S (30-35)	S (35-40)	S (40-45)	S (40-45)	S (45-50)
IR20 *(Xa-4)*	R (3-5)	S (30-35)	S (30-35)	MR (15-20)	R (1-3)	S (40-45)
Cas 209 *(Xa-10)*	S (40-45)	R (1-3)	S (35-40)	S (40-45)	R (1-3)	S (45-50)
IR1545-339 *(xa-5)*	R (3-5)	R (5-10)	R (3-5)	S (30-35)	R (3-5)	S (40-45)
DV85 *(xa-5, Xa-7)*	R (1-3)	R (1-3)	R (1-3)	R (3-5)	R (1-3)	MS (25-30)

[a] Plant age = 40 d after sowing. [b] R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible. Numbers in parentheses are ranges of % lesion area.

Before Xco races were clearly established, the bacterium was reported to vary greatly in bacteriological characters, although as a species it was considered homogeneous (Reddy and Ou 1976, Vera Cruz et al 1984) based on a few isolates from different Asian countries in which virulence to differential varieties was unknown. We therefore attempted to study the relationship between virulence of Philippine Xco races and bacteriological characters or phage sensitivity with a view to finding key diagnostic features of the races determinable in the laboratory, aside from host specificity.

NUMERICAL ANALYSIS OF PHENOTYPIC FEATURES

Fifty-two Xco strains representing races 1 to 6 (race 1, 7; race 2, 12; race 3, 7; race 4, 5; race 5, 7; race 6, 9), the weakly virulent strains (4), and NCPPB2446 were compared by computer-assisted numerical taxonomy, a relatively objective method in bacterial classification. The characters subjected to analysis were based on classical bacteriological tests following the *Manual of microbiological methods* (Society of American Bacteriologists 1957) and other standard tests. One hundred thirty-two unit characters such as cell morphology (4 unit characters), biochemical tests (20), acid production from carbohydrates (20), growth on various C sources (49), growth under different conditions (16), and resistance to antibiotics (23) were examined to allow a detailed comparison of the similarities and differences among the races. In the numerical analysis, only 103 morphological, physiological, and biochemical features were coded as 2 (positive) or 1 (negative) to calculate the similarity coefficient of Sokal and Michener (1958) (S_{SM}) using the program of Bonham-Carter (1967). Missing features were not included in the analysis. Clustering of the strains was done based on the group average method (Sneath and Sokal 1973) using the Clustan 1 C program (Wishart 1978) with the IBM 370 computer of the Agricultural Research Center, University of the Philippines at Los Baños.

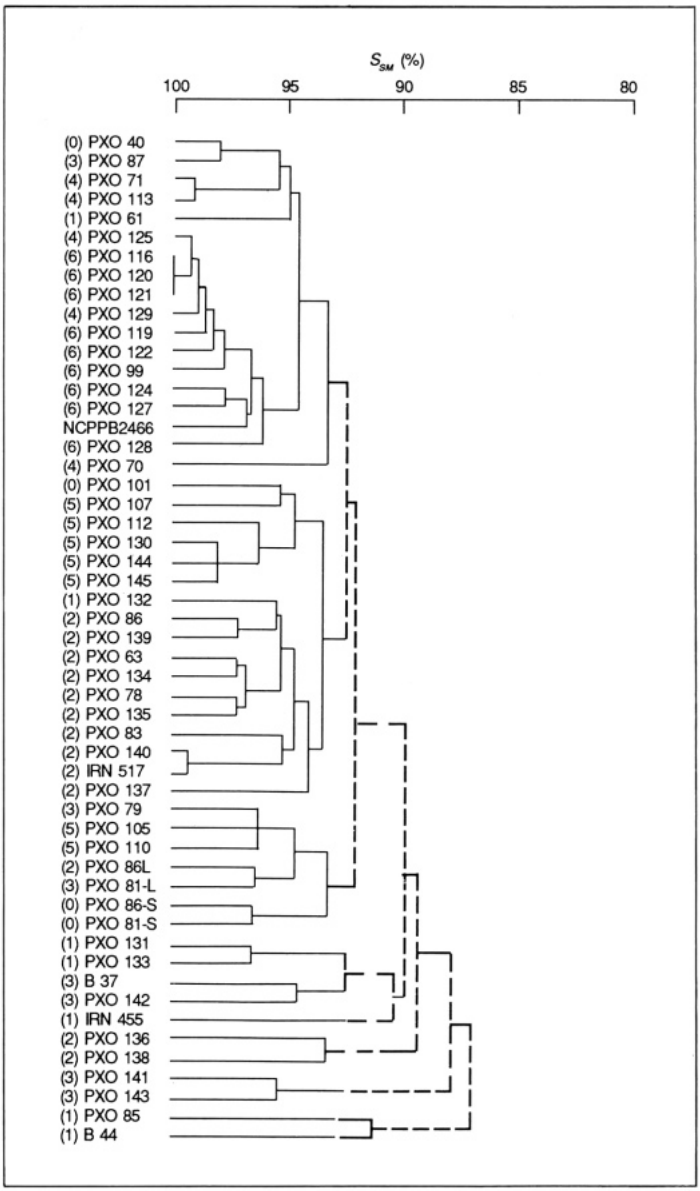
The dendrogram, considered the most useful representation of a taxonomic structure, was obtained after numerical analysis of 103 unit characters of 52 strains. Based on the phenetic groups formed at about 86.0% S_{SM} level, Xco strains representing different races clearly belong to a homogeneous group in species *campestris*. It is commonly found that phenetic groups formed at about 80% similarity level are equivalent to bacterial species (Sneath 1984). Hifni et al (1975) tested 14 isolates from Japan belonging to pathotypes I, II, and III, and 16 isolates from Indonesia belonging to pathotypes III, IV, and V, with selected cultural and physiological tests for taxonomical studies of plant pathogenic bacteria. They concluded that variations in cultural or physiological characteristics in Xco do not have any relationship to the pathotypes established in Japan and Indonesia. In the present study, however, there was a tendency for differentiation of 2 subphenons, as indicated in the cluster formed by the majority of races 4 and 6 and the majority of races 2 and 5 at the 93% S_{SM} level (Fig. 1). These subphenons were not necessarily discrete groups, but rather noteworthy areas of relative strain homogeneity apparent from the dendrogram. Race 1 was distributed widely in the dendrogram, while the majority of race 3 clustered at a lower similarity level than the rest of the races. It appears that race 3 is composed of a mixture of strains either similar to or different from those of other races. Because of the apparent strain homogeneity of other races, we referred back to the original table of strain data for possible descriptions of the likely groups in Xco races. The characters showing variable reactions were analyzed individually. Trehalose, a disaccharide, supported growth of all races except the majority of race 1, while the organic acid cis-aconitic, neutralized to pH 7 with 1N NaOH, was not utilized by all strains in races 1, 4, and 6. All of race 5 and the majority of race 2 utilized sodium aconitate.

To confirm these results, we studied more strains in each race (race 1, 58; race 2, 53; race 3, 56; race 4, 6; race 5, 22; race 6, 14) for their ability to utilize trehalose and sodium aconitate. The original set of 52 strains was included in this study. Standard procedures for media preparation and inoculation were followed.

A similar result was obtained from this confirmation test (Table 2); 84.3% of race 1 could not utilize trehalose as a C source, while 100% of races 4 and 6 grew luxuriantly on medium containing trehalose. The majority of races 2 (94.3%), 3 (92.8%), and 5 (96.4%) also grew on the same medium. Sodium aconitate inhibited the growth of races 4 and 6, and 98.2% of race 1.

None of the earlier studies included trehalose and sodium aconitate to compare Xco strains. It appears that the differentiation was not absolute, and that reactions among strains overlapped in other races in their utilization of trehalose and sodium aconitate. However, these characters showed a high degree of association with some of the Xco races in the Philippines (Vera Cruz and Mew 1986). Their relationship to the virulence of Xco in other countries should be studied further.

Mukoo and Isaka (1964) correlated pathogenicity with the intensity of liquefying gelatin. A further study by Isaka (1973) indicated a close relationship between both properties. Highly pathogenic isolates belonging to groups II and III based on Kozaka's (1969) system liquefied gelatin more than the less pathogenic ones (group I). Hifni et al (1975) found no relationship between gelatin liquefaction and



1. Dendrogram obtained by numerical analysis of phenotypic features of 52 strains of Xco with S_{SM} coefficient and group-average method. The race group of strains is given in parentheses.

pathogenicity; all isolates liquefied gelatin slowly and slightly starting at 14 d, regardless of pathotype. Our study also indicated no relationship between these characters. Gelatinase activity started slightly in races 2 and 4, and did not appear in race 1 except in isolate B44, while races 5 and 6 showed very slight to slight

Table 2. Frequency of isolates within Xco races showing positive reaction on selected phenotypic features.

Feature	Frequency (%)[a]					
	1	2	3	4	5	6
Growth on						
Trehalose	15.7	94.3	92.8	100	96.4	100
Sodium aconitate	1.8	94.3	91.3	0	9.5	0

[a]Isolates (no.) tested in each race group: group 1, 58; 2, 53; 3, 56; 4, 6; 5, 22; 6, 14.

liquefaction 12 d after incubation. After 21 d, gelatinase activity was fast, and a scale of 0-5 was adopted to compare the amount of gelatin liquefaction in strains of each race. No difference was observed among races; each strain, regardless of the race, was able to liquefy gelatin slightly, its ability increasing with prolonged incubation.

LYSOTYPE IN RELATION TO VIRULENCE

Several studies have reported no relationship between phage sensitivity and virulence (Fujii et al 1974, Kuhara et al 1965, Wakimoto 1960). However, Choi et al (198 1) reported that virulence was closely correlated with sensitivity to phage OP_2. The correlation was not distinct with other phages, but the correlation could be demonstrated by using suitable phage strains.

We therefore attempted to type Xco isolates using a set of phages to correlate lysotypes with Xco races. Initially, we selected 21 Xco isolates belonging to 6 races based on similarity of reaction to a set of phages. These Xco isolates were tested against 106 phage isolates used in previous tests. Fourteen phage isolates were then chosen and separated into five groups—namely groups A (P146), B (P44, P47, P45, P43, P48), C(P149, P154, P137), D (P81, P93), and E (P125, P118, P116)—based on the same or most similar reaction pattern to the races. Those that showed clear plaque formation were multiplied from a purified single plaque of each phage isolate on the most sensitive bacterium of each race: PXO 13 (race 1) for group A, IRN801 (race 2) for B, IRN702 (race 3) for C, PXO 71 (race 4) for D, and PXO 111 (race 5) for E; none of race 6 formed plaque from any of the phages tested. The mixtures of single plaques and their 72-h-old indicators in CaVf media were shaken overnight at room temperature (approx. 28 °C), followed by centrifugation at 15,000 rpm for 20 min. The supernatants were purified by shaking with 1 ml chloroform, and the upper layers of the mixtures were collected as stock cultures of phages.

Screening of 225 Xco (race 157; race 2,53; race 3,58; race 4,6; race 5,20; race 6, 14; unclassified, 17) isolates for phage typing was done using 5 groups of 14 phage isolates. Forty-nine *X. campestris* pv. *oryzicola* isolates were included to determine the specificity of the phage isolates for Xco. After determining the routine test dilution (RTD) of 14 phage isolates, the phage suspensions (RTD x 100) were inoculated in duplicate solidified peptone sucrose agar plates with 1 ml each of bacterial isolate using a multipoint inoculator. Plaque formation was observed 24 h later. Four types of reactions were recorded: C = very clear plaque, + = less clear or slightly turbid plaque, ± = turbid plaque, and - = no plaque formation.

The types of plaque formation observed for each bacterium-phage combination were converted to numerical data. Types C and + were coded 1; ± was also coded 1 because of incomplete resistance of bacteria to the phage as in the reaction of race 4 to most of the phages. The similarity of the bacterial isolates based on their reaction to the phage groups was calculated by cluster analysis with the Clustan software.

The dendrogram obtained from the cluster analysis of 225 Xco and 49 *X. campestris* pv. *oryzicola* revealed 8 clusters of lysotypes (Table 3). The majority of race 1 (56.1%) fell in cluster 1; 50.9% of race 2 and 72.1% of race 3 fell in cluster 3; 100% of race 4 fell in cluster 8; 35 and 40% of race 5 fell in clusters 5 and 6, respectively; and 100% of race 6 showing no plaque formation with all phage groups fell in cluster 7 (Table 4).

The lysotypes except those in clusters 2 and 4 showed similar reactions within phage groups. In cluster 2, the reaction of group E phage P125 (+) was different from that of P118 and P116 (-). In cluster 4, the reaction of group D phage P81 (+) was different from that of P93 (-), whereas that of group E phage P125 (-) was different from P118 and P116 (+).

From the distribution of lysotypes, some can be used to distinguish some races, such as the lysotype of cluster 8; 100% of race 4 isolates gave the same reaction to all phage groups, while all race 4 isolates showed positive reaction to both P81 and P93 only—however, plaque formation was turbid, probably due to incomplete resistance of race 4 to the phage group. Presently, race 4 isolates are being used to study temperate phages that are likely to show a reductive response. One hundred percent of race 6 was resistant to all Xco phages, while the majority of races 2 and 3 were susceptible to all phage isolates tested. The majority of race 1 (77.2%) was resistant to group E phages. Race 5 was distributed in several clusters overlapping with some strains in races 1, 2, and 3. The majority (58.8%) of unclassified Xco strains were negative to all phages, while 100% of *X. campestris* pv. *oryzicola* were also not infected by any of the phage isolates, indicating specificity of the phages to Xco. Indeed, typing Xco by phage can be demonstrated by using suitable phage strains (Choi et al 1981).

VARIATION IN VIRULENCE OF Xco IN RICE-GROWING COUNTRIES

A comparative study of five differential varieties from Japan and four from IRRI, all carrying specific genes for resistance, was conducted in Japan and the Philippines against races 1-4 in the Philippines and against pathogenic groups I-V in Japan. The nine bacterial groups were distinct in specific virulence, while each of the differential varieties had a gene or genes different from the others in specific resistance (Horino et al 1981).

In Thailand, three groups of bacteria (except those that had lost their virulence) were identified on IR8, IR20, IR1545, DV85, RD7 (gene unknown), and RD9 (no gene for BB resistance) (Eamchit and Mew 1982). Group I was virulent only to IR8 and RD9, group II was virulent to RD7 but not to IR20, and group III was virulent to RD7 and IR20. All groups were virulent to IR8 and RD9 but not to DV85.

In southern China, a weak interaction between bacterial isolates from Asia and differential varieties from China was observed (Wu et al 1981, unpubl. data).

Table 3. Lysotypes of 225 Xco and 49 *X. campestris* pv. *oryzicola*[a] classified in 8 clusters obtained from the dendrogram of their phage reactions to 14 phage isolates.

Lysotype (cluster)	Reaction to phage[b]													
	A	B					C			D		E		
	P146	P44	P47	P45	P43	P48	P149	P154	P137	P81	P93	P125	P118	P116
1	+	+	+	+	+	+	+	+	+	+	+	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+	-	-
3	+	+	+	+	+	+	+	+	+	+	+	+	+	+
4	-	+	+	+	+	+	+	+	+	+	+	-	+	+
5	+	-	-	-	-	-	+	+	+	-	-	-	-	-
6	+	-	-	-	-	-	+	+	+	+	+	-	-	-
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	+	+	-	-	-

[a]All *X. campestris* pv. *oryzicola* were in lysotype 7. [b]+ = positive reaction, - = negative reaction.

Table 4. Distribution of 6 Xco races and unclassified Xco, and *X. campestris* pv. *oryzicola* in 8 lysotypes.

Race	Total isolates (no.)	Percentage of isolates							
		L1	L2	L3	L4	L5	L6	L7	L8
1	57	56.1	15.8	7.0	0	1.8	19.3	0	0
2	53	0	5.7	50.9	9.4	24.5	7.6	1.9	0
3	58	3.4	0	74.1	1.7	5.2	3.4	12.1	0
4	6	0	0	0	0	0	0	0	100
5	20	0	10.0	0	10.0	35.0	40.0	5.0	0
6	14	0	0	0	0	0	0	100	0
Unclassified Xco	17	0	17.6	0	0	23.5	0	58.8	0
X. campestris pv. *oryzicola*	49	0	0	0	0	0	0	100	0

Recently, bacterial isolates from northern China were tested on differential varieties from IRRI, Japan, and Korea, and on commercial varieties from China. A strong interaction was noted among commercial varieties and isolates (Zhang Qi, Institute of Crop Breeding and Cultivation, pers. comm.).

In Indonesia, nine bacterial groups were recognized on Japanese and IRRI differentials (Horino 1981).

Isolates from India, Nepal, and Bangladesh appeared to have a broader spectrum of virulence than those from Southeast Asia.

Isolates from Australia and the United States have narrow virulence and seem to be specific to some varieties. In Africa, the virulence of the pathogen is not known.

The races of Xco in different countries may be a product of long evolution stabilized through a long period of gene interaction with the host. Whether this is also related to the ecology of rice cultivation and to environmental factors such as temperature and relative humidity deserves further study.

DISCUSSION

It is obviously necessary to know the full range of variability of which this bacterial rice pathogen, Xco, is capable with regard to bacteriological properties and pathogenicity to rice varieties. Variations in physiological and biochemical characteristics have been identified in Xco, but they are within the range of variability expected within a species and are not enough to suggest the existence of biochemical types as recognized in *Pseudomonas solanacearum* or *X. campestris* pv. *malvacearum.* Xco is so distinct and homogeneous that it has been suggested as a distinct species in the genus *Xanthomonas* (Swings 1989, this volume). In fact, although it is morphologically and in other instances physiologically very similar to *X. campestris* pv. *oryzicola,* few characters, such as growth in L-alanine as the sole C source and in 0.001% cupric nitrate, distinguish Xco very clearly from *X. campestris* pv. *oryzicola.* Within pv. *oryzae,* two characters—growth in trehalose, and sodium aconitate as C source—were found closely associated with all races except race 1 for

trehalose and races 2,3, and 5 for sodium aconitate. The association, however, was not absolute; overlapping was noted. On the other hand, earlier studies did not include these characters, which should be tested against isolates from other countries to determine their association. From the comparison of phenotypic features, races 1 and 3 isolates were highly heterogeneous and appeared to cluster at a lower similarity level than the others. This group of isolates should be studied in detail, as it may be a mixture of isolates. So far, no phenotypic feature can differentiate race 3 from race 2 except its specificity to Cas 209.

Although many workers have claimed no distinct relationship between phage sensitivity and virulence, our results indicate that isolates of some race groups have similar phage sensitivity and may fall into one lysotype. Isolates in races 1, 2, 3, and 5, however, overlap in their phage sensitivity. Race 4 appeared to be less sensitive to all phages tested; thus, it is at present being used to study temperate phages. Plaques formed by the temperate phages are usually turbid because their center is occupied by the growth of lysogenized cells (Goto 1965). These phages affect the genetic make-up of the infected bacterium by adding the phage DNA in the so-called prophage state, which gives rise to a lysogenized bacterium without causing lysis. Race 6 is different from the rest of the races in phage sensitivity; it is resistant to all phages tested.

Xco races, comparable to fungal pathogens and other bacteria, have been clearly and distinctly demonstrated. However, the spectrum of variability cannot be quantified unless a differential system useful in different countries is developed. Nonetheless, various methods and techniques are being developed to study the observed variation in relation to other characters aside from host specificity. These techniques presently being used as the bases for comparative analysis among races may be considered artificial, since the similarities are based entirely on phenotypic characteristics rather than genetic relatedness; however, the similarities and differences seem to agree with the findings obtained from preliminary studies of Xco races employing molecular genetic techniques to study variation in Xco. Using all available techniques, and relating one with the other, may improve our knowledge of just how variable Xco is as a pathogen in infecting rices with inherent resistance.

Another aspect of variation that Xco may exhibit is the possible occurrence of ecotypes, which at present has not been fully studied. Ecotypes are forms of the bacterium arising by selection in a particular habitat. In the Philippines, race 5 is found only in Banaue at an elevation of approximately 3,000 m above sea level. Phenotypically, it can be differentiated from races 1, 4, and 6 by its ability to utilize sodium aconitate, but not from races 2 and 3; it is virulent only on IR8 and the native varieties from Banaue. The remarkable homogeneity of characters with other races is also consistent with a view that there may be ecotypes in the BB pathogen. Southeast Asian isolates have different virulence from South Asian isolates. It is unclear whether this phenomenon is related to the intensity and ecology of rice cultivation, or to environmental factors such as temperature and relative humidity, and interaction among these factors that may lead to the evolution of ecotypes.

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Salient findings from the multilocation evaluation of the International Rice Bacterial Blight Nursery

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The International Rice Testing Program (IRTP). coordinated by the International Rice Research Institute, has established a global network for exchanging elite rices among rice scientists and evaluating those rices under diverse environments. The IRTP nurseries have been designed for evaluation under different cultural systems and for screening for varietal resistance to important biological, physical, and chemical stresses. One of the nurseries screens for resistance to bacterial blight (BB) caused by *Xanthomonas campestris* pv. *oryzae.* Evaluation of this nursery since 1981 in several Asian countries has identified several breeding lines and varieties with moderate to good resistance to BB. Some of these have shown tolerance for other stresses, and some have demonstrated high yield potential under either irrigated or rainfed conditions in IRTP multilocation trials. Differential reactions are evident across test sites and across years for some lines. The pathogenic strains from South Asia are more virulent than those from East and Southeast Asia, as evidenced by the high proportion of susceptible entries in East Asia.

The International Rice Testing Program (IRTP), coordinated by the International Rice Research Institute and funded by the United Nations Development Programme, was initiated in 1975 to facilitate the exchange of elite genetic material among rice scientists around the world and to provide a mechanism for testing the breeding material generated by individual rice improvement programs.

The international nurseries are broadly grouped into two categories: target environment nurseries and stress screening nurseries. Target environments for which nurseries are composed and tested include irrigated and rainfed. The rainfed category in turn includes upland, lowland (shallow water), deepwater, floating, and tidal wetland rice. The stress nurseries include screening sets for evaluating tolerance for or resistance to diseases, insects, adverse soils, and suboptimal temperatures.

Bacterial blight (BB) caused by *Xanthomonas campestris* pv. *oryzae* is one of the diseases for which a screening nursery was designed. This paper summarizes salient findings from the International Rice Bacterial Blight Nursery (IRBBN) screened across locations in Asia from 1981 to 1987.

MATERIALS AND METHODS

The test material included several improved semidwarf breeding lines and some tall traditional varieties. Table 1 gives the IRBBN test sites from 1981 to 1987. The number of entries evaluated and the number of test sites during the period are given in Table 2.

The procedures for BB screening were as follows:

Greenhouse test (seedling resistance)

- The test entries were pregerminated for 48 h in petri dishes.
- Seedlings (10-15) for each entry were seeded in rows 1 cm apart in 35- × 26- × 10-cm wooden boxes or plastic trays. Each entry was planted in a 10-cm row. Each tray was planted to 10 entries in single rows, and susceptible checks were planted in the center.
- Virulent local isolates were used for the inoculum.

Table 1. International Rice Bacterial Blight Nursery test sites, 1981-87.

Country	Sites (no.)	Location
Bangladesh	4	Comilla, Joydebpur, Rajshahi, Sonagazi
Burma	2	Gyogon, Yezin
China, all provinces except Taiwan	8	An Ren County, Changsha, Fuyang, Fuzhou, Guangzhou, Hangzhou, Nanning City, Nanjing
China, Taiwan	1	Changhua
India	13	Aduthurai, Coimbatore, Cuttack, Hyderabad, Kapurthala, Karjat, Kaul, Ludhiana, Pantnagar, Patna, Pusa, Raipur, R. S. Pura
Indonesia	3	Lanrang, Sukamandi, Ujung Pandang
Korea	1	Iri
Malaysia	1	Bumbong Lima
Nepal	3	Kankai, Parwanipur, Tarahara
Pakistan	1	Kala Shah Kaku
Philippines	1	Los Baños
Thailand	9	Bangkhen, Khonkaen, Kuan Gut, Pathumthani, Phrae, Rangsit, San Patong, Suphanburi, Ubon Rachathani
Vietnam	3	An Khanh, Omon, Tan Hong

Table 2. Entries evaluated in the International Rice Bacterial Blight Nursery and test sites, 1981-87.

Year	Entries (no.)	Test sites (no.)	Countries (no.)
1981	54	13	5
1982	94	21	10
1983	181	22	8
1984	213	23	9
1985	215	27	10
1986	89	27	11
1987	44	33	11

- The inoculum concentration was suggested to be about 10^8 cells/ml.
- Plants were inoculated 18-21 d after sowing (DAS). If more isolates were tested, separate boxes were used for individual isolates.
- Rating the reactions of the plants was based on lesion area (9%) 12-14 d after inoculation (DAI) or when the susceptible check showed maximum disease incidence. The following scale was used for scoring the disease:

Score	*Lesion area (%)*
1	0-3
2	4-6
3	7-12
4	13-25
5	26-50
6	51-75
7	76-87
8	88-94
9	95-100

Field test

- Seedlings were transplanted at 1 plant/hill at 21 DAS. Each entry was planted to a single row of 2-3 m with a spacing of 20 cm between plants within a row as well as between rows.
- The susceptible check TN1 was planted as every 10th row.
- The inoculum was prepared from a locally collected virulent isolate. Plants were inoculated at panicle initiation to booting by dipping sterilized scissors in the bacterial suspension and clipping off the leaves 2-3 cm from their tips; 5-10 hills of each plot were inoculated.
- Rating the disease reaction was based on a modified 0-9 scale of the *Standard evaluation system for rice* (IRRI 1980). Scoring was at 21 DAI or when the susceptible check showed maximum disease incidence. The scoring system was as follows:

Score	*Lesion area (%)*
0	0
1	1-5
3	6-12
5	13-25
7	26-50
9	51-100

RESULTS AND DISCUSSION

Promising entries

Entries frequently rated resistant (0-3 score) across sites in different years (1981-87) are listed in Table 3. Among them, the following were rated resistant at most sites and over different years: RP633-76-1, IR54, DV85, and IR444246-3-3-3, RP633-76-1 has two sources of resistance in its parentage: BJ1 and IR22. Some of the derivatives of the entries that were frequently rated good were also among the best entries in different years. For example, IR54 is one of the parents of resistant lines

Table 3. Promising entries in the International Rice Bacterial Blight Nursery (IRBBN), 1981-87.

Year	Entry	Cross[a]	Days to flowering	Plant height (cm)
1981	IR2798-88-3-2	IR1529-680-3/IR1913-41-2//IR1514A-E666	119	81
	IR4442-46-3-3-3	IR2061-464-2/IR1820-52-2	111	95
	RP633-519-1-3-8-1	IR8/BJI//IR22	107	84
	RP633-76-1	IR8/BJI//IR22	105	84
	IR13423-17-1-2-1	IR2863-38-1/IR2588-132-1-2//IR4417-177-1-4	101	83
	IR54	Nam Sagui 19/IR2071-88//IR2061-214-3-6-20	106	84
	IR9209-48-3-2	IR2061-465-1-5-5/IR2053-521-1//IR36	94	73
1982	DV85	–	89	115
	BR161-2B-25	Chandina/IR425-1-1-38-3	98	78
	BR171-2B-8	IR442-2-50-2/Mala	95	87
	IR4442-46-3-3-3	IR2061-464-2/IR1820-52-2	112	98
	RP633-76-1	IR8/BJI//IR22	103	87
	IR54	Nam Sagui 19/IR2071-88//IR2061-214-3-6-20	96	84
1983	BR51-74-6/J1	IR20/IR5-114-3-1	119	115
	IR15723-45-3-2-2-2	DV85/IR2061-522-6-9//IR36	93	89
	IR25560-132-2-3	IR54/IR48	111	104
	IR27325-63-2-2	IR17491-5-4-3-3/IR4570-74-2-2-3-3	115	101
	IR40	IR20*2/O. nivara//CR94-13	106	94
	IR4442-46-3-3-3	IR2061-464-2/IR1820-52-2	116	108
	IR54	Nam Sagui 19/IR2071-88//IR2061-214-3-6-20	108	95
	IR9830-26-3-3	IR36*2/Nam Sagui 19	1 08	89
	RP633-76-1	IR8/BJI//IR22	98	89
1984	BR161-2b-53	Chandina/IR425-1-1-3-8-3	93	76
	BR171-2b-8	IR442-2-50-2/Mala	95	86
	B4143d-Pn-51-4	B2360-8/2*Pelita I-l//IR36	100	98
	IR2798-88-3-2	IR1529-680-3/IR1913-41-2//IR1514A-E666	103	78
	IR54	Nam Sagui 19/IR2071-88//IR2061-214-3-6-20	100	88
	RP633-76-1	IR8/BJI//IR22	96	75
1985	DV85	—	89	104
	IR13423-17-1-2-1	IR2863-38-l/IR2588-132-1-2//IR4417-177-1-4	101	85
	IR22082-41-2	IR54/IR5657-33-2	99	93
	IR26717-1-1-2-1-1	G. Heenati/BR51-91-6//IR48	106	91
	IR29341-85-3-1-3	IR54/IR46	104	92
	IR54	Nam Sagui 19/IR2071-88//IR2061-214-3-6-20	107	91
	IR8192-166-2-2-3	IR2070-747-6/IR2055-219-1-3//IR2061-213-2-16	1 09	100
	RP633-76-1	IR8/BJI//IR22	100	82
1986	IR54	Nam Sagui 19/IR2071-88//IR2061-214-3-6-20	105	91
	RP2151-173-1-8	IR8/BJI//IR22///CR98-7216	105	90
	RP2151-192-1	IR8/BJI//IR22///CR98-7216	115	89
	RP2151-192-2-5	IR8/BJI//IR22///CR98-7216	118	91
	RP2151-33-2	IR8/BJI//IR22//ICR98-7216	100	100
	RP2151-40-1	IR8/BJI//IR22///CR98-7216	98	95
1987	RP2151-21-22	IR8/BJI//IR22///CU98-7216	99	109
	RP2151-173-1-8	IR8/BJ1//IR22///CR98-7216	107	104
	RP2151-40-1	IR8/BJI//IR22I//CR98-7216	99	106
	AC19-1-1	-	108	126
	DV85	-	93	116
	IR29341-85-3-1-3	IR54/IR46	113	107
	Camor	-	130	128

[a] - = unknown.

IR25560-132-2-3, IR22082-41-2, and IR29341-85-3-1-3; DV85 is one of the parents of resistant line IR15723-45-3-2-2-2; and RP633 is one of the parents of resistant lines RP2151-40-1 and RP2151-173-1-8.

Entries that showed resistance to other stresses in addition to BB in tests at selected locations were as follows:

Leaf blast (Los Baños, Philippines)	DV85
Brown spot (Raipur, India)	IR29341-85-3-1-3
Sheath blight (Kapurthala, India)	Anak Putih, BR51-282-8-HR50, BR51-49-6-HR63, IR13146-45-2, IR32307-75-1-3-1
Green leafhopper (Los Baños)	IR4613-54-5, IR13423-17-1-2-1, IR9209-48-3-2. IR9729-67-3, IR9828-91-2-3, IR54, MRC603-303

Some of the IRBBN entries are also tested in yield nurseries. Those that showed moderate to good resistance to BB and gave good yield performance across locations in different target environment nurseries were the following:

Irrigated nurseries

- Very early group — IR25588-7-3-1, IR9729-67-3, IR50, IR19728-9-3-2
- Early group — KAU1727, IR64, IR9828-91-2-3, C1322-28, MRC603-303
- Medium group — IRl9672-140-2-3-2-2, IR54, IR21820-154-3-2-2-3, IR28118-138-2-3, BR51-282-8, Cisadane, IR13540-56-3-2-1, IR13423-17-1-2-1

Rainfed lowland nurseries — BR51-282-8, Cisadane, BR51-74-6/Jl, IR13146-45-2-3

Upland nurseries — IR43

Entries rated good (0-3 score) in selected tests in different years at high disease pressure sites (mean test score higher than 5.6) are listed in Table 4. Among them, DV85, RP633-76-1, RP2151-40-1, RP2151-173-1-8, IR54, Java 14, BR171-2B-8, and IR13423-17-1-2-1 were rated resistant in three or more tests.

Differential reactions

Differential reactions at different test sites were evident for some IRBBN entries. The reactions of entries common to 3 yr of testing(1984, 1985, 1986) at selected sites in East Asia (EA), Southeast Asia (SEA), and South Asia (SA) are summarized in Table 5. For some entries at some sites, the reactions were not consistent from year to year, possibly because different strains of the pathogen were involved in different years. Inadequacy of disease pressure could also have contributed to some discrepant reactions. However, in all 3 yr it was evident that BB strains from SA are more virulent than those from EA and SEA, judging from 1) the low proportion of resistant entries in SA (Figs. 1-3), and 2) the fact that entries resistant in EA and SEA may or may not be resistant in SA, but those resistant in SA are rarely susceptible in EA and SEA.

Table 4. IRBBN entries rated good at some sites where disease incidence was high.

Year	Location	Site mean disease score	Entries rated resistant (0–3)
1981	Joydebpur	6.1	BR51-282-8, RP633-519-1-3-8-1, BR171-2B-8, IR13423-17-1-2-1, IR54
	Hyderabad	7.4	DV85, IR9801-9-3
	Pusa	6.6	Cas 209, IR20, Java 14, IR4613-54-5, MRC603-303
1982	Joydebpur	7.0	DV85, BR161-2B-25. Cisadane, BR171-2B-8, BR319-1-HR12, IR2798-88-3-2, IR4442-46-3-3-3, RP633-76-1, IR62, IR54
	Cuttack	6.7	IR54[a]
	Hyderabad	7.2	DV85, RP2151-40-1 (local check)
	Pusa	6.9	Nigeria 5, BR161-2B-25, IR13423-17-1-2-1
1983	Joydebpur	6.2	Java 14, 70X-46, IR13423-17-1-2-1, RP633-76-1
	Patna	7.8	IR25560-.132-2-3
	Pantnagar	8.6	DV85, IR20
	Coimbatore	6.5	Nigeria 5, BR161-2B-25. C1321-9, IR25571-31-1, IR25588-7-3-1, IR54, UPR82-1-7
1984	Joydebpur	5.7	BR160-93-2-4, BR319-1-HR28, IR5793-55-1-1-1, MR1
	Patna	6.2	Nigeria 5, Tetep, IR13539-100-2-2-2-3, IR13564-95-1, IR9217-58-2-2, RD7
	Kankai	6.4	BR171-2B-8, B4108d-Pn-69-8, IR19661-63-1-2-3, IR2798-88-3-2
	Birganj	6.6	B4126d-Pn-2-1, IR19670-263-3-2-2-1, IR21820-154-3-2-2-3, IR28128-45-2, IR36, IR43, IR60, IR9171-60-2-2, M61b-32-1, RTN187-1-1
1985	Kapurthala	8.4	DV85, IR18349-22-1-2-1-1, RP633-76-1
	Kankai	6.1	IR29295-70-1-1-1-1-31, IR29692-65-2-3, IR4819-77-3-2, IR54
1986	Omon	6.2	IR1545-339-2-2, Java 14, Tetep, CNM539, RP2151-173-1-8, RP2151-33-2, RP2151-40-1
	Joydebpur	6.4	Java 14, C712315, BR161-2B-58, BR319-1-HR28, IR15529-253-3-2-2-2
	Rajshahi	6.8	IR33360-5-3-2-3, RP2151-173-1-8, RP2151-192-1, RP2151-192-2-5, RP2151-2244, RP2151-33-2, RP2151-40-1
	Hyderabad	7.2	IR21188-87-3-3-2-2, IR28224-21-2-2-1, RP2151-192-1, RP2151-192-2-5, RP2151-2244
	Patna	5.8	Kuntlan, Nigeria 5, Tetep, BR51-50-34, IR54
1987	Los Baños	5.9	Kuntlan, AC19-1-1, BR118-38-17, Camor, DV85, IRAT109, RP2151-40-1
	Kapurthala	5.9	Nigeria 5, AC19-1-1, DV85, IR33360-5-3-2-3, Kachamota, Kalimekri 77-5, RP2151-173-1-8, RP2151-21-22, RP215140-1
	Pantnagar	6.6	BR315-12-14-1
	Raipur	5.8	BR118-38-17, BR319-1-HR28, DV85, IR11288-B-B-445-1, IR24632-145-2-2-2-3, IR29341-85-3-1-3, IR33360-5-3-2-3, RP2151-173-1-8, RP2151-21-22, RP2151-40-1

[a] Resistant to all 5 isolates.

Table 5. Differential reactions[a] to bacterial blight of IRBBN entries across locations in Asia, 1984-86.

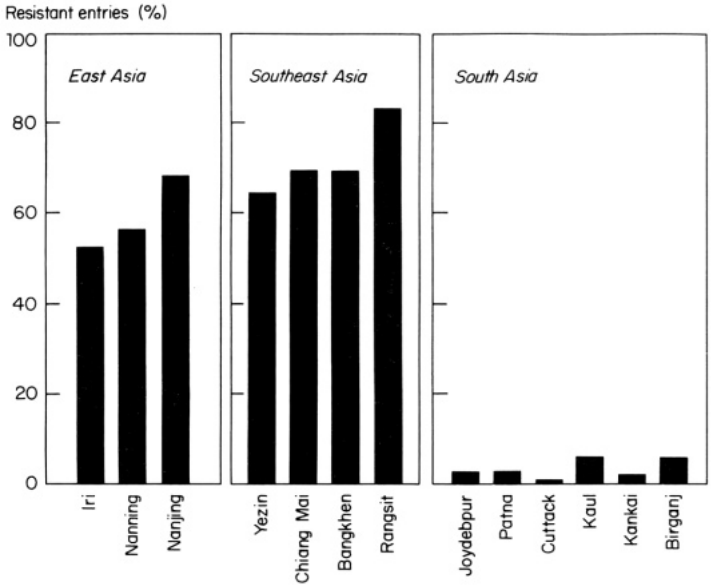
Designation	Year	East Asia			Southeast Asia				South Asia		
		Iri	Nanning	Guangzhou	Yezin	Los Baños	Rangsit	Joydebpur	Kapurthala	Pusa	Parwanipur
BR316-15-4-4-1	1984	R	R	R	R	I	I	I	S	I	S
	1985	I	R	R	I	I	I	S	S	I	R
	1986	R	R	R	S	I	S	S	R	I	I
B3894-22C-Sm-5-1-1	1984	S	R	I	S	R	I	I	S	S	S
	1985	I	R	R	R	S	I	I	S	S	S
	1986	-	R	R	R	-	I	-	R	-	I
B4143d-Pn-51-4	1984	S	R	R	R	I	R	I	S	R	I
	1985	S	R	R	R	S	I	S	I	R	S
	1986	R	R	I	I	S	I	S	R	R	S
Cas 209	1984	S	S	S	I	S	S	I	S	S	S
	1985	S	S	S	S	S	S	S	S	S	S
	1986	S	S	S	S	R	S	S	S	I	S
IR13564-95-1	1984	S	R	R	R	S	R	I	S	S	S
	1985	S	I	R	R	S	I	S	S	I	S
	1986	R	R	R	R	I	I	S	R	R	I
IR1475386-2	1984	S	-	I	R	S	I	I	S	S	S
	1985	I	I	R	R	S	S	I	S	S	S
	1986	R	R	I	I	I	I	S	R	S	R
IR1545-339-2-2	1984	S	R	R	R	R	I	S	S	S	S
	1985	S	R	R	R	R	I	S	S	S	S
	1986	R	I	R	R	R	I	S	S	S	S
IR20	1 984	S	R	R	R	S	R	I	S	R	I
	1985	S	R	R	R	S	I	S	S	R	I
	1986	R	R	R	R	S	I	S	R	A	I
IR4619-57-1-1-2-1	1984	S	I	S	R	I	S	I	S	S	S
	1985	S	S	S	I	S	S	S	S	S	S
	1986	R	I	S	I	I	S	S	R	S	S

continued on next page

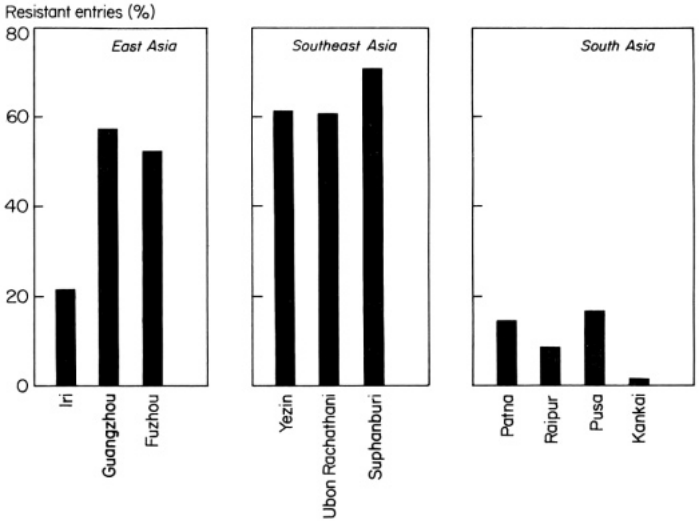
Table 5 continued.

Designation	Year	East Asia			Southeast Asia			South Asia			
		Iri	Nanning	Guangzhou	Yezin	Los Baños	Rangsit	Joydebpur	Kapurthala	Pusa	Parwanipur
IR48	1984	S	R	I	R	S	I	I	S	S	S
	1985	S	I	R	I	R	S	S	S	S	I
	1986	I	R	R	I	I	S	S	R	S	S
IR4819-77-3-2	1984	I	R	R	S	I	I	I	S	I	I
	1985	I	I	R	I	S	I	S	S	I	R
	1986	R	R	I	R	I	I	S	R	S	R
IR54	1984	I	R	R	R	S	R	I	S	R	S
	1985	S	R	R	R	S	I	I	S	R	I
	1986	R	R	R	R	I	R	I	R	R	R
IR60	1984	I	I	I	R	R	R	I	S	I	R
	1985	S	I	I	S	S	S	I	S	R	S
	1986	S	R	I	S	S	I	S	R	I	S
IR8	1984	S	S	S	S	S	S	S	S	S	S
	1985	S	S	S	I	S	S	S	S	S	I
	1986	S	S	S	-	-	S	S	S	S	-
IR8073-65-6-1	1984	S	I	I	I	I	I	S	S	S	I
	1985	S	I	R	R	S	S	S	S	S	I
	1986	S	R	I	R	S	I	S	I	I	S
Java 14	1984	I	R	S	I	R	-	-	-	S	S
	1985	I	I	R	I	R	I	-	-	S	-
	1986	R	R	R	R	-	R	R	S	-	S
Kuntlan	1984	R	R	S	S	R	-	I	S	S	S
	1985	S	R	I	R	R	R	I	S	S	I
	1986	R	I	S	R	R	R	S	R	I	S
Nigeria 5	1984	S	R	R	I	R	I	S	I	R	I
	1985	S	R	R	R	S	I	S	I	R	S
	1986	R	R	I	R	R	S	S	S	R	S
Tetep	1984	S	I	S	R	S	S	I	S	S	S
	1985	S	S	S	I	S	S	S	I	S	I
	1986	I	S	S	R	S	S	S	S	I	S

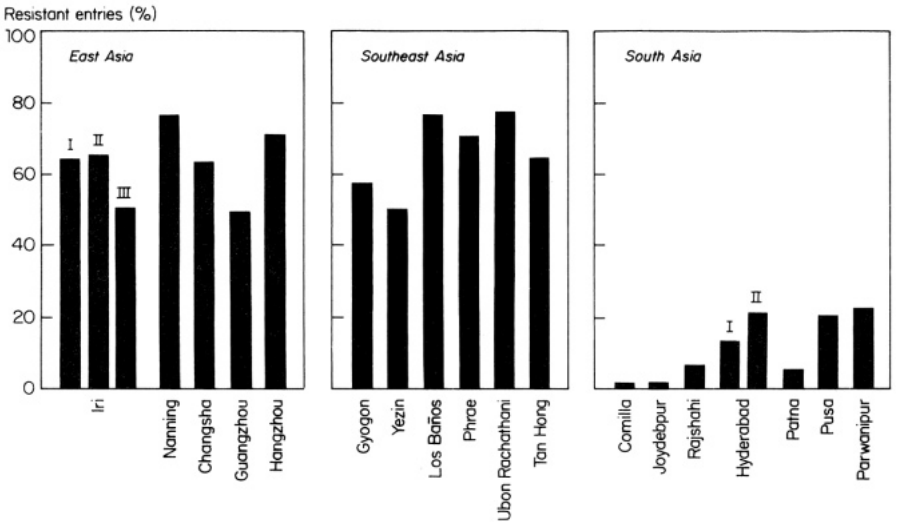
[a] R (resistant) = scores 0-3, I (intermediate) = scores 4-6, S (susceptible) = scores 7-9.



1. Severity of bacterial blight strains as reflected by the proportion of resistant entries, by region, 1984 International Rice Bacterial Blight Nursery.



2. Severity of bacterial blight strains as reflected by the proportion of resistant entries, by region, 1985 International Rice Bacterial Blight Nursery.



3. Severity of bacterial blight strains as reflected by the proportion of resistant entries, by region, 1986 International Rice Bacterial Blight Nursery.

CONCLUSION

The multilocation evaluation of the IRBBN over the past few years has led to the identification of several promising breeding lines that have either location-specific or broader resistance to BB. Some have tolerance for other stresses, and some have good yield potential. The genetic pool of BB-resistant varieties so identified can be appropriately utilized by national breeding programs to mitigate the losses caused by the pathogen. Meanwhile, screening efforts should be intensified to identify alternate sources of resistance.

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Notes

Author's address: D.V. Seshu, International Rice Research Institute, P.O. Box 933, Manila, Philippines.
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Major genes for resistance to bacterial blight in rice

T. Ogawa and G. S. Khush

This paper reviews oligogenic resistance to bacterial blight (BB) in rice. The major genes identified in Asian countries are discussed. Then the results of the Japan-International Rice Research Institute (IRRI) collaboration on BB resistance are reported. Under the collaboration, Japanese and IRRI differentials were genetically analyzed using Japanese and Philippine BB isolates. As a result, the gene symbols for BB resistance were rearranged according to the rules for gene symbolization in rice, and two new resistance genes were recently identified. Based on the results, the nature of major genes for BB resistance is discussed; some major genes have relatively wide spectra of resistance.

Disease resistance is usually classified into two categories, such as specific and nonspecific resistance, overall and adult resistance, vertical and horizontal resistance, qualitative and quantitative resistance, or overall and partial resistance. Qualitative resistance is generally considered to be controlled by major genes, while quantitative resistance is thought to be controlled by polygenic factors. However, genetic analysis of resistance is sometimes at variance with these definitions.

We deal with major gene resistance to bacterial blight (BB) of rice in this paper.

INITIAL GENETIC ANALYSIS

During his studies on reciprocal translocation in rice in Japan, Nishimura (1961) reported that BB resistance in varieties Kogyoku and Koganemaru was controlled by one dominant gene. He also showed that the resistance gene was located on chromosome 11 in his system of numbering chromosomes.

Washio et al (1966) deduced three major factors for BB resistance from analyzing the cross between varieties Shimotsuki and Kogyoku. He proposed that the genetic constitution of Kogyoku was $X_1X_1X_2X_2X_3+X_3+$ while that of Shimotsuki was $X_1+X_1+X_2+X_2+X_3X_3$. In his analysis, gene X_1 was the basic resistance factor but could not act by itself. He considered X_2 and X_3 complementary genes for the action of X_1. He also reported that resistance in Norin 27 and Kanto 60 was controlled by one dominant gene, and that a line of Wase Aikoku 3 showed resistance to all BB

groups known at that time; resistance in this line was controlled by one dominant gene.

Kuhara et al (1970) also concluded that resistance in the five varieties of the Kogyoku group (in the Japanese differential system) (Kozaka 1969) was controlled by one dominant gene.

The International Rice Research Institute (IRRI) began evaluating tropical varieties for BB resistance in the 1960s, and a number were found resistant to Philippine isolates. After identifying the donors, IRRI initiated genetic studies.

Resistance in Sigadis was reported to be controlled by an incompletely dominant gene (Heu et al 1968). Murty and Khush (1972) and Murty et al (1973) reported that resistance in BJ1 appeared to be controlled by one recessive gene, while resistance in DZ192 seemed to be conditioned by two recessive genes.

Resistance in a Lacrosse/Zenith-Nira selection in India was thought to be governed by a single recessive gene (AICRIP 1969).

SYSTEMATIC IDENTIFICATION OF RESISTANCE GENES

In extensive studies on the genetics of BB resistance using 27 resistant varieties, Sakaguchi (1967) concluded that resistance to Japanese bacterial group I was conditioned by gene *Xa-1* while resistance to Japanese bacterial group II was controlled mainly by gene *Xa-2* (originally designated as genes *Xa-1* and *Xa-2,* respectively). From linkage analysis, he concluded that the two genes were located on Nishimura's (1961) chromosome 11 ("pl" linkage group), and that the two genes were linked with a recombination value of 2-16%.

Gene *Xa-1* appears to be the same as those identified by Nishimura (1961) and Kuhara et al (l970).

Ezuka et al (1975) found that resistance in the Wase Aikoku group to Japanese bacterial groups I, II, and III was conditioned by one dominant gene, which they designated *Xa-w* (later changed to *Xa-3* according to the nomenclature rules for rice genes). They also indicated that resistance conditioned by *Xa-3* at the seedling stage was not expressed as completely as that of the adult stage. Toriyama (1972), Ezuka et al (1974), and Ezuka and Horino (1976) considered resistance in Wase Aikoku to be adult resistance.

Gene *Xa-3* was also shown to be independent of *Xa-1* in Kogyoku and of *Xa-2* in Rantai Emas 2 (Ezuka et al 1975). These three genes— *Xa-1, Xa-2,* and *Xa-3* —were identified using Japanese isolates of the BB pathogen in Japan.

Thereafter, IRRI scientists began gene identification of BB-resistant cultivars in the Philippines. Petpisit et al (1977) concluded that resistance in IR20, IR22, and IR1529-680-3 showed incomplete dominance, and that these cultivars carried the Same gene for resistance, which they designated *Xa-4.* They also reported that IR1545-284 and RP291-7 carried the same recessive gene for resistance, designating it *xa-5.* Genes *Xa-4* and *xa-5* segregated independently.

Olufowote et al (1977) also reported that some varieties carried genes *Xa-4* or *xa-5,* and that breeding lines IR944-102 and IRl698-241 carried another gene, closely linked to *Xa-4.*

Librojo et al (1976) analyzed additional varieties possessing *Xa-4* or *xa-5* by allelic tests with IR22 (*Xa-4*) and IRl545-339 (*xu-5*). Furthermore, they reported that Semora Mangga had an allelic gene for resistance at the *Xa-4* locus but showed somewhat different expression than the other allele at that locus; Semora Mangga showed resistance only at booting and flowering, while IR22, Sigadis, TKM6, Syntha, and Hom Thong were resistant at all plant growth stages. Thus, they designated the dominant gene of Semora Mangga $Xa\text{-}4^b$ to distinguish it from the $Xa\text{-}4^a$ of other cultivars.

Sidhu and Khush (1978) identified another new gene, *Xa-6,* in IR944-102, IR1698-241, Zenith, Malagkit Sungsong, Nagkayat, and Dayaggot. *Xa-6* was reported to be linked with *Xa-4,* with a recombination value of 26% but to be independent of *xa-5.*

Sidhu et al (1978, 1979) analyzed additional BB-resistant varieties. Of 74 cultivars analyzed, 19 were found to have $Xa\text{-}4^a$, 20 had $Xa\text{-}4^b$, and 32 had *xa-5* (Sidhu et al 1978). In addition, three cultivars-DV85, DV86, and DZ78—were found to carry one recessive gene, *xa-5,* and one dominant gene that was independent of *Xa-4, xa-5,* and *Xa-6.* Therefore, they designated the dominant resistance gene of DZ78 as *Xa-7* and conjectured, from morphological similarity and perhaps because they come from common stock, that both DV85 and DV86 also carried *Xa-7.* Gene *Xa-7* conveys resistance only at flowering. In addition to *Xa-7,* they identified one recessive gene in PI 231129 and designated it *xa-8;* it is independent of *Xa-4, xa-5,* and *Xa-6.*

Sidhu et al (l979) carried out further genetic studies on the resistance of 70 rice varieties from Indonesia. From allele tests, they identified a variety, Majaer, carrying the recessive gene *xa-5,* but all other cultivars carried the single dominant gene *Xa-4.* From these, 69 cultivars were susceptible at the seedling and maximum tillering stages but were resistant at booting and flowering. Therefore, they concluded that these cultivars have the $Xa\text{-}4^b$ allele.

On the basis of genetic analysis of 58 resistant varieties for BB resistance, Singh et al (1983) identified a new recessive gene, designating it *xa-9.* Gene *xa-9* was linked with *Xa-6,* with a recombination value of 5.9%, but no linkage was detected between *xa-9* and *Xa-4.* Gene *xa-9* was also independent of *Xa-3, xa-5, Xa-7,* and *xa-8.*

These studies on the genetics of BB resistance were carried out at IRRl using only one isolate of Philippine race 1. However, four races of the BB pathogen in the Philippines were identified based on their virulence to differential varieties (Mew and Vera Cruz 1979, Mew et al 1982). Among the differential varieties, Cas 209 showed a specific reaction to the four races, i.e., it was resistant only to race 2. Yoshimura et al (1983) reported that the resistance of Cas 209 was controlled by a single dominant gene that was independent of *xa-5* but was linked with *Xa-4,* with a recombination value of 27.4%. They designated the gene *Xa-10.*

Recently in Japan, Ogawa and Yamamoto (1986) genetically analyzed three breeding lines—IR1529-680-3-2,IR944-102-2-3, and RP9-3—for BB resistance using Japanese races. They reported that one of the two genes of IR944-102-2-3 that confer resistance to Japanese races IA, II, and IIIA is either the same as the *Xa-3* of Chugoku 45 or very closely linked to it, and the second dominant gene of IR944-102-

2-3 for resistance to Japanese races II and IIIA is independent of *Xa-1* of Kogyoku and *Xa-3* of Chugoku 45. Thus, they designated the second dominant gene of IR944-102-2-3 as *Xa-11,* and postulated from their pedigree records and reaction patterns that RP9-3 and IR8 also carry *Xa-11.*

Yamamoto et a1 (1977) found distinct evidence of race specialization in the causal organism of BB in Indonesia. They found an inverse relationship between the reaction to races III and V and the reaction to races II and V (Table 1). Using an Indonesian isolate, Xo7306 of race V, found by Yamamoto et al (1977), Ogawa et al (1978) reported that the resistance of Kogyoku and Java 14 to race V was controlled by one dominant gene, which is closely linked with *Xa-1.* This *Xa-kg* gene was recently redesignated as *Xa-12* following the rules for rice gene symbolization (Ogawa 1987). After the discovery of *Xa-12,* Yamada (1984) indicated that IRRI varieties IR28, IR29, and IR30 were resistant to bacterial groups I (T7141) and V (H75304) at all stages of plant growth, while Kogyoku showed resistance only at the mature stage. From allele tests between the resistance genes of Kogyoku and the IRRI cultivars, he reported that resistance genes of the IRRI cultivars, which confer resistance to groups I and V, were allelic to *Xa-1* and stronger than *Xa-12.* Thus, he designated them as $Xa\text{-}1^h$ and $Xa\text{-}12^h$ (originally $Xa\text{-}kg^h$), respectively. Moreover, from the segregation data in the crosses of the IRRI varieties with Toyonishiki, the recombination values were estimated as $Xa\text{-}1^h\text{-}Xa\text{-}12^h = 2.0 \pm 0.65$, $Xa\text{-}1^h\text{-}Ph = 2.8 \pm 0.77$, and $Xa\text{-}12^h\text{-}Ph = 3.7 \pm 0.87$.

GENE IDENTIFICATION IN OTHER COUNTRIES

From F_2 and F_3 analyses of resistant varieties in Sri Lanka, Watanabe (1976) estimated that Wase Aikoku 3 carried two genes, *Xa-a* and *Xa-h;* PI 209938 and Zenith carried *Xa-p* and *Xa-i;* and RL Gopher carries *Xa-i;* and these genes differ in reaction. Moreover, Bluebonnet/ Rexark was considered to have an incompletely dominant gene, *Xa-b,* for resistance.

Jayaraj et a1 (1972) reported that the resistance of BJ1 to Indian isolates was controlled by three complementary genes, X_1, X_2, and X_3, but the resistance gene X_1 was inhibited by one inhibitor, $I\text{-}X_1$, in IR8.

Table 1. Relationship between varietal groups and bacterial blight races in Indonesia (Yamamoto et al 1977).

Varietal group	Reaction to Indonesian races of BB				
	I	II	III	IV	V
Kinmaze	S	S	S	S	S
Kogyoku	R	S	S	S	R
Rantai Emas	R	R	S	S	R
Wase Aikoku	R	R	R	S	S
Java 14	R	R	R	S	R

[a]R = resistant, S = susceptible.

Moses et al (1974) carried out genetic studies of resistance in cultivars IRRI 69/469, IRRl70 /470, BJ1, Lacrosse / Zenith-Nira, and Malagkit Sungsong against two Indian isolates. As a result, they proposed two complementary dominant genes, *A* and *B*, in Malagkit Sungsong; one dominant gene, *R,* in IRRI 69/469 and IRRI 70/470; and one dominant gene, P_1 , in Lacrosse/Zenith-Nira.

Yu et al(1986) related that Zhao et a1 (1985) had reported resistance of IR28 to Chinese strains of BB governed by two genes, *Xa-a* and *Xa-h.* Then, Yu et a1 (1986) found that *Xa-a* and *Xa-h* were linked to *la* (lazy growth habit). The recombination values were calculated to be 27.9% between *Xa-a* and *la,* and 33.7% between *Xa-h* and *la.*

Genetic studies on resistance of rice varieties to BB have also been carried out in Korea, China, Bangladesh, and Indonesia. However, there are no designations of major genes for BB resistance. The identified major genes for resistance to bacterial blight are summarized in Table 2.

Table 2. Summary of BB resistance genes originally identified.

Gene identified	Cultivar analyzed	Isolate used	Note	Reference
		Japanese isolates		
X_1	Kogyoku	Giken 44		Washio et al (1966)
X_2, X_3	Shimotsuki	Nara Himeji	Complementary gene for *X1*	
Xa-1	Kogyoku Koganemaru Pi No. 1	X-I7	Chromosome 11 (of Nishimura 1961)	Sakaguchi (1967)
Xa-2	Rantai Emas 2	X-17 X-14	linked with *Xa-1* (2.16%)	Sakaguchi (1967)
Xa-3	Wase Aikoku 3 Java 14 Koentoelan Nagomasari	Q6808 (I) 07102 (I) T7174 (I) H5809 (II) T7147 (II) Q6809 (III) T7133 (III)	Adult resistance	Ezuka et al (1975)
Xa-1^h	IR28 IR29 IR30	T7141 (I)		Yamada (1984)
Xa- kg^h	IR28 IR29 IR30	H75304 (V)	linked with *Xa-1^h*: (2.0±0.65%)	
Xa-11	IR944-102-2-3-RP9-3	T7174 (I) T7147 (II) T7133 (IIIA) IVA7505 (IV)		Ogawa and Yamamoto (1986)
		Philippine isolates		
Xa-4	IR20 IR22 IR1529-680-3	PXO 25 (I)		Petpisit et al (1977)

continued on next page

Table 2 continued.

Gene identified	Cultivar analyzed	Isolate used	Note	Reference
xa-5	IR1545-339 RP291-7	PXO 25 (1)		Petpisit et al (1977)
Xa-4[a]	IR22 Sigadis TKM6, etc.	PXO 61 (1)		Librojo et al (1976)
xa-4[b]	Semora Mangga	PXO 61 (1)	Adult resistance	Librojo et al (1976)
Xa-6	Malagkit Sungsong Zenith, etc.	PXO 61 (1)		Sidhu and Khush (1978)
Xa-7	DZ78 DV85 DV86	PXO 61 (1)	Adult resistance	Sidhu et al (1978)
xa-8	PI 231 129	PXO 61 (1)		Sidhu et al (1978)
xa-9	Khao Lay Nhay Sateng	PXO 61 (1)	linked with *Xa-6* (5.9%)	Singh et al (1983)
Xa-10	CAS209	PXO 61 (1) PXO 86 (2) PXO 79 (3) PXO 71 (4)	linked with *Xa-4* (27.6 ± 0.2) Chromosome 5 of Shastry et al (1960)	Yoshirnura et al (1983)
		Sri Lankan isolates		
Xa-a, *Xa-k*	Wase Aikoku 3	CAR 1	Multiple gene	Watanabe (1976)
Xa-i	PI 209938 Zenith RL Gopher		Multiple gene	
Xa-p	PI 209938 Zenith		Multiple gene	
Xa-b	Bluebonnet/ Rexark		Incomplete	
		Indonesian isolates		
Xa-kg	Kogyoku Java 14	Xo-7306 (V)	linked with *Xa-1* (2%)	Ogawa et al (1978)
		Indian isolates		
X1	BJ1	H14		Jayaraj et al (1972)
x2		H89		
x3		H 146		
I-x1	IR8	H14, H89, H146	Inhibitor to *X1, X2,* and *X3*	Jayaraj et al (1972)
A	Malagkit	X010		Moses et al (1974)
B	Sungsong	X032		
P1	Lacrosse/ Zenith-Nira			
R	IRRI 69/469 IRRI 70/470			
1p	IR8		Inhibitor to *P1*	
		Chinese isolates		
Xa-a	IR28			Zhao et al (1985)
Xa-h			linked with *Xa-a* (17%)	Yu et al (1986)

COMPARISON OF IDENTIFIED RESISTANCE GENES

The genes for BB resistance identified in each country, except for *Xa-12,* were reported on the basis of analysis using local bacterial isolates. They could not be compared directly with each other. Therefore it was necessary to reanalyze the key varieties using a uniform set of races, and to compare the results of the previous studies in each country, since it is important to set up a common base to define the relationships between the groups of BB pathogens and the resistance of rice varieties to the races.

For this purpose, IRRI and the Ministry of Agriculture, Forestry, and Fisheries of Japan, through the Tropical Agriculture Research Center (TARC), initiated collaborative research on BB resistance in 1982. Since then, Japanese and IRRI differentials have been analyzed using Japanese races at TARC as well as using Philippine races at IRRI. The results of this collaboration follow.

Resistance of Kogyoku (*Xa-1, Xa-12*), Tetep (*Xa-1, Xa-2, Xa-12*), and Cas 209 (*Xa-10*)

Kogyoku and Tetep are susceptible to Philippine races 1-4, while Cas 209 is susceptible to 7 Japanese races (Ogawa and Yamamoto 1987). These results are similar to those of Horino et al (1981). Therefore, Kogyoku, Tetep, and Cas 209 probably do not carry additional genes against Japanese and Philippine BB races. Also *Xa-1, Xa-2, Xa-10,* and *Xa-12* are different from any other identified gene in Japan or at IRRI (Ogawa and Yamamoto 1987).

Resistance of Wase Aikoku 3 (*Xa-3*), Chugoku 45 (*Xa-3*), and Java 14 (*Xa-1, Xa-3, Xa-12*)

Chugoku 45 and Java 14 showed resistance to Philippine races 1–4 (Horino et al 1981, Ogawa and Yamamoto 1987). We have done genetic analysis of resistance in Chugoku 45 and Java 14 using Japanese races at TARC and Philippine races at IRRI. The studies at IRRI showed that F_1 plants of Toyonishiki/Chugoku 45 and Toyonishiki, Java 14 are resistant to Philippine races 1-4. The F_2 population of these two crosses showed a segregation ratio of 3 RRRR (resistant to races 1–4):1 SSSS (susceptible to races 1-4). The F_2 population of Wase Aikoku 3/ Chugoku 45 and Chugoku 45/ Java 14 showed no segregation for susceptibility (Table 3).

The F_3 lines from the cross Toyonishiki, Chugoku 45 derived from F_2 plants that were resistant to Philippine races were inoculated with Japanese races at TARC. Of 133 lines tested, 33 were homozygous resistant, 100 were segregating, and none was completely susceptible for resistance. On the other hand, 53 F_3 lines derived from susceptible F_2 plants were homozygous in susceptibility. Similar results were obtained when the reaction of F_2 plants of this cross at TARC was compared with the F_3 reaction at IRRI.

Thus, we concluded that gene *Xa-3* in Wase Aikoku 3, Chugoku 45, and Java 14 conveys resistance to Philippine races 1-4 (Ogawa et al 1986e). Trisomic analysis of gene *Xa-3* located it on chromosome 11 of the chromosome numbering system of

Table 3. Reaction[a] to BB of F_1 and F_2 progenies at booting to flowering (Ogawa et al 1986e).

Cross	F_1 reaction to 4 races	F_2 reaction to 4 races		X^2	P
At IRRI		*RRRR* (no.)	*SSSS* (no.)		
Toyonishiki/ Chugoku 45	RRRR	193	65	0.005	>0.9
Toyonishiki/Java 14	RRRR	187	78	2.779	0.5-0.7
Wase Aikoku 3/ Chugoku 45	RRRR	226	0		
Chugoku 45/Java 14	RRRR	547	0		
At TARC		*R (no.)*	*S (no.)*		
Wase Aikoku 3/ Kinmaze	R	174	55	0.118	0.8-0.9
Chugoku 45/Kinmaze	R	172	62	0.279	0.5-0.7
Toyonishiki/ Chugoku 45	R	232	75	0.053	0.8-0.9
Kinmaze/Java 14	R	98	33	0.002	> 0.95
Toyonishiki/Java 14	R	275	75	2.381	0.1 -0.2
Wase Aikoku 3/Java 14	R	289	0		
Chugoku 45/ Wase Aikoku 3	R	266	0		
Chugoku 45/Java 14	R	160	0		

[a]R = resistant, S = susceptible. For reaction of cross at IRRI, the first letter stands for reaction to Philippine race 1, the second to race 2, the third to race 3, and the fourth to race 4. For cross at TARC, Japanese isolate T7133 (race IIIA) was used for inoculation.

Shastry et al (1960), and it showed a recombination value of 22.3% with *d-27* from linkage analysis using gene markers at IRRI (Ogawa et a1 1987b).

Resistance of Zenith (*Xa-6*), Sateng (*xa-9*), Semora Mangga (*Xa-4^b*), and Cempo Selak (unknown gene)

Zenith, Sateng, Semora Mangga, Cempo Selak, and Java 14 (*Xa-3*) showed similar reactions to Japanese and Philippine races; that is, they were resistant to most BB races, showed a browning reaction around the lesion (Kaku and Kimura 1978), and were evaluated as moderately resistant to BB races (Table 4) (Ogawa and Yamamoto 1987). Semora Mangga, which had earlier been reported to carry *Xa-4^b,* showed a different reaction from that of *Xa-4* to Philippine races 1–4. All these cultivars are classified as javanicas based on plant morphology.

Thus, we have conducted allele tests between resistance gene *Xa-3* (using Chugoku 45 and Java 14) and the resistance genes of the above varieties.

From the analysis of F_1 hybrids and F_2 populations between susceptible cultivars and the resistant cultivars mentioned above, the resistance of Zenith, Sateng, and Cempo Selak to Japanese and Philippine races was found to be controlled by one dominant gene (Table 5). In earlier studies (Singh et al 1983), Sateng was thought to carry a recessive gene *xa-9,* but our results show it has a single dominant gene.

Table 4. Reaction[a] of representative rice cultivars to 4 Philippine races of BB at booting (Ogawa et al 1986b).

Cultivar	Race 1		Race 2		Race 3		Race 4	
	Lesion length[b] (cm)	Reaction	Lesion length[b] (cm)	Reaction	Lesion length[b] (cm)	Reaction	Lesion length[b] (cm)	Reaction
IR24 (susceptible check)	24.7	S	18.5	S	20.6	S	18.4	S
IR20	1.8	R	13.8	S	14.8	S	7.4	MR
Semora Mangga	1.9	R	0.7	R	0.7	R	0.8	R
Wase Aikoku 3	1.8	R	0.7	R	0.6	R	0.6	R
Chugoku 45	2.0	R	1.5	R	1.1	R	0.7	R
Java 14	2.1	R	1.2	R	1.0	R	0.8	R
Zenith	3.4	R	1.2	R	0.5	R	0.5	R
Sateng	6.9	MR	8.7	MR	7.8	MR	3.4	MR
Cempo Selak	1.7	R	1.8	R	0.7	R	0.3	R

[a]Average lesion length in 15 inoculated leaves (3 leaves from 5 plants) at 14 d after inoculation (DAI). R = resistant, MR = moderately resistant, S = susceptible.

Table 5. Reaction[a] to BB of F_1 and F_2 progenies at booting to flowering (Ogawa et al 1986b), c,d).

Cross	Reaction of F_1 to race IIIA	Reaction of F_2 to race IIIA		X^2 (3: 1)	P
At TARC		*R (no.)*	*S (no.)*		
Kinmaze/Zenith	R	282	109	1.726	0.1-0.2
Toyonishiki/Zenith	R	306	92	0.800	0.3-0.5
Chugoku 45/Zenith	R	398	0		
Toyonishiki/Sateng	R	299	98	0.021	0.8-0.9
Chugoku 45/Sateng	R	356			
Sateng/Zenith	R	302			
At IRRI	*Reaction of F_1 to races 1, 2, 3, 4*	*Reaction of F_2 to races 1, 2, 3, 4*			
		RRRR (no.)	*SSSS (no.)*		
IR24/Zenith	RRRR	165	47	0.960	0.3-0.5
Chugoku 45/Zenith	RRRR	230	0		
Java 14/Zenith	RRRR	277	0		
IR24/Sateng	RRRR	139	58	2.073	0.1-0.2
Toyonishiki/Sateng	RRRR	172	51	0.539	0.3-0.5
Chugoku 45/Sateng	RRRR	253	0		
Java 14/Sateng	RRRR	339	0		
Sateng/Zenith	RRRR	355	0		
Java 14/Semora Mangga	RRRR	331	0		

[a] See Table 3.

Furthermore, in the F_2 populations from the crosses between Chugoku 45 or Java 14 and either Zenith, Sateng, Semora Mangga, or Cempo Selak, no susceptible plants appeared (Table 5).

These results show that Zenith, Sateng, Semora Mangga, and Cempo Selak have *Xa-3* for resistance. Gene symbols *Xa-4*^{b}, *Xa-6,* and *xa-9* are therefore redundant (Ogawa et a1 1986b,c,d).

Resistance of IR20 (*Xa-4*)

IR20 is highly resistant to Japanese races IA, IB, and V, and resistant to II, IIIA, IIIB, and IV (Ogawa and Yamamoto 1987). We analyzed F_2 populations of IR24/IR20 at TARC and IRRI, and the F_3 lines derived from F_2 plants grown at TARC were evaluated for resistance at the seedling stage. We also analyzed the F_2 population of Kogyoku/ IR20, because IR20 showed high resistance—similar to that of Kogyoku—to races IA, IB, and V.

Data from the F_2 analysis at TARC show that the resistance of IR20 is controlled by one dominant gene, *Xa-4,* which confers moderate resistance to all known Japanese races. Allele tests with Kogyoku showed that IR20 also possesses *Xa-1* and *Xa-12.*

Kaku and Kimura (1985) also reported that Sigadis and TKM6 (with *Xa-4*) showed moderate resistance to races II,III, and IV, and high resistance to races I and V.

We also analyzed the relationship between *Xa-3* and *Xa-4,* because *Xa-4* and *Xa-4*^{b} had been reported to be allelic (Librojo et a1 1976). The F_1 progenies of

IR20/ Java 14 were resistant to the four races. In the F_2 population of 344 plants, 254 were resistant to the 4 races and 90 were resistant to race 1, moderately resistant to race 4, but susceptible to races 2 and 3. However, no plants susceptible to all four races appeared in this population. Therefore, we concluded that *Xa-3* and *Xa-4* are very closely linked (Ogawa et al 1986b). The cultivars having *Xa-4* reported by Sidhu et al (1978, 1979) might be separated into two groups: some with *Xa-3* and others with *Xa-4*.

IR20 is moderately resistant to race 4. The nature of its resistance gene was reported by Yoshimura et al (1985), who indicated a continuous frequency distribution of lesion length to race 4 in the F_2 population.

Resistance of IR1545-339 *(xa-5)*

We confirmed that the resistance of IR1545-339 is controlled by one recessive gene, which conveys high resistance to Philippine races 1-3 and moderate susceptibility to race 4. This recessive gene is effective against Japanese races, and IR1545-339 apparently does not carry additional genes against Japanese races.

Resistance of DVS5 *(xa-5, Xa-7)*

DV85 was confirmed to have one recessive gene and one dominant gene as reported by Sidhu et al (1978): The dominant gene, *Xa-7,* conveys high resistance to Philippine races 1-3, but DV85 is susceptible to race 4. *Xa-7* confers high resistance to race 1 in the homozygous genotype, but moderate susceptibility or susceptibility in the heterozygous genotype.

DV85 may have additional recessive genes, but this is still being investigated. DV85 also appears to have one dominant gene and one recessive gene for resistance to Japanese races.

Resistance of PI 231129 *(xa-8)*

PI 231129 was confirmed to have one recessive gene for resistance or moderate resistance to Philippine races 1-3. However, whether or not *xa-8* is effective against race 4 has not been confirmed. PI 231129 also showed resistance to all Japanese races, and this resistance was considered to be controlled by one recessive gene.

NEW GENES FOR RESISTANCE

Rice cultivars were classified into three major groups based on their reaction to four Philippine races (Ogawa et al 1986a). Varieties of the Java 14 group are resistant to all four Philippine races at booting and have a single dominant gene, *Xa-3,* for resistance. Varieties of the TKM6 group are resistant to race 1, moderately resistant to race 4, but susceptible to races 2 and 3; they have a single dominant gene, *Xa-4,* for resistance. Varieties of the DZ192 group are resistant to races 1-3 and moderately resistant to race 4; they are resistant at the seedling stage and have a recessive gene, *xa-5,* for resistance.

Recently, we observed that varieties showing the reaction pattern of Cas 209 to Philippine races 14 appeared to be a fourth major group in the results of our screening tests.

In addition to the four major groups, we are finding several minor groups of varieties, such as varieties carrying *Xa-4* and *Xa-10; xa-5* and *Xa-7;* and *Xa-4* and *xa-5*. One of the minor groups showed resistance to all six Philippine races (races 5 and 6 were recently found at IRRI). The F_1 hybrids of these varieties with IR24 showed susceptibility to all six races. Varieties BJ1, AC19-1-1, Aus 274, Chinsurah Boro 11, and Kalimekri 77-5 belong to this group, which we have designated the BJ1 group.

The F_1 progenies of the crosses of these varieties with IR1545-339 were resistant to races 1 and 2, moderately resistant to race 4, but susceptible to race 6. These results show that varieties of the BJ1 group have the recessive gene *xa-5* for resistance.

The F_2 plants from the crosses of IR24 with BJ1 group varieties can be classified into four groups based on their reaction patterns to the six races. Plants of group 1 are susceptible to races 1-4. Plants of group 2 show a reaction pattern similar to that of IR1545-339. The reaction of the plants in group 3 is similar to that of their resistant parents. The fourth group of plants shows resistance to race 6 only.

The F_2 plants from the crosses of IR1545-339 with BJ1 group cultivars did not segregate for susceptibility to races 1 or 2 but segregated in a ratio of 1 resistant:3 susceptible to race 6.

These results indicate that the five varieties of the BJ1 group have two recessive genes; one is *xa-5* and the other is the new recessive gene. We designated this gene *xa-13* (Ogawa et al 1987a). Gene *xa-13* conveys resistance only to race 6 but has a complementary effect with *xa-5* in imparting resistance to race 4.

Variety TN1 was resistant only to race 5 (PXO 112) but was very susceptible to the other 5 races. This reaction is different from any other differential (Table 6). The F_2 populations of Toyonishiki/TNl, Milyang 23/TN1, and TN1/Tetep, and the backcross progeny of IR24/ TN1// IR24 were analyzed (Table 7). The resistance of TN1 to race 5 was controlled by one dominant gene, which we designated as *Xa-I4* (Taura et al 1987).

The genes for resistance to BB identified so far are listed in Table 8 (Ogawa 1987).

A COMMON BASE FOR GENETIC RESEARCH INTO BACTERIAL BLIGHT RESISTANCE

We are now developing continuously near-isogenic lines with monogenic resistance under the Japan-IRRI collaboration. We have just developed near-isogenic lines with *Xa-3, Xa-4, xa-5, Xa-7, xa-8, Xa-10,* and *Xa-11;* near-isogenic lines with *Xa-1, Xa-2, Xa-12, xa-13,* and *Xa-14* are in the final stages of development.

Systematic research on varietal resistance to BB is difficult in Asian countries because we do not have a common basis for genetic research. After developing near-isogenic lines with monogenic bases, we might be able to start systematic identification of resistance genes to BB.

NATURE OF MAJOR GENE RESISTANCE TO BACTERIAL BLIGHT

Major genes or qualitative genes confer a high level of resistance, but the resistance may break down due to the evolution of new races of the pathogen (Ezuka and

Table 6. Reaction of differential rice cultivars to Philippine races of BB at booting (Taura et al 1987).[a]

Cultivar	Race 1		Race 2		Race 3		Race 4		Race 5		Race 6	
	LL (cm)	RE	LL (cm)	RE	LL (cm)	RE	LL (cm)	RE	LL (cm)	RE	LL (cm)	RE
IR24 (+)	25.7	S	35.6	S	32.5	S	31.6	S	22.7	S	36.0	S
Milyang 23 (+)	15.3	S	26.5	S	28.5	S	31.3	S	24.9	S	43.5	S
Toyonishiki (+)	8.3	MS	17.0	S	16.2	S	11.6	S	12.9	S	21.2	S
TN1	19.6	S	35.2	S	37.4	S	31.5	S	4.9	R	30.4	S
Tetep (*Xa- 7, Xa-2*)	14.5	S	30.7	S	29.1	S	26.5	S	17.0	S	17.0	S
Chugoku 45 (*Xa-3*)	0.4	R	1.9	R	2.2	R	1.3	R	3.5	R	11.5	S
Java 14 (*Xa-1, Xa-3, X-12*)	0.6	R	2.3	R	1.4	R	2.9	R	6.9	R	24.4	S
IR8 (*Xa-11*)	17.0	S	25.6	S	28.7	S	14.9	S	13.6	S	19.5	S
IR20 (*Xa- 7, Xa-4*)	6.9	R	22.5	S	24.5	S	9.7	MS	3.9	R	18.3	S
IR1545-339 (*xa-5*)	1.2	R	2.6	R	1.1	R	17.9	S	4.4	R	30.0	S
OV85 (*xa-5, Xa-7*)	0.4	R	0.4	R	0.5	R	6.0	MR	2.0	R	6.5	MR
Cas 209 (*Xa-10*)	24.2	S	3.5	R	45.3	R	40.0	S	1.7	R	50.4	S
BJ1 (*xa-5, xa-13*)	0.6	R	3.8	R	2.8	R	4.7	R	0.6	R	3.7	R
PI 231 129 (*xa-8*)	3.5	R	8.0	R	6.5	R	12.2	MS	5.5	R	4.5	R

[a]LL = average lesion length from 15 inoculated leaves (3 leaves from 5 plots) at 14 DAI. RE = reaction: R = resistant, MR = moderately resistant, MS = moderately susceptible, and S = susceptible.

Table 7. Reaction[a] of F_1 and F_2 populations from the crosses of TN1 with 4 cultivars to 4 races (races 1,2, 4,5) of BB of rice (Taura et al 1987).

Cross	Reaction of F_1 progenies	Reaction of F_2 populations		x^2 (3:1)	P
		SSSR	*SSSS*		
Toyonishiki/TN1	SSSR	185	84	5.563	0.01 -0.02
Milyang 23/TN1	SSSR	200	70	0.123	0.7 -0.8
TN1 /Tetep	SSSR	208	57	1.722	0.1 -0.2

[a] R = resistant, S = susceptible.

Sakaguchi 1978). However, our data indicate that major gene resistance to BB is variable; it is not always of a high level.

Xa-1, Xa-2, Xa-10, and *Xa-14* convey high resistance to a few Japanese or Philippine races of the BB pathogen. These genes are considered to confer typical qualitative resistance.

Gene *xa-5* conveys high resistance to Philippine races 1-3 and 5, and *Xa-7* to Philippine races 2 and 3.

The resistance conferred by *Xa-4* to Philippine race 1, by *Xa-11* to Japanese races, and by *Xa-3* to Japanese and Philippine races appears to be moderate

Table 8. Gene symbols for resistance to BB of rice (Ogawa 1987).

Gene symbol[a]	Original designation	Representative cultivars
Xa-1	*Xa-1*	Kogyoku, Java 14
Xa-1^h (t)	*Xa-1*^{h}	IR28, IR29, IR30
Xa-2 (t)	*xa2*	Rantai Emas 2, Tetep
Xa-3	*Xa-w*	Wase Aikoku 3, Chugoku 45, Java 14
	Xa-4	Sernora Mangga
	Xa-6^b	Zenith
	xa-9	Sateng
Xa-4	*Xa-4*	TKMG, IR20, IR22
	Xa-4^a	
xa-5		DZ192, IR1545-339
Xa-7		DV85
xa-8		PI 231 129
Xa-10		Cas 209
Xa-11		Elwee, IR8, RP9-3
Xa-12 (t)	*Xa-kg*	Kogyoku, Java 14
Xa-12^h (t)	*Xa-kg*^{h}	IR28, IR29, IR30
xa-13		EJ1, Chinsurah Eoro II
Xa-14		TN1

[a](t) = tentative symbol pending further confirmation.

compared with the genes described above, and these genes express incomplete resistance in the heterozygous condition. Genes *Xa-4* and *xa-5* convey only low level resistance to Philippine race 4.

Among the above genes, *Xa-3, xa-5,* and *xa-8* appear to convey a wide spectrum of resistance to Asian isolates of the BB pathogen.

Quantitative resistance is usually considered to be governed by minor genes or polygenes, because it does not show a clear-cut Mendelian segregation ratio in the hybrid population. On the other hand, quantitative resistance is believed not to be broken down by the shift of races of the pathogen. However, there is no clear evidence that major gene resistance to BB in rice is not useful in controlling the disease. Similarly, there is no proof that minor gene resistance (polygene resistance) is always effective in protecting the rice crop from BB. Therefore, we need more basic research on the comparative effectiveness of the two types of resistance in controlling BB as well as on the genetics of so-called quantitative resistance.

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Genetic behavior of quantitative resistance to bacterial blight in rice

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Bacterial blight resistance in rice is generally categorized into two types. qualitative and quantitative resistance, from the viewpoint of assessing disease development. Because of its nonspecificity to pathogen race, quantitative resistance has become of general interest in breeding programs. Although the genetic behavior of quantitative resistance is not well understood, recent studies have shown that the quantitative differences in resistance observed in some rice varieties are inherited and that the resistance is controlled by polygenes or by several genes. In hybrid progenies, quantitative resistance is not race-specific. One example of major gene control of quantitative resistance is described.

Bacterial blight (BB) of rice caused by *Xanthomonas campestris* pv. *oryzae* is a major disease, now widely distributed in most rice-growing countries. Its incidence has become more severe in Asia since the introduction of modern agricultural practices such as cultivation of high-yielding varieties and heavy application of N fertilizers. Although bactericides are applied to control BB in Japan, none are highly effective or economical. Therefore, using resistant varieties appears to be the most appropriate control method.

BREEDING STRATEGIES AND IMPORTANCE OF QUANTITATIVE RESISTANCE

Work on varietal improvement for BB resistance has been carried out in Japan for the last 50 yr and at the International Rice Research lnstitute (IRRI) for the last 20 yr, and many varieties and breeding material with resistance to this disease have been developed (reviewed by Ezuka and Sakaguchi 1978, Khush and Virmani 1985). National breeding programs of the other Asian countries are also endeavoring to incorporate adequate BB resistance into improved varieties. The efforts to breed resistant cultivars have minimized disease damage in Asian regions where varieties with adequate resistance are grown (Mew and Khush 1981). In past breeding programs, however, high-level resistance, specific to the race of the pathogen, was used as the main genetic source of resistance. Recent reports on pathogenic variation in the bacterium suggest the existence of broad-spectrum pathogenic races and

shifting of the races of the bacterial population (Eamchit and Mew 1982, Horino 1981, Horino et al 1983, IRRI 1985. Mew and Vera Cruz 1979, Mew et al 1982, Yamamoto et al 1977), which threaten to break down varietal resistance.

Several breeding strategies for BB resistance have been proposed to cope with the breakdonwn of varietal resistance, concisely summarized by Ezuka and Sakaguchi (1978) as follows:

- use of horizontal resistance,
- incorporation of horizontal resistance into vertical resistance,
- mixed cultivation of varieties with different vertical resistance,
- rotation of varieties with different vertical resistance, and
- accumulation of a large number of resistance genes in a single variety.

To determine which breeding strategy is applicable and to actualize that strategy, careful prediction of the pathogen race and detailed genetic, pathological, and epidemiological information about the resistance of rice varieties are essential.

Rice varieties differ in degree of resistance or susceptibility to BB. From the viewpoint of assessing disease development, BB resistance is generally categorized into two types: qualitative and quantitative. Qualitative resistance is high-level resistance; comprehensive assessments of this type of resistance by using many isolates and subsequent genetic analysis have indicated that it is usually race-specific and under major gene(s) control, several of which have been identified. On the other hand, quantitative resistance is low-level resistance that generally shows no pathogen race specificity; that is, it is horizontal, although the evidence so far (Ando et al 1973, Kaku et al 1980, Ogawa and Sekizawa 1980. Yamamoto et al 1977) is incomplete. The horizontal nature of quantitative resistance is important for a breeding program to avoid the breakdown of varietal resistance. Therefore, quantitative resistance to BB has lately become of general interest.

POLYGENE-CONTROLLED RESISTANCE

Genetic analysis supplies basic information to support practical breeding. The most effective and careful selection for a certain character can be achieved only when breeders know both the mode of inheritance of a character—whether major genic or polygenic—and the characteristics of gene expression. Quantitative resistance to BB in some rice varieties should be considered major genic or polygenic.

Quantitative resistance of rice varieties to BB controlled by polygenes is a complicated topic for genetic analysis, because there is usually continuous variation, with no distinct classes, in segregating populations. Therefore, biometrical genetics and selection trials in successive generations have been used for analysis.

Washio et al (1966) first reported that the slow lesion-developing type of resistance (quantitative resistance) in some Japanese varieties to Japanese bacterial group I was controlled by polygenes. In Sri Lanka, Watanabe (1976) applied the biometrical genetics approach in several cross combinations including varieties showing moderately resistant to susceptible reactions. Mid-parent values of resistance in F_1 plants and normal distribution for resistance in F_2 populations suggested that the moderate resistance of some rice varieties involved in the crosses

was governed by polygenes, and high heritability values were estimated. Nagaraju et al (1977) pursued the segregation pattern of disease resistance up to the F_6 generation derived from the cross between the resistant indica variety BJ1 and the susceptible japonica variety Jukkoku. Their results showed that the inheritance of resistance in BJ1 was polygenic. Although early studies thus gave possible evidence that the quantitative nature of BB resistance in some rice varieties was under polygenic control, the information was fragmentary.

Wasano and his colleagues systematically conducted genetic analysis for BB resistance in the progenies from cross combinations including only rice varieties without any major gene(s) showing substantially low but different degrees of resistance (Wasano 1982, Wasano and Dhanapala 1982, Wasano et al 1979). The genetic analysis of early generations (F_1, F_2, and F_3) clearly revealed that the mode of inheritance for resistance was polygenic, and the dominance effect contributed more to genetic variation in the early generations than did the additive effect. These results suggested that selection for resistance in early generations was not effective, and that selection should be done in later generations. Several kinds of heritability estimated in their studies also supported this conclusion. In a selection experiment for polygenic resistance, the resistant lines selected were more resistant than the resistant parent, and the lines showed the same level of resistance to five strains belonging to different bacterial groups. Thus, Wasano and his colleagues clearly demonstrated polygenic inheritance of BB resistance.

Comprehensive analysis of quantitative resistance to the disease controlled by polygenes or by several genes was conducted by Yamada and his colleagues. First, Horino and Yamada (1979) evaluated the resistance of IRRI varieties including IR28 to representative strains of the five Japanese bacterial groups. They found that most IRRI varieties showed qualitative resistance to groups I and V and a high degree of quantitative resistance to groups II-IV. Yamada et al (1979) and Yamada (1984) analyzed the genetic behavior of IR28 quantitative resistance to bacterial groups II-IV. The mid-parent values of disease scores in F_1 plants from the cross of IR28 with susceptible varieties, and the normal distribution for disease scores in the F_2 population. showed that quantitative resistance of IR28 to groups II-IV was controlled by several genes or by polygenes. Analysis by the additive-dominance model using square root transformations of original disease scores revealed that the additive gene effect contributed very significantly to the segregation population mean, and that the dominance gene effect was relatively less important. The researchers also suggested that some other more complicated factor(s) may contribute an effect to the segregating population mean. High values of broad-sense and narrow-sense heritabilities estimated in their studies suggested that quantitative resistance to the three bacterial groups could successfully be selected for in early generations. High positive correlation coefficients among disease scores for the three pathogen groups suggested simultaneous selection of plants resistant to those three groups in segregating populations. In addition, Yamada (1986) examined the relationship between quantitative resistance and the *Xa-l*^{h} gene controlling qualitative resistance (Yamada and Horino 1981). Mean comparison of disease scores for bacterial groups II-IV in three genotypic groups for *Xa-l*^{h} in F_2 plants and

F_3 lines showed that the groups homozygous and heterozygous for *Xa-1*^{h} were significantly more resistant than the group lacking *Xa-1*^{h}. Yamada (1986) also investigated the effect of quantitative resistance to bacterial groups I and V by using F_3 lines lacking *Xa-l*^{h} and *Xa-kg*^{h}, and found genetic variations for quantitative resistance to all bacterial groups, and high positive correlation among disease scores to all five pathogen groups. Thus, Yamada and his colleagues intensively analyzed the quantitative resistance of IR28 by biometrical genetics and provided useful information about the genetics of and breeding for quantitative resistance to BB in rice.

Most IRRI varieties, including IR28, have been bred through screening for *Xa-4* controlling resistance to race 1 of Philippine isolates and show a similar reaction pattern to the five Japanese bacterial groups. The relation between IR28 quantitative resistance to Japanese isolates and *Xa-4* resistance is not yet clear.

MAJOR GENIC CONTROL OF QUANTITATIVE RESISTANCE

There is only one case of major genic control of quantitative resistance to BB of rice: Yoshimura et al (1984,1985) found that IR1545-339, a rice differential for classifying Philippine bacterial strains into races, conveyed not only high resistance to races 1-3 but also weak resistance to strain PXO 71 of race 4. Subsequently, they analyzed the genetic behavior of resistance in a cross combination of the fully susceptible variety TN1 and IR1545-339, and further examined the relationship between the weak resistance and the high resistance to races 1-3 governed by a recessive gene xa-5 in the F_2 and F_3. The F_1 plants showed the same level of susceptible reaction to PXO 71 as did TN1 . The distribution of lesion lengths caused by PXO 71 in the F_2 population deviated from normal and showed two modes at the parental values. In the same F_2 population, the plants that were homozygous for xu-5 were distributed around the resistance mode without exception; and the plants that were heterozygous for *xa-5* were distributed around the susceptibility mode. F_3 tests showed the same tendency. These results demonstrated that xu-5, which conveys high resistance to races 1-3, is also responsible for weak resistance to PXO 71 of race 4. This pleiotropic effect of a major gene should be considered when studying the genetics of quantitative resistance to BB. More such studies are needed.

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The genetics of *Xanthomonas campestris* pv. *oryzae*

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Conjugal transfer systems for introducing broad host-range cosmid cloning vectors are now available for several *Xanthomonas campestris* pathovars including *X. campestris* pv. *oryzae* (Xco), the causal agent of bacterial blight of rice. Extrachromosomal DNA can also be introduced into Xco by transformation, but at low frequencies. Once in the bacteria, cosmids pLAFR5 and pSa747 are stably maintained in the absence of antibiotic selection. Thus, we now have a genetic system in Xco that will enable us to analyze the structure and function of genes involved in pathogen-plant interactions.

Although considerable advances have been made in molecular genetic techniques, their application to *Xanthomonas campestris* pv. *oryzae* (Xco) has been limited by the lack of genetic systems in the pathogen that are amenable to molecular analysis. Without such tools, our investigations of the interactions (both compatible and incompatible) between the bacterial blight pathogen and rice are severely limited. Our major efforts have been to develop DNA cloning systems in Xco. This paper describes the current status of genetic research in *Xanthomonas* spp. and the progress made toward developing a genetic system for Xco.

XANTHOMONAS GENETICS

Conjugal DNA transfer techniques

Xanthomonads, in general, are latecomers in terms of classical and molecular genetics. However, within the last 5 yr, several reports have been published involving the cloning of various genes from different *Xanthomonas* pathovars and their use in complementation of mutant phenotypes (Daniels et a1 1984b, Gabriel et al 1986, Harding et a1 1987, Swanson et a1 1988). These accomplishments were made possible by using vectors adapted for other plant bacteria such as pLAFRl, which was developed for use with *Rhizobium* (Friedman et al 1982), or pSa747, which was developed for use with Enterobacteriaceae, Rhizobiaceae, and Pseudomonadaceae (Shaw et al 1988). Cosmid pLAFRI, for example, is an IncP plasmid that is not self-transmissible but can be mobilized into some xanthomonads by trans-acting

functions on the narrow host range plasmid pRK2013 (Ditta et al 1980). pRK2013 cannot replicate in *X. campestris* pvs. *campestris* (Turner et al 1984), *vesicatoria* (Swanson et al 1988), or *oryzae* (Kelemu and Leach, unpubl. data). Thus, using triparental matings (Ditta et al 1980), DNA cloned in pLAFR1 can be conjugally transferred from *Escherichia coli* (where the library is maintained) to xanthomonads. Daniels and coworkers (for review, see Daniels et al 1987) used these tools to investigate genes involved in the interactions of *X. campestris* pv. *campestris* and crucifers. Their strategy involved the initial isolation of chemically induced *X. campestris* pv. *campestris* mutants altered in pathogenicity (path$^-$) to turnips (Daniels et al 1984a). In parallel, a genomic library of wild-type *X. campestris* pv. *campestris* DNA was constructed in pLAFR1 (Daniels et al 1984b). To identify specific clones containing genes involved in pathogenicity, the library was introduced conjugally into the path$^-$ mutants, and the resulting transconjugants were screened on turnip seedlings for restoration of pathogenicity. Some of the genes that complemented the mutants were further characterized by restriction mapping and Tn5 mutagenesis in *E. coli* (Turner et al 1985). The fragments containing Tn5 insertions were introduced into the wild-type *X. campestris* pv. *campestris* genome by marker exchange mutagenesis, thereby constructing defined mutants deficient in pathogenicity. Detailed study of these nonpathogenic Tn5 mutants indicated that they had reduced ability to excrete extracellular enzymes (Daniels et al 1987). Thus, by using recombinant DNA techniques combined with transposon mutagenesis, genes important in pathogenicity were identified and, in some cases, a function has been ascribed to the gene product. Other nonpathogenic mutants were not defective in production or excretion of the enzymes tested, suggesting that factors not yet known are involved in pathogenicity (Dow et al 1987). Recently, *X. campestris* pv. campestris genes that are expressed differentially following entry of the pathogen into turnip plants have been isolated (Osbourn et al 1987). It will be interesting to determine if such genes or their products play a role in pathogenicity.

Others have reported the isolation of genes involved in incompatibility, or avirulence genes, from *X. campestris* pvs. *malvacearum* and *vesicatoria* (Gabriel et al 1986, Swanson et al 1988). These pathogens, like Xco, exhibit pathogenic specialization on their host plants. Genomic libraries from isolates that conferred incompatibility in association with particular host genotypes were constructed and mobilized into compatible pathogens. Transconjugants were screened for a change from a compatible to an incompatible response on given host cultivars. For example, beginning with such a strategy, Swanson et al (1988) isolated a DNA fragment from *X. campestris* pv. *vesicatoria,* which contains an avirulence gene avrBs$_1$. When a plasmid containing avrBs$_1$ was introduced into spontaneous race-change mutants (incompatible to compatible on pepper), the ability of the mutants to confer incompatibility was restored on pepper containing the Bs$_1$ locus for resistance to *X. campestris* pv. *vesicatoria.* The identification and isolation of genes that confer incompatibility suggests that induction of resistance is the result of positive gene functions in the pathogen. Thus, the avirulence genes will be useful tools in understanding race-specific resistance.

Transformation

Transformation of narrow or broad host-range plasmids into *X. campestris* pathovars has been unsuccessful (Turner et a1 1984). Recently, Murooka et al (1987) developed a plasmid vector (pBXC12) from a *X. campestris* pv. *citri* plasmid (pXCL6) and pBR328 that could be transformed into several *Xanthomonas* pathovars, including *oryzae* and *campestris.* It appeared critical that cells be grown to the mid-logarithmic phase, because late logarithmic- or stationary-phase cells produced large amounts of polysaccharide, which presumably interfered with cell competency. To avoid restriction systems, plasmid preparations used for transformation were reisolated from transformed *X. campestris* pv. *citri.*

Mutagenesis

Transposon (Tn) mutagenesis of bacterial genomes is a better method for obtaining mutants than chemical mutagenesis because mutants with Tn insertions on the bacterial chromosome can be readily selected using the drug resistance conferred by the transposon. In addition, the transposon can be used as a probe to identify those mutants having a single Tn insertion (and thus, only one inactivated cistron). Daniels et al (1984a) used chemical mutagenesis—by methyl-N-nitro-N-nitrosoguanidine (NTG)—to isolate the path$^-$ mutants used in their investigations because transposon mutagenesis with Tn5 or Tn7 was unsuitable for use in *X. campestris* pv. *campestris* due to low transposition frequency, instability, or preferential insertion sites (Turner et al 1984). Recently, Shaw et al (1988) constructed a transposon (Tn*4431*) carried by the suicide vector, pSa325, which can be introduced randomly into *X. campestris* pv. *campestris.* Tn*4431* is derived from Tn*1721* and contains the promoterless luciferase (*lux*) operon of *Vibrio fischeri* and a tetracycline resistance gene. This vector system enables the in vivo construction of transcriptional fusions between the lux operon and bacterial promoters. Using Tn*4431,* a nonpathogenic mutant that could not grow in detached cauliflower leaves was isolated. A clone that restored pathogenicity was identified from a cosmid genomic library of the parent strain.

XANTHOMONAS CAMPESTRIS PV. *ORYZAE* GENETICS

Conjugal introduction of cosmid DNA into Xco

Although vector systems have been described for several other *X. campestris* pathovars, a major constraint to genetic analysis of Xco remained in the inability to introduce extrachromosomal DNA into the pathogen. Therefore, we determined conditions for efficient cosmid transfer into Xco. Based on the ability of members of the genus *Xanthomonas* to accept and maintain broad host-range IncP (pLAFRI) (Ditta et a1 1980) and IncW (pSa747) (Tait et al 1983) plasmids, we concentrated on conditions facilitating the introduction of plasmids from these groups into Xco. Furthermore, Murata (1982) reported that the IncP plasmid RP4::Mu could be conjugally introduced into Japanese Xco isolates, suggesting that conjugal introduction of vectors derived from the IncP group might be possible. In our initial

attempts using published mating procedures, no plasmid transfer could be detected with any of more than 50 Philippine and Indian Xco isolates tested. In these experiments, nutrient media—peptonesucrose agar (PSA) (Tsuchiya et al 1982), nutrient agar (Difco), and several minimal media described as suitable for Xco culture (Ou 1985)—were tested (with appropriate antibiotics added) for transconjugant selection. Cephalexin (20 µg/ml) was added to inhibit *E. coli* growth. Several characteristics of Xco may have contributed to the inability to recover transconjugants. First, the organism produces a large amount of slime in media, which may interfere with the cell-cell contact needed for cosmid transmission. Second, Xco grows very slowly not only in minimal media but also in nutrient media; thus, overgrowth by surviving donor *E. coli* cells occurred. Also, Xco is relatively fastidious and would not grow to single colonies on minimal media, hindering selection away from *E. coli.* Finally, we found that Xco was inhibited by *E. coli* if the *E. coli* cells were grown on Luria-Bertani (Miller 1972) or some other yeast-containing media. If *E. coli* was grown on nutrient medium (Difco), inhibition of Xco was not detectable.

To enhance selection away from *E. coli,* rifampicin-resistant Xco isolates were selected. When these strains were used in matings, pLAFR5 (a derivative of pLAFR1 from N.T. Keen, University of California, Riverside) and other pLAFR derivatives could be introduced into a rifampicin-resistant Xco isolate at low frequency (Table 1). pSa747 and pSF6 (IncW cosmid vector) (Selvaraj et al 1984) could be introduced into PXO 86^{rif} or PXO 61^{rif}, but again, at low frequency. The conjugation frequencies reported are from experiments done under conditions considered optimal for introducing cosmid DNA into these isolates. The liquid mating procedure used was adapted from that described by Panopoulos et al (1975). This procedure was not only simple and rapid, but it yielded more transconjugants

Table 1. Conjugal transfer frequencies of cosmid vectors pLAFR5, pSa747, and pSF6 into rifampicin-resistant or Czapek-selected *Xanthomonas campestris* pv. *oryzae* (Xco) isolates.

Xco recipient		Transfer frequency per recipient cell[b] with cosmid/helper		
Race	Isolate[a]	pLAFR5/pRK2013	pSa747/pSa322	pSF6/pRK2013
1	PXO 61^{rif}	NTD	2×10^7	2×10^7
1	PXO 61^{Cz}	NTD	2×10^5	2×10^5
2	PXO 86^{rif}	2×10^7	2×10^7	2×10^7
2	PXO 86^{Cz}	6×10^4	3×10^4	2×10^4
6	PXO 99^{rif}	NTD	NTD	NTD
6	PXO 99^{Cz}	2×10^7	2×10^5	2×10^5
5	PXO 112^{Cz}	7×10^4	5×10^4	3×10^4

[a]rif = rifampicin resistant, Cz = selected to grow on Czapek Dox minimal medium (pH 7.1) amended with 0.2% L-glutamic acid. [b]20 µl each of 1×10^9 cfu/ml *E. coli* HB101 (pLAFR5) and HB101 (pRK2013), HB101 (pSa747) and HB101 (pSa322), or HB101 (pSF6) and HB101 (pRK2013) were mixed with 150 µl recipient cells (10^9-10^{10} cfu/ml) in microtiter plate wells. The mixture was incubated at 28 °C for 16 h. Transfer frequencies of pLAFR5, pSa747, and pSF6 cosmids were calculated from the numbers of Tc^r (2.5 µg/ml), Km^r (50 µg/ml), or Sp^r S, m^r (50 µg/ml each) transconjugants, respectively, per number of recipients. NTD = no transconjugants detected.

than did filter paper matings. *E. coli* donor cells (4- to 6-h cultures in nutrient broth, 10^8-10^9 colony-forming units [cfu]/ml) containing a cosmid (pLAFR5 or pSa747) and helper plasmid (pRK2013 or pSa322, respectively) were mixed with the recipient Xco cells in microtiter dishes. The age of Xco cells (12-17 h, about 10^9-10^{10} cfu/ml) and length of mating (16 h) were critical. Although some rifampicin-resistant isolates could receive cosmid DNA via conjugation, the frequencies remained low.

To enhance mating frequencies and facilitate selection against *E. coli,* Xco isolates that grew to single colonies on a minimal medium (Czapek Dox supplemented with 0.2% L-glutamic acid, pH 7.1, hereafter called Cz) were selected. After adaptation on Cz medium, single colonies appeared within 4-7 d. Furthermore, on this medium, Xco produced less slime. Because continuous culturing of Xco often leads to loss of virulence on rice (T. W. Mew, IRRI, Los Baños, Philippines, 1987, pers. comm.), only those antibiotic-resistant and Cz-selected isolates that maintained pathogenicity on appropriate rice cultivars were used in subsequent studies. Both rifampicin resistance and ability to grow on Cz medium were stable after transfers through plants or on culture media such as nutrient agar or PSA.

Cz-selected isolates were very efficient recipients (10^{-4} -10^{-5} transconjugants/ recipients) of pLAFR5, pSa747, and pSF6 (Table 1). We do not know why the Cz-adapted isolates are better recipients than the rifampicin-resistant strains. It is unlikely that enhanced mating frequencies were due only to improved selection against *E. coli* cells, since the rifampicin effectively (and cephalexin less effectively) inhibited *E. coli* donors. The Cz-selected strains produce much less extracellular polysaccharide on Cz than on nutrient agar; a reduction in slime may enhance cell-cell contact and improve cosmid transmission frequency. This phenomenon is not exclusive to Cz-selected isolates; isolates adapted to grow on modified Miller's minimal medium A are also efficient recipients.

If transconjugants are to be useful in understanding the interactions between Xco and rice, it is critical that the cosmid remain stable in the bacterium in the absence of antibiotic selection in plants. The cosmids pLAFR5 and pSa747 were stable both in culture without antibiotic selection and in plants for up to 15 d, the time necessary to assay symptom expression. pSF6 was not stable in the absence of antibiotic selection. By 4 d after inoculation (DAI), the ratio of Xco cells containing pSF6 to the total number of cells had greatly diminished (to 0.1). No pSF6-containing cells could be isolated from plants or media at II DAI. The presence of pLAFR5 or pSa747 in the pathogen did not affect bacterial growth in culture or pathogenicity toward rice differential cultivars.

Transformation

Yamasaki et al (1966) transformed wild-type Xco chromosomal DNA into a leucine auxotroph and restored the ability to synthesize leucine. As described above, Murooka et al (1987) reported a protocol to transform Xco (and other pathovars) with DNA or a plasmid derived from a cryptic *X. campestris* pv. *citri* plasmid and pBR328. The frequency of transformation in Xco, however, was very low (10^2 -10^3 transformants/µg of plasmid DNA). Furthermore, the need to pass the plasmid through pv. *citri* before transformation introduces another inconvenient step in the procedure. We have preliminary evidence that a modified *E. coli* procedure

(Morrison 1979) can be used to transform cosmid DNA (pLAFR5, isolated from *E. coli* cells) into Xco. Again, a critical variable appears to be the age of the bacterial cells used to produce competent cells (10 h). The transformation efficiency, however, was very low (10^2 transformants/μg of cosmid DNA), Thus, the use of transformation for genetic analysis in Xco remains questionable.

IDENTIFICATION OF GENES INVOLVED IN PATHOGENICITY IN Xco

With the development of genetic tools in Xco, we now have the capability to search for genes involved in pathogenicity and race specificity. Genomic libraries of an isolate representing each of the six Philippine races have been constructed in the cosmid pLAFR5 or pSa747. The libraries have been characterized by auxotrophic or Rec A^- complementation in *E. coli* HB101 as well as by acquisition of drug resistance (e.g., when rifampicin-resistant Xco isolates were used to construct the library).

A pSa747 library constructed from a pathogenic Xco isolate was mobilized from *E. coli* into a spontaneous nonpathogenic mutant. Two cosmid clones were identified that restored pathogenicity on rice to the nonpathogenic mutant. The clones are now being characterized. These preliminary experiments demonstrate that mutant phenotypes can be restored by introducing cloned DNA into Xco. A more comprehensive analysis of pathogenicity genes will use a series of NTG-induced, $path^-$ mutants.

Transposon mutagenesis in Xco has to date been unsuccessful. We suspect that this is because the plasmid vector fails to enter the bacterium and thus cannot deliver the transposon. The broad host-range plasmid pSF6 can be transferred into Xco but cannot be maintained (Table 1). Thus, this plasmid may be useful as a suicide plasmid to deliver transposons. New vectors reported to yield transposon mutants in other *X. campestris* pathovars (e.g., Tn*4431*) (Shaw et al 1988) will be tested with Xco.

The lack of a suitable transposon mutagenesis system in Xco, however, can be circumvented by other approaches. NTG mutagenesis has been used to create color and $path^-$ mutants in Xco (Ardales and Leung, unpubl. data; Tsuchiya et al 1982). Four NTG-induced mutants of a race 2 isolate (IRN793) are especially interesting because the mutation alters the interaction of the isolate on one differential cultivar (Cas 209) from incompatible to compatible (Table 2). Mutants with altered specificity are valuable tools for identifying avirulence genes.

Certain genes, such as the avirulence genes, can also be identified without the use of mutants (Gabriel et al 1986, Staskawicz et al 1984, Swanson et al 1988). Tn-mutated cloned fragments can then be introduced into bacteria using marker exchange mutagenesis (Turner et al 1985).

CONCLUSION

Within the last 5 yr, the methodology and tools of molecular genetics have become applicable to Xco. We now can study genes controlling or enhancing compatibility or incompatibility. A major objective of our research is to identify and clone genes

Table 2. Interaction of nitrosoguanidine-induced mutants of *Xanthomonas campestris* pv. *oryzae* isolate IRN793 with differential rice cultivars.

Isolate[a]	Average lesion length (cm) on cultivars[b]				
	IR8	IR20	IR545-339	Cas 209	DV85
IRN793	11	9.5	1.0	2.0	1.8
132 (NTG)	13	8.5	1.4	17.4	1.3
123 (NTG)	6.3	4.9	1.1	8.6	2.0
135 (NTG)	14.6	12.2	1.7	17.4	1.6
93 (NTG)	18.1	16.0	2.9	22.5	5.8

[a]Nitrosoguanidine (NTG)-induced mutants were obtained by treating broth cultures overnight with 50 µg NTG for 10-30 min, grown overnight in peptone sucrose broth and plated on nutrient media. [b]Plants 35-40 d old were inoculated by the leaf clipping method using cell suspensions from 3-d-old slant cultures, Lesion lengths were measured 14 d after Inoculation,

involved in race-specific incompatibility and in the parasitic ability of the pathogen. With knowledge of the pathway of pathogenesis, we can then manipulate the plant genotype to counteract critical points in the pathway to achieve stable resistance. Once race-specific genes are identified, it will be possible to obtain translational products. Antibodies to these proteins will be useful as diagnostic tools in epidemiological research.

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Notes

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Breeding rice for resistance to bacterial blight

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Resistance to bacterial blight (BB) is one of the most important objectives for rice breeders in Asia. Although found in all rice-growing environments, BB is particularly severe in irrigated and rainfed lowland areas. At least 12 resistance genes have been identified. The earliest breeding work was begun in Japan, in 1923, where many resistant cultivars were developed and grown. The discovery of pathogenic specialization and the "breakdown" of resistance in cultivar Asakaze led to the incorporation of different resistance genes into improved cultivars. Polygenic or horizontal resistance has been used more recently in Japanese breeding programs. At the International Rice Research Institute (IRRI), the resistance gene *Xa-4* has been incorporated into most elite lines and cultivars. This gene has provided excellent resistance in East and Southeast Asia. Elite breeding lines with *xa-5* and *Xa-7* have also been developed. A gene from *Oryza longistaminata* conferring broad resistance against Indian races has been transferred to improved breeding lines. IRRI breeding lines and cultivars with resistance genes have been widely used by national programs, either directly as varieties or in hybridization programs. In addition, national programs are using their own sources of resistance to develop improved cultivars. Use of major gene resistance and avoidance of highly susceptible parents have proven effective in obtaining durably resistant cultivars. In the future, more emphasis may need to be placed on polygenic or partial resistance, particularly if more intensive rice production increases the incidence of breakdown of major gene resistance.

Bacterial blight (BB) of rice, along with blast (B1), tungro, and grassy stunt, is a disease of international importance. It occurs in all the countries of Asia where rice is grown, which encompass 92% of the world's total production. BB is found in all rice-growing environments, but its control is particularly important in irrigated and rainfed lowland areas. Whereas it did not receive much attention from breeders outside Japan before the 1960s, in a survey of rice breeders in 10 Asian countries in the late 1970s, BB was listed as the second most important disease, closely following Bl (Hargrove and Cabanilla 1979). In a survey of breeders working on rainfed

lowland rice, BB control was listed as the single most important breeding objective in terms of rice area (Mackill 1986).

Incorporation of BB resistance is one of the important objectives of rice improvement programs in Asia. Excellent screening techniques for evaluating germplasm and for segregating populations have been developed (Kauffman et al 1973), and a large volume of germplasm has been evaluated. Numerous donors for resistance have been identified. On the basis of genetic analysis of these donors, at least 12 major genes have been identified (Ogawa and Khush 1989, this volume). Several resistant varieties have been developed and are widely grown. The status of host resistance programs for BB in Asia is reviewed below.

JAPAN

The breeding program for BB resistance was started in 1923, and a resistant variety, Zensho, from the cross Shiga Sekitori II/Shoyu was released in 1932 in Aichi Prefecture. Zensho 26, a reselection of Zensho, was further crossed with Jukkoku and Kanto 53 at the Kyushu and Miyazaki Agricultural Experiment Stations in Kyushu region, where BB was prevalent. By 1964, several resistant varieties such as Hoyoku, Kokumasari, Shiranui, and Oyodo had been selected from these crosses. Among these, Hoyoku was most widely grown.

Another resistant variety, Kogyoku, was developed in 1932 from a cross between Shobei and Shiro Senbon made at the Aichi Agricultural Experiment Station in 1926. Kono 35 was another native resistant variety used as a donor parent. It was crossed with Asahi 1 at the Kumamoto Agricultural Experiment Station in 1936, and Norin 27 was selected from this cross at the Kyushu Agricultural Experiment Station in 1946. Asakaze, another resistant variety, selected from the cross Norin 27/Takara, was released in 1957. Asakaze is the first resistant variety known to have "broken down" because of the occurrence of a more virulent strain.

After the discovery of pathogenic specialization in the causal bacterium, rice varieties Lead Rice (an indica) and Wase Aikoku 3 (a native Japanese variety) were found to have wide spectra of resistance to prevalent races, which had been classified into three groups by that time (Sakaguchi 1967). The resistance gene of Lead Rice was transferred to the background of japonica varieties by five or six backcrosses. The resistance gene of Wase Aikoku 3 was incorporated into Chugoku 45 and Chukei 314 by Washio et al (1966). Chugoku 45 and Chukei 314 were widely used as donors for resistance in the breeding programs in Japan.

In recent years, rice breeding programs in Japan have paid special attention to incorporating horizontal or polygenic resistance to BB. Variety Asominori, developed in 1973 at the Kyushu Agricultural Experiment Station in cooperation with the Kumamoto Prefectural Agricultural Experiment Station, has an excellent level of horizontal resistance (Sato et a1 1977). Nishikari, another variety with horizontal resistance, was developed in 1981 from the cross Miyakei 322/ Asominori at the Miyazaki Prefectural Agricultural Experiment Station. Other donors for horizontal resistance to BB being used in Japan are Akamochi, IR28, Gomashirazu, IR26, and Pelita I-1.

INTERNATIONAL RICE RESEARCH INSTITUTE

The breeding program for BB resistance at the International Rice Research Institute (IRRI) was begun in 1965 when several donors—initially TKM6, Tadukan, Sigadis, and W1263—were used in the hybridization program (Khush 1977). Numerous resistant breeding lines were developed. IR20 and IR22, both released in 1969, were the first resistant IR varieties and inherited their resistance from TKM6 and Tadukan, respectively. IR26, released in 1973, and IR28, IR29, and IR30, all released in 1974, also inherited their resistance from TKM6. All subsequent IR varieties released by the Philippine Seed Board except IR56 are BB-resistant (Table 1). From the genetic analysis and from the genealogy of these resistant varieties, as weli as from their reaction patterns to Philippine races of BB, it is clear that all have the *Xa-4* gene for resistance. However, IR48 is also resistant or moderately resistant to races 2, 3, 4, and 5 and may have an additional resistance gene.

Table 1. Reaction of IR varieties to 6 races of bacterial blight in the Philippines.

Variety	Reaction[a] to race					
	1	2	3	4	5	6
IR5	S	S	S	S	S	S
IR8	S	S	S	S	S	S
IR20	R	MS	S	MR	R	S
IR22	R	MS	S	MR	R	S
IR24	S	S	S	S	S	S
IR26	R	MS	MS	MR	R	S
IR28	R	S	S	MR	R	S
IR29	R	MS	MS	MR	R	S
IR30	R	MS	MS	MR	R	S
IR32	R	MS	S	MR	R	S
IR34	R	MS	S	MR	R	S
IR36	R	MS	S	MR	R	S
IR38	R	MS	MS	MR	R	S
IR40	R	MS	S	MR	R	S
IR42	R	S	S	MR	R	S
IR43	R	MS	S	MR	R	MS
IR44	R	S	S	MS	R	S
IR45	R	MS	S	MR	R	S
IR46	MR	S	S	MS	R	S
IR48	R	R	MR	MR	R	MS
IR50	R	S	S	MR	R	S
IR52	R	S	S	MS	R	S
IR54	R	MR	MR	MR	R	S
IR56	S	S	S	S	S	S
IR58	R	MS	MS	MR	R	S
IR60	R	MS	MS	MR	R	S
IR62	R	S	S	MR	R	S
IR64	R	MS	MS	MR	R	S
IR65	R	S	S	MR	A	S
IR66	R	S	S	MR	R	S

[a]S = susceptible, R = resistant, MR = moderately resistant, MS = moderately susceptible.

Xa-4 conveys resistance to the prevalent races of BB in East and Southeast Asia but not to those in South Asia. Therefore, IR varieties with *Xa-4* are not resistant to BB in India. However, IR54, which inherited *Xa-4* from Nam Sagui, is resistant in several regions of India, indicating that IR54 has an additional resistance gene. Some of the progenies of IR54 such as IR28224-66-2, released as PR109 in Punjab State of India, are also resistant. Similarly, another progeny line of IR54, IR29341-85-3-1-3 (IR54/IR46), did not score higher than 5.0 at any of the locations in the 1985 International Rice Bacterial Blight Nursery (IRRI 1986).

IR varieties are resistant to BB race 1, the most prevalent in the Philippines in the late 1960s and early 1970s. However, races 2 and 3 have now become more widespread in the Philippines. We have, therefore, incorporated into our breeding material genes *xa-5* and *Xa-7,* both of which convey resistance to Philippine races 1, 2, and 3. Elite breeding lines with *xa-5* (Table 2) and *Xa-7* (Table 3) are now in advanced stages of testing. During our evaluation of elite breeding lines, we have found that some have resistance to races 1 and 2 in the Philippines (Table 4), although those lines have *Xa-4.* We are analyzing these lines further.

Table 2. Some elite breeding lines with *xa-5* for resistance.

Line	Parents
IR44962-7-6-2-2	IR4563-52-1-3-6/IR21820-154-3-2-2-3
IR44962-22-1-3-2-3	IR4563-52-1-3-6/IR21820-154-3-2-2-3
IR44962-161-2-4-4-2	IR4563-52-1-3-6/1R21820-154-3-2-2-3
IR45965-57-2-3-2-2	IR48/1R13540-56-3-2-1//1R4563-52-1-3-6
IR47741-19-2-1-3	IR4563-52-1-3-6/IR5533-13-1-1//IR29506-60-3-3-2
IR47743-5-1-2-3	IR4563-52-1-3-6/1R17491-5-4-3-3//IR29506-60-3-3-2
IR47751-144-1-3-2	IR4563-52-1-3-6/IR25588-32-2//IR59506-60-3-3-2
IR47761-27-1-3-6	IR4563-52-1-3-6/5218//IR21820-154-3-2-2-3
IR47787-116-1-2-2	IR13540-56-3-2-l/IR4563-52-1-3-6//1R31809-108-3-3
IR48012-20-1-3-2-3	IR4563-52-1-3-6/Kinandang Patong// IR19660-274-3-3-1-3
IR48777-6-1-2-3	IR13146-45-2-3/1R4563-52-1-3-6
IR48787-7-2-1-3	IR26760-27-1-3-2-1/1R4563-52-1-3-6
IR51099-68-1-3	IR26760-27-1-3-2-1/IR456552-1-3-6//1R54

Table 3. Some elite breeding lines with *Xa-7* for resistance.

Line	Parents
IR52370-87-3-2-1	S1-5-78-6-2/IR28222-9-2-2-2-2//IR35546-52-3-3-2
IR52370-87-5-1-1	S1-5-78-6-2/IR28222-9-2-2-2-2//IR35546-52-3-3-2
IR52371-28-3-2-1	S1-5-78-6-2/IR31892-46-3-2//IR28222-9-2-2-2-2
IR52371-43-1-3-1	S1-5-78-6-2/IR31892-46-3-2//IR28222-9-2-2-2-2
IR52371-50-3-6-1	S1-5-78-6-2/IR31892-46-3-2//IR28222-9-2-2-2-2
IR52372-10-1-2-1	S1-5-78-6-2/IR31899-30-1-1-3//IR31892-46-3-2
IR52372-12-6-3-1	S1-5-78-6-2/IR31899-30-1-1-3//IR31892-46-3-2
IR52373-76-3-1-1	S1-5-78-6-2/IR34583-19-3-3//IR35546-52-3-3-2
IR52373-76-4-2-1	S1-5-78-6-2/IR34583-19-3-3//IR35546-52-3-3-2

Table 4. Some elite breading lines with resistance to races 1 and 2 of bacterial blight in the Philippines.

Line	Parents
IR5657-33-2-2-3	IR2006-P3-31-3/IR2146-68-1//IR2031-724-2-3/ IR1702-158-4
IR21848-65-3-1-2-2	IR4570-124-3-2/1R4432-52-64//IR4570-124-3-2-2-2
IR24594-204-1-3-2-6-2	IR2843-79-2/IR54//IR48
IR32829-5-2-2	IR4683-54-2/RPW6-17/IR17494-32-2-2-1-3
IR40720-72-1-2	IR17494-32-3-1-1-3/IR25863-61-3-2
IR42029-38-1-3-3-2	IR17491-5-4-3-3/New Sabarmati (BAS)// IR25621-94-3-2
IR31179-6-1-2-2	IR4568-86-1-3-2/KLG6986-161-7//IR13168-143-1

We have also employed a strain of *Oryza longistaminata* in our BB resistance program. This strain was found highly resistant in 17 states of India (S. Devadath, Central Rice Research Institute, Cuttack, 1985, pers. comm.). It is also resistant to all BB races in the Philippines. We transferred this gene to IR24 by making four backcrosses and utilized the progenies in our regular hybridization program. Elite breeding lines with this gene are being evaluated.

IR varieties and IR breeding lines are regularly exchanged with national rice improvement programs on the basis of requests from national program scientists or through International Rice Testing Program nurseries. Thus, many IR breeding materials with BB resistance have been utilized by national rice programs in Asia either as direct introductions or in hybridization programs. These genes for BB resistance have been widely dispersed and utilized internationally.

The elite breeding lines at IRRI are evaluated for BB resistance twice a year by clip inoculation with Philippine races 1 and 2. Our breeding lines with *Xa-4* rarely show a disease reaction higher than 7 on a scale of 1-9, and many have scores of 5-6 to race 2. These lines have no major genes for resistance to race 2. Although races 2 and 3 are now widespread, varieties with *Xa-4* such as IR36, IR42, IR54, IR60, and IR64 rarely show severe disease symptoms in the field. These observations suggest that our varieties and breeding lines have a good level of horizontal resistance. We have utilized numerous parents from tropical Asia in our hybridization program, and they have probably contributed minor genes for horizontal resistance. Even varieties with *Xa-4* such as TKM6, W1263, Sigadis, and Tadukan have minor genes for resistance besides *Xa-4*.

CHINA

Most of the native indica rices in China are susceptible to BB. *Xa-4* conveys resistance to most of the prevalent races. Thus, varieties and breeding lines with *Xa-4* such as IR20, IR22, IR26, IR30, IR36, IR1561-228-3-3, and IR2061-464 have been widely used as donors, and several resistant varieties have been developed. Most of the provincial academies of agricultural sciences are paying special attention to developing BB-resistant varieties. Some of the resistant varieties developed in China

Table 5. Rice varieties with resistance to bacterial blight developed in China.

Variety	Donor parent	Institute where developed
Hua Zhu 40	IR20	Guangdong Academy of Agricultural Sciences
Ching-Hua-Ai 6	IR20	Guangdong Academy of Agricultural Sciences
Wan-Hua-Ai 4	IR20	Guangdong Academy of Agricultural Sciences
Zhu-Bao-Ai 384	IR20	Guangdong Academy of Agricultural Sciences
P20-5	IR29	Guangdong Academy of Agricultural Sciences
Er-Jiu-Feng	IR29	Jiaxing Agricultural Institute
Yang-Shen 6	BG90-2	Yangzhou Agricultural Institute
5144	IR2061	Jiangxi Academy of Agricultural Sciences
HA79317-7	IR36	Hunan Academy of Agricultural Sciences
Chuan Mi 1	C632082	Sichuan Academy of Agricultural Sciences
Tian Rui 410	IR22	Yunan Academy of Agricultural Sciences
Han 86	IR30	Guangdong Academy of Agricultural Sciences
Gui Lu Zao	IR26	Guangdong Academy of Agricultural Sciences
Qui Qing 2	IR20	Guangdong Academy of Agricultural Sciences
Qing-Hua-Ai	IR20	Guangdong Academy of Agricultural Sciences

Table 6. IRRI rice varieties and breeding lines with *Xa-4* released as varieties in China.

Variety or line	Where released	Name
IR26	Hupeh, Anhwei	IR26
IR28	Hunan	IR28
IR36	Anhwei	IR36
IR1561-228-33	Hunan	32 Xuan 5
IR9129-102-3	Guangdong	Guo-Ji-You-Zhan
IR9965-48-2	Guangdong	Waiyin 35
IR15853-89-7	Guangxi	N90
IR21015-80-3-3-1-2	Guangxi	N304
IR21929-12-3-3	Fujian	Minkang 108

are listed in Table 5. In addition, several IR varieties and breeding lines with *Xa-4* have been released as varieties in China (Table 6).

Several IR varieties and breeding lines with *Xa-4,* such as IR26, IR30, and lR9761-19-1, have been used as restorer parents in the hybrid rice program in China, and, because of the dominant nature of *Xa-4,* the hybrids are resistant to BB.

VIETNAM

Varieties with *Xa-4* are highly resistant to prevalent races of BB in Vietnam. At least 25 IR varieties and breeding lines (Table 7) with *Xa-4* have been released and are planted to about 40% of the rice area in the country. Many IR breeding lines and varieties are being used as sources of resistance in Vietnamese breeding programs.

THAILAND

Varieties with *Xa-4* are resistant to most of the prevalent races of BB. None of the released varieties are resistant. However, elite breeding lines having *Xa-4* for

Table 7. IRRI breeding lines and varieties with *Xa-4* released as varieties in Vietnam.

Breeding line	Variety name
IR532E576 (IR20)	TN20
IR579-160-2 (IR22)	NN22
IR1561-228-3	TN73-2
IR1529-680-3-2	TN73-1
IR1541-102-7	IR26
IR2153-159-1-4	IR30
IR2070-747-6-3-2	IR32
IR2070-199-3-6-6	NN8A
IR2070-734-3	NN4A
IR2070-423-2-5-6	IR38
IR2071-625-1-252 (IR36)	NN3A
IR2071-586-5-6-3 (IR42)	NN4B
IR2071-179-3	NN5A
IR2307-247-2-3	NN6A
IR2823-399-5-6	NN2B
IR2797-115-3	NN3B
IR4570-83-3-3 (IR48)	NN5B
IR8423-132-6-2-2	CR203
IR9129-192-2-3-5	NN7A
IRl3240-10-1	NN8B
IR1820-210-2	IR1820
IR18348-36-3-3	IR64
IR19746-11-3-3	CN2
IR1251-96-1-5-3	IR2151
IR2153-26-3-5-6	IR2153

resistance have been selected from crosses involving IR28, IR32, IR42. IR48, and other IRRI varieties and breeding lines.

MALAYSIA

Twenty improved rice varieties have been released in Malaysia since 1964. Three of them—MRl (Setanjung), MR77 (Seberang), and MR73 (Makmur)—are resistant to BB. However, it is not known which genes convey resistance in these varieties. Many of the elite breeding lines are resistant. The resistant donors used include TKM6, DV85, Zenith, IR22, and many IRRl breeding lines.

INDOSESIA

Many IR varieties and breeding lines with *Xa-4* have been released in Indonesia (Table 8) and are widely grown. IR36, with *Xa-4,* has been the most widely grown for the last 10 yr. However, varieties with *Xa-4* are not resistant to all the races of BB in Indonesia. Cisadane. another widely grown variety, is resistant to pathotype 3, the common race in Java. IR26, Krueng Aceh, and Citanduy (IR5657-33-2-2-3) are also resistant to pathotype 3. Other donors for resistance being used in Indonesia are Zenith, DV85, Java 14, IR1545-339-2-2, Dular, ARC5756, Balai Kambang, Malaman, Pelita I-1, Sigadis, and Semora Mangga.

Table 8. IRRI lines and varieties with *Xa-4* released in Indonesia.

Breeding line	Variety name
IR1541-102-7	IR26
IR2061-214-38-2	IR28
IR2153-159-1-4	IR30
IR2070-747-6-3-2	IR32
IR2061-213-2-17	IR34
IR2071-625-1-252	IR36
IR2071-621-2	Asahan
IR2070-423-2-5-6	IR38
IR2071-586-5-6-3	IR42
IR2058-78-1-2-3	IR46
IR2307-247-2-2-3	Semeru
IR4570-83-3-3	IR48
IR4744-295-2-3	Tajum
IR9224-117-2-3-3-2	IR50
IR5657-33-2-2-3 [a]	Citanduy
IR5853-162-1-2-3	IR54
IR13429-109-2-2-1	IR56
IR13543-66	Kelara
IR15529-253-2-2	Bahbalon
IR18348-36-3-3	IR64
IR19743-46-2-3-3-2	Jangkok
IR28128-45-3-3-2	Dodokan
IR21015-196-3-1-3	IR65

[a]Has an additional gene for resistance.

NEPAL

BB is the second most important rice disease in Nepal. Several improved rice varieties released in the post- IR8 era such as Laxmi, Janaki, Durga, Sabitri, Chaite 2, Chaite 4, Makwanipur 1, and Barkh 2 have adequate levels of resistance. Among the known genes for resistance, *Xa-3* and *xa-13* convey resistance to local races. IR26 and IR60 show good levels of resistance to local races.

BANGLADESH

Among the 20 varieties released in the post-IR8 era, none are highly resistant. However, BR4, BR5, BR12, BR14, BR15, BR16, and BR20 have some level of tolerance. Several breeding lines such as BR817-1, BR168-2B-23, BR171-2B-8, BR161-2B-25, BR315-12-1-4-1, BR319-1-HR28, R2829-89-2, IR33209-22-1-1-1, and BR51-282-8-HR29 are resistant and are being used as resistance donors. Some of the native donors of resistance to isolate BXO 9 include Akhnisail, Horibhog, Ghunshi, Fulbaria, Kachamota, and Bagdor. Camor from Indonesia is highly resistant to local races and is being used as a donor. Isolate BXO 5 is also prevalent, and the donors of resistance to this isolate are DV85, Bazail 414, Nuncha, and Lal aman.

INDIA

The traditional tall varieties grown in India were susceptible. Improved semidwarf varieties recommended in late 1960s and early 1970s were also susceptible. However, several improved varieties with some level of resistance have now been released in several states (Table 9). Three of these—PR4141, PR109, and HKR120—are highly resistant. Sona Mahsuri, Vijaya Mahsuri, Samba Mahsuri, and Swarna are suitable

Table 9. Some improved bacterial blight-resistant rice varieties released in India.

Variety	Year released
All-India	
Sasyasree	1979
IR36	1981
Andhra Pradesh	
Sona Mahsuri	1982
Vijaya Mahsuri	1982
Samba Mahsuri	1986
Swarna	1982
Bihar	
Janaki	1978
Radha	1984
Sujata	1984
Haryana	
HKR120	1987
Karnataka	
Mandya Vijaya	1986
Madhya Pradesh	
Asha	1980
Deepti	1980
Usha	1980
Maharashtra	
Ratnagiri 68-1-1	1976
Orissa	
Rama Krishna	1980
Udaya	1985
Pondicherry	
Bharathidasan	1985
Punjab	
PR4141	1982
PR109	1986
Tamil Nadu	
CO 43	1982
CO 44	1982
IR50	1982
Uttar Pradesh	
Saket-4	1971
Prasad	1978
Govind	1982
Pant Dhan 4	1983
Pant Dhan 6	1986
West Bengal	
Biraj	1982
Suresh	1982

Table 10. Donors for resistance to bacterial blight in India.

Donor	Origin
AC19-1-1	Bangladesh
DV85	Bangladesh
DV86	Bangladesh
DZ78	Bangladesh
Hashikalmi	Bangladesh
Kachamota	Bangladesh
BJ1	India
Chinsurah Boro II	India
Syaphal	India
TKM6	India
Semora Mangga	Indonesia
Nam Sakouy	Laos
Patong 32	Malaysia
Nam Sagui	Thailand
Lua Ngu	Vietnam
PI 231 129	United States
Lacrosse-Zenith Nira	United States
IR54	IRRI

for rainfed areas. HKR120, PR109, PR4141, Pant Dhan 4, and Pant Dhan 6 are suited to the lowiand irrigated rice areas in Punjab, Haryana, and western Uttar Pradesh; IR36 and IR50 are suitable for irrigated transplanted as well as direct seeded rainfed areas.

Several donors with a high level of resistance are now being used (Table 10). Of these, DV85, DV86, DZ78, BJl, and Chinsurah Boro II have a recessive gene (Sidhu et al 1986) that may be identical to *xa-13* (Ogawa and Khush 1989). Similarly, Lua Ngu, Nam Sagui, Nam Sakouy, and Patong 32 have a dominant gene (Sidhu et al 1986) that appears to be closely linked to *Xa-4.* IR54, which inherited its resistance from Nam Sagui 19 is being widely used as a donor for resistance. Ptb 33 and its derivatives such as IR13427-40-2-3, IR19661-23-2-2. and IR19661-150-2-2-1 are highly resistant in Central and North India. Some of the improved breeding lines with BB resistance are listed in Table 11.

CONCLUSIONS

Varietal resistance has proven very effective in controlling BB in the tropics. Using major genes for resistance and avoiding parents with high susceptibility have resulted in varieties showing durable resistance in many areas. In areas where BB races appear to be more virulent, such as South Asia, major genes with resistance to the prevalent pathogen populations have been identified. These genes are being transferred into improved breeding lines.

As the use of improved varieties with major gene resistance increases, and as more intensive management and cropping patterns are employed, it is possible that the breakdown of existing varieties will become a major problem. In this case, partial resistance will become a more attractive approach in developing resistant varieties, as it has in the case of Bl. Partial resistance is apparently common in rice, and has, in fact, already been incorporated into improved varieties. In some cases it appears to

Table 11. Some promising rice breeding lines with resistance to bacterial blight in India.

Designation	Cross	States where found suitable
RP2095-5-8-38	Vikram/Andrewsali	Maharashtra
RP2095-5-8-3	Vikram/Andrewsali	Andhra Pradesh, western Uttar Pradesh
OR131-5-8	Kumar/OR57-49	Karnataka, western Uttar Pradesh
OR447-20	OR158-5/Rasi	Orissa, Andhra Pradesh, Punjab
RP2151-40-1	IET4141/CR98-7216	Punjab, Haryana
RP2151-21-22	IET4141/CR98-7216	Andhra Pradesh, Uttar Pradesh, Bihar, Orissa, Maharashtra
RAU77-2	IET140026/IR5201-127-1	Madhya Pradesh, Bihar, Uttar Pradesh, Punjab, Haryana
IR18348-36-3-3	IR5657-33-2-1/IR2061-465-1-5-5	Andhra Pradesh, Tamil Nadu, Orissa, Madhya Pradesh, Bihar
PAU726-8-1-1-3	Improved Sona/RP633-76-1-1-2	Punjab
PAU853-13-1-2-1	PR4141/PAU50-B-51-1-1	Punjab
PAU1126-1-4	PAU50-B-25/IR54	Punjab, Haryana
PAU1126-17-5	PAU50-B-25/IR54	Punjab
PAU1126-64-4	PAU50-B-25/1R54	Punjab

be polygenic (Yoshimura 1989, this volume). More basic studies are needed on this type of resistance, since its importance is likely to increase.

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Bacterial blight in North Queensland, Australia

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Rice has been cultivated in the Mareeba district (17° S, 145° E) of North Queensland since the early 1970s and. to date, this is the only area in North Queensland where *X. campestris* pv. *oryzae* (Xco) has been identified. Bacterial blight (BB) caused by Xco has not been recorded in Australia's major rice-producing district, the Murrumbidgee Irrigation Area in New South Wales. An outbreak in the Northern Territory in the early 1970s was confined to native and experimental materials. But BB is the major disease of rice in the Mareeba district. The disease was first recorded on summer-planted Starbonnet in March 1985. Since then it has occurred in both summer and winter plantings. The current commercial cultivars Starbonnet, Bluebonnet, and Lemont—albred in the U.S.—are highly susceptible to BB. A severe epidemic developed following a tropical cyclone in February 1986. Grain yield in the 1986 summer crop was reduced 30-50% to an average of 3.5 t/ha, mostly attributed to an increase in the proportion of unfilled grains rather than a reduction in 1,000-grain weight. Poor whole grain milled rice and increased harvesting costs due to lodging exacerbated the financial loss to producers. The strain of Xco in North Queensland is probably indigenous to Australia. BB has been recorded on *Oryza rufipogon* growing in ricefields in the Mareeba district. *O. rufipogon* and *O. australiensis* are endemic to Northern Australia and could be native hosts for Xco. The pathogen from North Queensland has an unusual virulence pattern on differential hosts, although the average virulence is low. A similar virulence pattern has been ascribed to strains from the Northern Territory. The rice breeding program in North Queensland has incorporated germplasm from the International Rice Research Institute and the International Center for Tropical Agriculture into Starbonnet and Bluebonnet. These breeding lines, along with imported cultivars, are being evaluated for Xco resistance.

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Current status of breeding for rice bacterial blight resistance in Guangdong, China

Wu Shangzhong

The level of bacterial blight (BB) resistance of rice varieties bred in Guangdong from 1960 to 1970 was evaluated. All high-yielding and good plant type varieties were either susceptible or highly susceptible. Five BB strains were found in Guangdong, showing different levels of virulence: I, II, III, IV, and V. Groups III and IV are the predominant strains in South China. These groups were quite stable from 1984 to 1986. Comparative studies on the pathogenic reactions of BB isolates collected from Southeast Asia indicated that they may also be placed in five groups by their reactions to the Chinese differential hosts. Group IV from Guangdong is similar to PXO 61 from the Philippines. Genetic analysis and an examination of heritability indicated that up to 90% of the tested cultivars had the *Xa-4* gene. More than 10,000 sources of rice germplasm were examined, of which 2,012 cultivars were intensively assessed by the clipping method for resistance against group IV in Guangdong; 28 were found highly resistant, 187 resistant, and 285 moderately resistant. Forty-five selected cultivars were evaluated individually for multiple resistance. A series of single-, double-, and triple-resistance donors were recommended for resistance breeding; the majority carried gene *Xa-4*. Several high-yielding, good grain quality varieties—namely, Ching-Hua-Ai 6, Wan-Hua-Ai 1, Zhu-Bao-Ai 384, P20-5,79006, and 79007—have been successively bred and released, and are grown on about 500,000 ha in Guangdong Province and Guang-Xi Autonomous District.

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Bacterial blight of rice in Tamil Nadu, India

S. R. Sree Rangasamy

Bacterial blight (BB) of rice can cause considerable yield losses during the first season in Tamil Nadu. Disease resistance has been reported to be governed by one to three genes that express dominance or recessiveness according to the host's genetic background. Polygenic inheritance has also been reported. At least five genotypes have been identified as promising donors conferring BB resistance. At Tamil Nadu Agricultural University, systematic evaluation of varieties and entries is continuously in progress under natural and artificial conditions. A breeding program incorporating BB resistance with high yield is also in progress. BB reduced yields severely in 1965-70, when it became epidemic for the first time, after the introduction of high-yielding varieties (HYVs), especially TN1. In subsequent years, however, a stream of HYVs with different genotypic backgrounds was developed and adopted for cultivation, and the seventy of BB was greatly reduced. Popular short- and medium duration varieties currently grown in Tamil Nadu are not very vulnerable to BB.

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Resistance breeding against bacterial blight of rice in Haryana, India

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Bacterial blight (BB) caused by *Xanthomonas campestris* pv. *oryzae* has been responsible for heavy yield losses in Haryana, India. The severity of the disease increases under high management levels. There is no chemical control for BB, and the development of resistant varieties is the most promising approach. Varieties HAUl18-154, HKR120, HKR122, IR54, and TKM6 having the *Xa-4* gene; IET4141, RP2151-21-1, and BJ1 having *xa-5*; and DV85 having *xa-5* and *Xa-7* have been identified as BB resistant. Resistant varieties have been used in the breeding program for developing strains with high grain yield and BB resistance. Crosses PR106/IR54, PR106/1ET4141, PR106/RP2151-21-1, PR106/HKR120, HKR101/IET4141, HKR1/IR54, HKRI/IET4141, HKRI/ RP2151-21-1, Palman 579/IR54, Palman 579/IET4141, IR36/RP2151-21-1, Jaya/RP2151-2l-I, and Jaya/HKR120 are promising. Variety HKR120, developed from line IR19661-150-2-2-2-1, has been released for general cultivation in the State, particularly for BB endemic areas.

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Genetic studies of adult plant resistance of rice to bacterial blight

Xie Yuefeng

Eleven adult plant resistant varieties of rice showed a clear-cut transition from susceptible to resistant responses from 55 d after seeding (DAS) (9-leaf stage) to 68 DAS (11-leaf stage). Nangen 15 and Wax Aikoku 3 showed resistance earlier than IRl695, Rathu Heenati, or Ketan Gundel. Specific interaction between some adult plant resistant varieties and isolates differing in virulence was observed. The adult plant resistant varieties and susceptible varieties were crossed with susceptible varieties Guang-Lu-Ai 4 and Mi-Quan-Mong to study the dominance reversal of the gene for adult plant resistance. At the 6- to 7-leaf stage (37 DAS), all the adult plant resistant parents, the F_1s, the F_2s, and the BCF_1s were susceptible. The segregation ratio of the F_2 population of Nangen 15 at the 7- to 8-leaf stage indicated that the resistance gene was recessive. At the 10- to 14- and 13- to 14-leaf stages, Nangen 15 and the F_1 were resistant, while the segregation ratio of the F_2 (3:l) and BC populations (1:l) revealed that the resistance gene was dominant. The behavior of Wase Aikoku 3 and Zenith was similar to that of Nangen 15. On the other hand, Bulu Putih and its F_2s were susceptible at the 7- to 8-leaf stage. At the 10– to 11-leaf stage, Bulu Putih was resistant, the F_1 and BC populations were susceptible, and the F_2 segregated into IR:3S. At the 13- to 14-leaf stage, Bulu Putih and the F_1 were resistant, and the F_2 and BC populations segregated into 3R:1S and 1R:1S, respectively. The resistance of Pulut Kamandi, Ketan Gundel, Ketan Gondopuro, Ketan Bajong, and Rathu Heenati was similar to that of Bulu Putih. These differences in response may be due to the expression of resistance in heterozygous and homozygous plants. The resistance of heterozygous plants was expressed 4-19 d or 1-5 leaves later than that of homozygous plants.

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Location of IR28 genes *Xa-a* and *Xa-h* resistant to Chinese strains of *Xanthomonas campestris* pv. *oryzae*

Xie Yuefeng

IR28, with genes *Xa-a* and *Xa-h* resistant to Chinese strains of *Xanthomonas campestris* pv. *oryzae* (Xco), was crossed with lines possessing marker genes *v-1, ws, vr, vrp, g, nl, la, z-2, tri, ch, bc-1, dl, gl,fgl,* and *pa* to determine the location of the resistant genes. The F_1 hybrids and F_2 populations were inoculated with Xco strains Jangling 691 from Hubei and Os 75 from Beijing. The linkage relationshig of *Xa-a* and Xa-h to the marker gene *la* was obtained from the analysis of F_2 populations of M9/IR28 and M10/IR28. *Xa-a* and *Xa-h* had no linkage relationships to the other marker genes. In the F_2 of M9/IR28, 200 plants were normal and 55 had lazy habit. The F_2 ratio of + R : +S : *la*R : *la*S was 168:38:28:31 in response to Jangling 691 and 184:39:37:25 in response to Os 75, both showing independent digenic segregation (9:3:3:1). The recombination values of *Xa-a* and *la,* and of *Xa-h* and *la* were calculated at 27.9 and 33.7%, respectively. Allelic tests between *Xa-a* and *Xa-h* showed that the two resistance genes are linked with a recombination value of 17%. Thus, genes *Xa-a* and *Xa-h* are both located on chromosome 9. However, the relationships of *Xa-a* and *Xa-h* to the resistant genes *Xa-4, Xa-1*^{h}*,* and *Xa-kg*^{h} in IR28 is unknown, since only Chinese strains of Xco were used in this study.

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Evaluation of wild type *Xanthomonas campestris* pv. oryzae for nutritional requirements and antibiotic resistance

S. H. Choi, Y. S. Choi, K. S. Jin, S. H. Lee, and E. J. Lee

Seventy strains of *Xanthomonas campestris* pv. *oryzae,* collected from field-grown rices, were evaluated for their nutritional requirements and antibiotic resistance using both M56 and WF-P-P minimal medium varying in 19 amino acids and 10 antibiotics. All 70 strains were cysteine or methionine auxotrophic, both amino acids being replaceable by the other. With few exceptions, most strains were highly sensitive to various antibiotics, specifically streptomycin, ampicillin, novobiocin, erythromycin, oleandomycin, vancomycin, rifampicin, and oxymycin at 5 μg/ml of medium. The minimal inhibitory concentration of penicillin was less than 40 μg/ml, but pimaricin did not inhibit growth of any of the 70 strains at 100 μg/ml.

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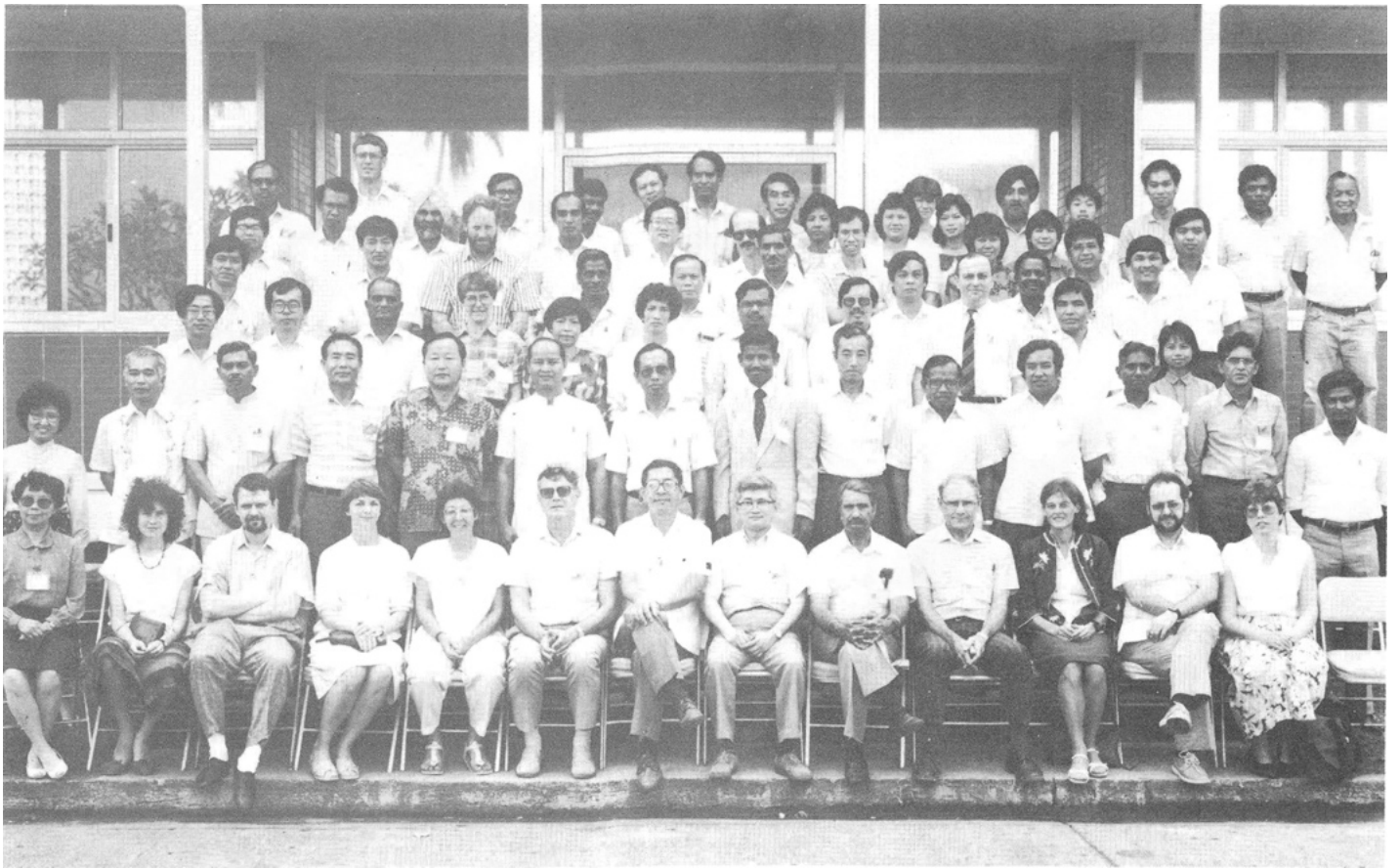

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