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T. T. Hebert.

Rice blast disease in Peru, by Hernando R. Huerta P.

Rice blast disease in Brazil, by Regina E. de Mello Amara1.

Rice blast disease in Africa, by R. J. Williams.

Phenotype stability of pathogenic races of *P. oryzae* and its implications  
for breeding of blast resistant rice varieties, by Frances M. Latterell.

Geographical distribution and predominant races of *P. oryzae* Cav., by  
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# Horizontal resistance to the plant disease in rice

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## FOREWORD

The need to find a more stable resistance of rice to the blast disease led the Centro Internacional de Agricultura Tropical (CIAT) to sponsor a Seminar on Horizontal Resistance to the Blast Disease of Rice. The meeting was held at CIAT headquarters, near Palmira, Colombia, October 8-12, 1971. The seminar, moderated by Dr. H. David Thurston of the New York State College of Agriculture, had participants from all the Latin American countries, and from Japan, South Africa and the United States.

Fifteen papers were presented dealing with the main topic of the meeting as well as with related topics important to explain the high variability of the causative pathogen, *Pyricularia oryzae*. Generally, topics included phenotypic variability, pathogenic variability, production of the perfect stage, and geographic situation of the disease in Latin America, Africa and Asia.

In addition to the scheduled papers, participants also discussed future plans and directions for continued work on rice blast disease. General conclusions were to strengthen the international search for sources of broad spectrum resistance to the disease; to define the high phenotypic and pathogenic variability to the fungus; and to increase research to find the perfect stage of *Pyricularia oryzae*, to help us understand the enormous variability of this fungus. These studies should indicate the best ways of breeding for the development of new rice varieties which possess a broad field resistance to the present and future races of blast.

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## CONTENTS

Horizontal resistance in plants: concepts, controversies and applications. <i>R. R. NELSON</i> .....	1
Horizontal resistance: six suggested projects in relation to blast disease of rice. <i>J. E. van der PLANK</i> .....	21
Techniques and philosophies on the development and use of perfect stages to understand pathogen variation and host resistance to plant diseases. <i>R. R. NELSON</i> .....	27
Variability of <i>Pyricularia oryzae</i> Cav. and its relation to varietal resistance. <i>S. H. OU</i> .....	49
Recent progress of studies on horizontal resistance in rice breeding for blast resistance in Japan. <i>KUNIO TORIYAMA</i>	65
Recent advances in studies on horizontal resistance to blast disease of rice in Japan. <i>TAKUJI KOZAKA</i> .....	101
Factors which may express general resistance in rice to <i>Pyricularia oryzae</i> Cav. <i>JOSE TADASHI YORINORI and H. DAVID THURSTON</i> .....	117
Indications of partial resistance of rice to the fungus <i>Pyricularia oryzae</i> Cav. <i>MARAT RODRIGUEZ and GUILLERMO E. GALVEZ</i> .....	137
Pathogenic variability and cytology of monoconidial subcultures of <i>Pyricularia oryzae</i> Cav. <i>RICHARD A. FREDERIKSEN</i>	155
Production of the perfect stage of <i>Pyricularia</i> from rice and other hosts. <i>T. T. HEBERT</i> .....	161
Rice blast disease in Peru. <i>HERNANDO R. HUERTA P.</i> ...	165
Rice blast disease in Brazil. <i>REGINA E. de MELLO AMARAL</i>	177
Rice blast disease in Africa. <i>R. J. WILLIAMS</i> .....	189
Phenotypic stability of pathogenic races of <i>Pyricularia oryzae</i> , and its implications for breeding of blast resistant rice varieties. <i>FRANCES M. LATTERELL</i> .....	199
Geographical distribution and predominant races of <i>Pyricularia oryzae</i> Cav. <i>SHOHEI MATSUMOTO</i> .....	235

## **Horizontal resistance in plants: concepts, controversies and applications**

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Man's efforts to control plant diseases by use of resistant varieties frequently have resulted in sudden and sometimes spectacular shifts in the racial make-up of plant pathogen populations. Such shifts have led to the increased frequency of races with unusual virulence for widely grown and presumably resistant varieties. The cyclic rise and fall of resistant germplasm is well known. Recent attempts to control the blast disease of rice with resistance genes is merely one of many examples of what often seems to be a futile effort to contain a highly plastic and potent parasite.

Man is fortunate that most species of agronomic plants possess adequate resistance genes to most of their parasites. If not, their wild counterparts usually do and man often has been able to utilize them in breeding programs. Simply stated, the important issue is how to most effectively utilize the resistance available to us. It seems inevitable that new races of the blast fungus and other important pathogens will arise periodically. Resistance genes can be used to best advantage by minimizing the consequences of newly appearing races by preventing their increased frequency and prevalence within the species. Several potential uses of resistance genes to negate extreme pathogen variability have been proposed. This paper examines one of them, the one that has prompted this meeting.

The terms "resistance" and "susceptibility" are used to depict those situations in which some measure of host-parasite interaction is evoked. Each term is relative and the two terms are relative to one another. They represent a continuum of interactions on one scale or spectrum. Because they are relative terms they are often used ambiguously and arbitrarily. When, for example, is a plant susceptible and when is it resistant? Plants are considered resistant or susceptible by different researchers according to the criteria they use.

The degree of resistance of a plant frequently is related to the relative incidence of infection and the relative extent of pathogenesis. In that sense the terms are not used correctly. The resistance of a plant is characterized by the amount of disease that it incurs or by the extent of damage that it sustains. Disease, however, is a product of the interrelationship of host and parasite under a given environment rather than a specific character of the host. A relative level of incompatibility between host and parasite usually results in a low level of disease and from this comes the conclusion that the host is resistant. A low level of disease could occur as a result of two different phenomena: the host has a sufficient ability to defend or the parasite has an insufficient ability to attack. Nonetheless, we do speak of genes for resistance in the host and we do "search" for host genes that govern disease responses. It is in this context that disease resistance is evaluated in this paper.

Disease resistance in plants to plant pathogens is characterized by one of two major kinds of host responses. The host either resists the establishment of a successful parasitic relationship by restricting the infection site and the infection process, or it resists the colonization and growth of the parasite in the host subsequent to a successful infection, although the infection process, culminated by reproduction of the parasite, is completed. Resistance is considered herein as an active, dynamic response of the host to a parasite and thus excludes the passive phenomena of immunity, tolerance and disease escape. Resistance to the successful establishment of a viable parasitic relationship classically is referred to by such terms as hypersensitivity, specific resistance, non-uniform resistance, vertical resistance or major gene resistance. Resistance to colonization is a host response characterized erroneously by the term tolerance and variably by the terms field resistance, generalized resistance, non-specific resistance, partial resistance, uniform resistance, horizontal resistance, multigenic or polygenic resistance and minor gene resistance. The fact that resistance is relative places these two kinds of resistance on a continuous scale. The difference between a pin-point fleck reaction and a small necrotic lesion is a relative indication of host reaction to a pathogen. The difference between a small necrotic lesion and a larger one is equally relative. Hypersensitivity at the cellular level, as an example, technically can be viewed as resistance to growth. The main point is that two major kinds of resistance phenomena are evidenced in host reactions.

The terms vertical resistance (VR) and horizontal resistance (HR) have been popularized by van der Plank (1968). Although they are probably not the best terms to use, since neither clearly depicts the kind of host response incited by a parasite or the relative effectiveness of the resistance to different races of a parasite, they will be used herein to conform with the title of the seminar.

Vertical resistance has been a major tool in efforts to control disease by genetic means. The merits of this kind of resistance have long been extolled; its demerits have become increasingly more clear. Resistance of this type is dramatically effective against one or more races of a pathogen and dramatically ineffective against others. It is an all or nothing resistance. Classically, VR resistance is expressed in the form of a hypersensitive response to the race or races against which the resistance is effective, although it does not necessarily have to be so. It is a resistance against the establishment of a successful infection site. Such resistance does not permit the continued colonization of the host and consequently curbs the amount of disease and thus the subsequent production of inoculum.

From a genetic standpoint, VR usually behaves as a single gene trait. More often than not VR is dominant over susceptibility. A single gene may confer resistance to one or many races of a pathogen. It is by far the most dramatic resistance reaction elicited by plants against their pathogens. The acceptance of race-specific resistance probably is due to the fact that man is philosophically and scientifically inclined to react most favorably toward the most dramatic host response. His search for the dramatic host response usually is prompted by the fact that currently used resistance has been matched by a new race of the pathogen. The search for a source of resistance to match the race is accomplished by matching the race against many potential sources of resistance in field or greenhouse breeding programs. This approach normally can yield only race-specific resistance. Vertical resistance is easily recognized and readily attained because of its relatively simple inheritance. Thus, by coincidence, what man has been inclined to seek is also the easiest to obtain.

The incapacity of races of a pathogen to overcome VR usually is a dominant trait, while the capacity to do so is recessive. Because VR functions only when a "resistant" host interacts with an "inable" pathogen, Flor (1955) suggested a gene-for-gene relationship as a result of his research with flax rust. Briefly, the theory states that for each gene conditioning race-specific resistance in the host there is a specific and related gene in the pathogen that conditions the ability to negate that resistance.

While VR reacts specifically against specific races, it is incorrect to assume that a gene-for-gene system exists in all cases of this kind of resistance. Vertical resistance may operate on a one-for-one basis and indeed does in several well documented instances. The test for genes for VR is not one-for-one but a

differential host reaction to different races. A differential reaction to different races would be the sole criterion for VR even if all examples of VR were shown to function on a gene-for-gene basis.

It is generally acknowledged that resistance of any kind is subject to breakdown when new virulent races arise. The pathogen usually requires a single genetic change to overcome a single gene for resistance. The instability of race-specific resistance in cereals to stem rust and in potato to late blight are ample testimony of the relative ease by which effective genetic changes occur among populations of plant pathogens. The relative instability of race-specific resistance can be expected if for no reason other than sheer probabilities that single gene changes occur more often than multiple gene changes.

From the standpoint of the onset and subsequent increase of disease among plant populations, VR functions by reducing the initial amount of inoculum available for disease onset. For example, if two races each comprised equal amounts of the initial available inoculum and the host possessed a vertical resistance gene for one race, the amount of effective initial inoculum would be reduced by one-half. The reduction of effective initial inoculum is one of the more significant features of multi-line varieties. The increased probability that portions of the available inoculum will come to rest on components of the multi-line with VR to the race(s) is directly proportional to the number of lines comprising the multi-line. It is probable that a synthetic, a variety of a cross-pollinated species produced by combining selected lines followed by normal pollination, operates similarly to a multi-line by selectively restricting the impact of initial inoculum.

When initial infection sites have become successfully established, pathogens proceed to further colonize their hosts. A period of colonization is usually followed by a period of reproduction which, from a disease standpoint, represents the production of inoculum for subsequent infection cycles and generations of disease. The exceptions to this total pattern are those pathogens whose primary infection and subsequent colonization are restricted to one generation of disease. Smut species colonizing kernels or seeds of cereal crops are examples of pathogens that accomplish the disease cycle in a single infection process.

Plant species may possess several types of resistance mechanisms that come into play after infection sites are established. These mechanisms generally tend to restrict the extent to which pathogens can colonize host tissue and the relative degree to which pathogens are able to produce inoculum for subsequent infection or disease cycles within a single growing season. Resistance of this type reduces the amount of disease that develops, usually by reducing the amount of disease that occurs within a single infection cycle and the rate at which disease develops from one infection cycle to another.

Plant resistance to colonization and reproduction of pathogens disrupts several different phases of pathogenesis. The incubation period of a pathogen is the time required from the initial stages of infection to the production of inoculum for a subsequent infection cycle. Plants may express resistance to pathogens by increasing the time required to complete the incubation period. Resistance may be expressed by restricting the amount of tissue that is colonized at a single infection site. The end results are smaller pustules or lesions, as example. Reducing the amount of inoculum produced frequently is a characteristic of plant species possessing post-infectious resistance. The time necessary for sporulation to occur may be extended in colonized tissue that has reached a stage where sporulation is possible and normally expected. These major effects by plants restricting the development of their pathogens all function to decrease the rate of infection within and among populations of plants.

There are certain general features of resistance mechanisms that affect infection rates. It is not a dramatic resistance to the casual observer. Disease is present and the impact of the resistance on the progression of disease is not evident when viewed at a single point in time. The virtues of resistance to disease increase are becoming increasingly evident. Resistance to increase and spread is not race-specific, at least in the sense of all or nothing effects on different races, as in the case with VR. It can be termed appropriately as horizontal resistance (HR) or non-specific resistance if certain general parameters are kept in mind. There is a growing misconception that HR reacts uniformly against all races of a pathogen. While the gross effects are the same, the degree to which HR functions against different races can vary to a considerable extent. A hypothetical model can make the point. Assume that there are only three races of a pathogenic species. These races are capable of inciting disease in a variety with neither vertical nor horizontal resistance genes. The variety is "susceptible" to the three races but not equally so because of inherent differences in virulence among the races. The most virulent race causes more disease and does so in less time. A second variety has no vertical genes for resistance to the three races but does possess resistance genes that reduce the rate of disease development by reducing infection rate within and among plants. The incubation period of two of the three races is lengthened by three days in the second variety as compared to their capabilities in the susceptible variety with no resistance genes. The incubation period of the third and most virulent race is lengthened by only one day in the second variety as compared to the first variety. The variety with resistance genes reacts uniformly against the three races in that the incubation period of all races is lengthened and yet reacts differentially to the three races with respect to the relative time that the incubation period of the three races is lengthened. In the broad sense, these resistance genes confer HR to the variety because disease increase is reduced against all races of the pathogen. The fact that HR is not uniformly effective against all races should

be expected in light of the known abilities of races or different isolates of races to differ in virulence.

Van der Plank (1968) depicts horizontal resistance as reacting uniformly against all races of a pathogen. In that sense, there appears to be no evidence that horizontal resistance exists in any plant species to any plant pathogen. At least that notion has never been put to an adequate test. There are, however, many examples in which plant selections or cultivars express a level of resistance to colonization or spread of disease. Whether this kind of resistance is effective against all races or uniformly so against all races is not as important from a practical standpoint as some would believe. A variety of wheat with HR to all races of stem rust occurring in the United States is valuable in that country even though a race exists in Australia which could completely overcome the resistance.

Horizontal resistance usually, if not always, is conditioned by the combined action of several genes. Its polygenic nature probably accounts for the relative stability of the resistance for long periods of time. It is a longer lasting resistance because races with the necessary genes to overcome it are less likely to arise. Whereas single genetic changes in the pathogen often are sufficient to overcome VR conditioned by a single resistance gene, several different genetic changes probably are needed by the pathogen to overcome resistance that is polygenic in nature. The probabilities that any given race can acquire, accumulate, and maintain all the necessary genetic changes are lessened. The stability of horizontal, polygenic resistance appears to be based on probabilities of sequential events occurring in the pathogen.

The "loss" of HR usually is gradual and seldom constitutes a complete loss. The stepwise loss of resistance can be subtle and may be obvious only to the keen observer. Whereas "complete" HR may function to increase the incubation period by three days, for example, "partial" non-specific resistance resulting from the loss of one or more of the components of the polygenic system may be effective in increasing the incubation period by two days. Similar relative efficiencies of complete and partial HR are evidenced in the effects of resistance on lesion size and number and on the various aspects of the sporulation process.

Certain potato (*Solanum* spp.) lines have exhibited stable HR in Mexico to races of *Phytophthora infestans* (Mont.) de Bary, the late blight organism. Some recent indication of a gradual erosion of the HR in some lines supports the idea that the pathogen must acquire several new genes to completely overcome HR. The future use of HR to curb population shifts seems very promising. The problems inherent in detecting and incorporating genes for HR into acceptable cultivars is another story.

The preceding portion of this paper has discussed different kinds of resistance mechanisms in a somewhat general manner. The remaining portion will treat some specific aspects that are important to any applied consideration of horizontal resistance.

### **The expression of horizontal resistance**

Apparently HR can have an influence on much of the disease cycle. The composite result is less disease and an increased time requirement for disease to progress. Researchers have observed that plants presumably possessing HR often sustain infections later than susceptible plants. Luke, Chapman and Barnett (1972) observed late-rusting in some oat cultivars, stating that crown rust symptoms appeared 10-14 days later than in susceptible cultivars. Late-rusting characteristics were associated with late and intermediate-maturing cultivars but not with early-maturing lines. Late-rusting was reported to be dependent upon spore concentration and environment. Late-rusting reduces the amount of disease by delaying disease onset, but has no apparent effect on disease increase in subsequent infection cycles.

Retarding the rate of disease increase appears to be one of the prime functions of horizontal resistance. Such effect has been noted by several workers and described and characterized variably as: slow-rusting by Luke, Chapman and Barnett (1972) where the percentage of crown rust infection increases more slowly; slow-rusting in wheat; and partial resistance in potatoes to late blight (Guzman 1964). Sporulation was less and slower to occur and lesions were smaller and fewer in number on potato clones showing high levels of partial resistance, which is apparently equitable to HR. Restricted lesion size and delayed sporulation are features of the resistance of certain maize lines to northern leaf blight caused by *Helminthosporium turcicum*. United States maize lines apparently possess effective HR to rust, since serious levels of the disease are rare. The epidemiology of slow-rusting in Mexico has been investigated by Mackenzie (personal communication) using the Colombian wheat variety Bonza 55 to represent the attribute of slow-rusting. Caldwell (1968) has discussed some aspects of horizontal resistance.

### **The detection of horizontal resistance**

Since HR manifests itself by decreasing the rate of disease increase over time, its full detection can only be made over time. Most forms of HR cannot

be recognized, for example, by observing host reaction to initial infection, as is the case in screening for vertical resistance. Similarly, seedling tests cannot disclose the full complement of HR. Plants with HR do sustain given amounts of disease; their defense is their ability to keep disease at a level of little, if any, consequence.

The portion of the disease cycle that is influenced by HR dictates the mechanics of screening and detection. If HR acts primarily to reduce or retard sporulation, its action could be masked by the presence of outside inoculum from adjacent susceptible plants. In such a case, the classical use of row plots of many lines to screen for resistance would be self-defeating and of no value. Lines suspected of having HR should be evaluated in the presence of disease in relatively isolated or protected plots and observed over the span of a growing season. The amount of disease at the time of harvest or at a time when disease can have an impact on yield is of prime importance in evaluating HR. Disease-free controls are useful in evaluating the amount of disease and yield in areas where it is possible to keep plants disease-free.

#### **The stability of horizontal resistance**

The stability of HR will be influenced largely by the number of resistance genes conditioning the character and by the relative ability of the pathogen to create new races with the necessary virulence genes to match the host. The first portion of this paper referred to the stability of HR in terms of genetic probabilities, i.e., the greater the number of host genes the lesser the probabilities are that the pathogen can create and accumulate the necessary virulence genes.

There are several examples that attest to the stability of HR. Certain potato clones in Colombia and others in Mexico have withstood serious levels of late blight for many years in areas where virulent races are present. Certain cultivars of some cereals have withstood damaging levels of rust for years, including stem rust of wheat in Colombia and crown rust of oats in the United States.

The true expression of disease resistance may not be achieved when one or more factors function below the optimum to create an inadequate selection pressure. The failure to detect the ultimate pathic response can promote serious consequences. An inferior selection pressure can lead to a greater instability of what is presumed to be an adequate measure of HR.

The pathogen may be the contributing factor to an inadequate selection pressure. Plant pathogens are as variable or more so than their hosts. Different

populations of a pathogen usually exhibit sharp contrasts in aggressiveness, pathogenicity or virulence. Selection among plant populations for the most resistant members must be tempered by the fact that such selected plants express resistance only to what they were evaluated against. A key and critical factor concerns the extent to which the isolates used as inoculum or present naturally in the field are representative of the range of variation within the species. It is difficult to formulate a general guideline concerning numbers and sources of isolates necessary to constitute an adequate inoculum, but the issue can be addressed in terms of probabilities. Fifty isolates are more likely to represent a wide range of variation than will a single isolate. Fifty isolates from fifty locations should be more representative than fifty isolates from one location and so on.

#### **Sources of horizontal resistance**

As a general rule the best sources of HR probably are germplasms that are not products of man's attempt to improve crop plants by modern breeding techniques. As a converse and more specific rule, HR is not likely to be found in host materials that are products of man's effort to combat disease by use of hypersensitivity. In addition to usually race-specific, a hypersensitive response to a pathogen is generally conditioned by a single gene. Breeding procedures designed to locate the resistance gene in a commercially and agronomically acceptable genotype can readily lead to a loss of genes contributing to HR, since HR is masked in the presence of race-specific resistance.

Wild, ancestral species are almost always an excellent source of HR if they have evolved in the presence of the pathogen. Natural selection seems to dictate the survival and increased frequency of plant populations best fit to withstand damaging levels of disease. In general, wild species appear not to have evolved to a hypersensitive, race-specific type of resistance. As a case in point, Niederhauser (1961) stated, "In Mexico we were forced to concentrate on the multigenic field resistance when it was found that no tuber-bearing *Solanum* species was immune or hypersensitive to the pathogen when exposed in the field." Mexico is considered to be the co-epicenter where *Solanum* and *Phytophthora* have co-evolved for centuries. Wahl's (1970) observations on the "abundance" of HR in *Avena sterilis* to the crown rust pathogen in Israel support the contention that wild species in their epicenters have evolved to HR. Interspecific sterility may be a limiting factor in attempts to transfer resistance from wild to cultivated species.

Old-line germplasm, such as open-pollinated maize varieties, which are the products of man's selection of seed from the better performing plants also

have proven to be good sources of HR. The same rationale applies to this case as to wild species, differing only in time and the nature of the selection pressure.

### **Incorporating Horizontal Resistance**

Standard breeding methods used to deal with any quantitative trait can be used to incorporate HR. There does seem to be a philosophical tendency to view breeding for HR as an impossible task. Agronomists, however, have worked successfully with quantitative characters for years e.g., yield. There are problems to be sure. For example, suppose that HR in a crop is conditioned by five genes and that these five genes are to be transferred into an acceptable agronomic background by back-crossing. It is unlikely that all five genes will appear in one genotype in one back-cross. Suppose that two of the five genes appear in one genotype. What level of HR, if any, will the genotype express? This is a difficult question to answer categorically, but hopefully a keen observer may obtain some indication of an intermediate level of HR. Subsequent back-cross generations to that genotype should gradually result in complete transfer of the HR genes.

### **On Genes for Vertical and Horizontal Resistance**

The concepts developed about the terms vertical vs. horizontal, major vs. minor, specific vs. non-specific resistance, etc. have led us to conclude either by direct statement, inference or deduction that certain genes condition one kind of resistance (i.e., vertical), while different genes condition the other kind of resistance (i.e., horizontal). This section presents and discusses the concept that the same resistance genes govern both kinds of resistance. As example, it will present evidence and evolutionary logic that genes for vertical resistance and genes for horizontal resistance are the same genes.

The *Solanum demissum* - *Solanum tuberosum* - *Phytophthora infestans* system will be used as a prime model for this discussion. Late blight of potatoes is a classic disease; it has been made more currently classic in its use by van del Plank (1968) to promote the concept of vertical vs. horizontal genes for resistance.

Niederhauser (1961) stated, "In Mexico we were forced to concentrate on the multigenic field resistance when it was found that no tuber-bearing *Solanum*

species was immune or hypersensitive to the pathogen when exposed in the field." Apparently no recent evidence disputes this statement. In the same study Niederhauser also stated, "Field resistance is defined here as the resistance exhibited by a plant towards all races capable of causing on it more than a hypersensitive reaction." The author considers Niederhauser's statement as an acceptable definition of field resistance. It is identical to van der Plank's concept of horizontal resistance. Niederhauser further stated that field resistance "is characterized by slow-spreading lesions in which sporulation is sparse. The lesions per plant are fewer, and tend to be on older, lower leaves."

The **Solanum - Phytophthora** system not only speaks effectively to all of the points that are relevant to this discussion, but also is among the most appropriate of examples, since **Solanum** and **Phytophthora** have co-evolved in Mexico over a long period of time. Co-epicenters most accurately depict the story of evolution when discussing the evolution of genes for resistance. Thus, co-epicenters as used in this sense, are geographic areas in which both host and parasite have co-evolved.

The late blight story supports the contention that genes for vertical resistance (hypersensitivity) and genes for horizontal resistance (field resistance) are the same genes. It is a pertinent story because of man's attempted exploitation of the resistance demonstrated by **S. demissum** to a multitude of races of **P. infestans** in the Toluca Valley of Mexico. Although **S. demissum** exhibited field resistance, genes were extracted singly from **S. demissum** to obtain a vertical or hypersensitive resistance to certain, but not all, races of the pathogen. Thus the birth of the R-genes. The acceptance of the vertical effects of R-genes probably was due to the fact that we are philosophical and scientifically schooled to react favorably to the most dramatic host response. Vertical resistance is more easily recognized and more readily attained because of its relatively simple inheritance. Thus, by coincidence, what we have been inclined to search for is also the simplest to attain.

Additional R-genes have been extracted from **S. demissum** and each confers hypersensitive responses to races lacking the necessary gene(s) to overcome the specific resistance of the host. Unfortunately, R-genes have been called major genes because they evoked a major dramatic response (hypersensitivity) by the host to certain races. Major and minor genes should not be viewed as genes with differential strengths or contrasting efficiencies until evidence supports a distinction.

**Solanum** in Mexico probably has co-evolved with **P. infestans** for a long time. Early in its evolution **Solanum** probably had no genes for resistance to **P. infestans**, if one postulates the origin of **Solanum** prior to the origin of **P.**

**infestans** or before the evolution of the fungus as a parasite of **Solanum**, and if one postulates no prior pleiotrophic functions of genes for resistance. At some point in the evolution of **Solanum**, **P. infestans** also evolved, perhaps from a saprophyte to a parasite, and placed a selection pressure on **Solanum** for resistance to **P. infestans**. It is simplest to assume that the original parasite population(s) of **P. infestans** was reasonably homogeneous and as a pathogen probably constituted a simple race. The validity of that assumption is not pertinent, however. It is simplest to assume that **Solanum** evolved to resistance at a single gene locus, although again such an assumption need not be made for this discussion. How then did **Solanum** react to **P. infestans**? Probably by hypersensitivity, since we associate field resistance with the collective action of many genes; probably like a modern-day variety with an R-gene for vertical resistance. Subsequently, members of the populations of **P. infestans** evolved and strains pathogenic to the then resistant **Solanum** were selected. Such must have been the case, since **P. infestans** still exists.

The process of co-evolution proceeded, perhaps stepwise in a gene-for-gene manner. Plants of **Solanum** with fewer genes for resistance probably were selected against and either dropped out of the population or remained in a low frequency. It is difficult to imagine that all genotypes of host and parasite that existed during their evolutions remain a part of the current members of their respective species.

How long such evolution or co-evolution has occurred and what stage of co-evolution currently exists are not important. What is important is how did **Solanum** acquire the field resistance so evident today in **S. demissum** and other wild species of **Solanum**. Perhaps the gradual incorporation of additional resistance genes, each of which at their time of origin and selection conferred a vertical response to different pathogen genotypes, ultimately gave **S. demissum** sufficient genetic materials to survive the parasitism of **P. infestans**, even of those races that could infect and colonize the plant. In a real sense, resistance restricting infection to a few cells and resistance restricting size of lesions are similar phenomena differing only in magnitude. If a vertical gene is not effective against a race in a vertical manner, it is assumed not to function at all in resistance. Similarly, if five vertical genes are not effective against a race, they are all assumed to be functionless. Such an assumption is difficult to accept. It seems more feasible that five singularly functionless genes can be collectively functional. If each contributes something in the way of resisting the parasite at a point beyond hypersensitivity, the net, collective result logically seems to be a collective resistance against colonization. Five genes able to react vertically to some races but not to others can collectively react in a horizontal way to the "other" races.

An important question relative to the understanding of the collective action of vertical genes can be raised. Consider a hypothetical example in which each

of eight vertical resistance genes conditioned a fleck reaction to one of eight races; one different vertical gene against one different race. When the eight vertical genes are combined into one host genome, how would this change the response of that host to the eight races? The host with the eight vertical genes would still react vertically to the eight races. The eight races are not involved in the concept. It is the ninth race to which the host with eight vertical genes has no vertical gene which is pertinent to the concept. The concept suggests that the existing eight vertical genes would collectively function in a horizontal resistance to the ninth race.

It is likely that the last resistance gene incorporated into plants of *S. demissum* with some resistance genes did not confer a hypersensitive response to the pathogen. The symbiotic relationship between the two species was no longer sharply antagonistic. They had learned the value of co-existence. They had learned that the price of co-existence was less than the price of alternate superiority and inferiority. A relaxing of selection pressures on both species paralleled their new relationship. Neither was in serious jeopardy of extinction.

Philosophical or teleological as these speculations may seem, they probably represent the scientific explanation embodied in the co-evolution and co-existence of host and parasite. They probably represent the means by which *S. demissum* acquired field resistance.

Thurston, Heidrick and Guzman (1962) observed the occurrence of late blight on 263 clones of *Solanum andigenum* and 200 clones of *Solanum phureja* for several years in the field in Colombia. All clones exhibited a field resistance which did not appreciably change from year to year. Apparently, hypersensitivity is not the trademark of these two ancestral species of *Solanum*.

It could be speculated, conversely, that *Solanum* species originally were resistant or immune to *P. infestans* and that portions of that resistance were lost over time. How *Solanum* evolved to field resistance is not as important as the fact that it did evolve to that point.

Field resistance is multigenic in nature. It is a quantitative trait. A quantitative trait is governed by many genes simply because no one gene can accomplish the task. There can be no other logical explanation for a multigenic control of a character. Genes controlling a quantitative trait are essentially polygenes. Each contributes something to a collective venture that none can accomplish alone. From an evolutionary standpoint, polygenes probably arose one at a time. At least it is unlikely that many genes arose simultaneously. Thus, it is equally unlikely that a quantitative trait arose as a result of a single change in the species. If a quantitative trait is not the result of a single change, it must be the result of several independent changes. Assume, as an example, that a quantitative trait is conditioned by five genes. The five genes are polygenes

and arose one at a time. How could or how did the first of the five polygenes survive in the population, let alone spread throughout other members of the same or different population? The first gene to evolve probably could not have survived as a polygene, unless one postulates that all polygenes are neutrally-selective alleles. At its time of origin, the first gene (polygene) probably conferred a more major effect, an effect that it alone could accomplish. It was maintained in the species because it was worth something to the species. This is to imply that the so-called minor genes in *S. demissum* were major genes at the time of their origin and still can exhibit major gene effects when they are separated from other genes which collectively govern a quantitative trait. Major genes (vertical) and minor genes (horizontal) are the same genes. Relative to the idea of the singly-major and collectively-minor effects of genes, it is known that many "mutant" genes exact small effects on quantitative traits. There is no doubt that mutations can affect quantitative traits or that removal of one gene from a group of genes can affect that expression of the trait. In that context, we are considering effects on an existing trait. My discussion concerns the sequences leading to the origin of a quantitative trait.

No one polygene drastically alters the expression of a character. To what extent each contributes by its presence or absence may depend on the extent that each was effective as a major gene earlier in its evolution. This point is made to acknowledge that a particular gene contributing to field resistance may not be recognizable by our current resources as a gene conferring hypersensitivity. It is inconceivable that all genes that arose in the pathogen during its evolution still exist today. Genes currently present in *S. demissum* may have conferred a hypersensitive response to a genotype (race) or the pathogen which no longer exists.

Hypersensitivity and hyposensitivity (tolerance *sensu* Gauman) are the extremes in the spectrum of co-evolution of host and parasite and are not the usual trademarks of wild or ancestral species that have co-evolved with their parasites for long periods of time. The fact that evolution is a dynamic, continuing process assures us that such extremes either exist currently or will exist in the future. At any one point in time, no one can speculate as to whether hypersensitivity that currently exists in a naturally evolving host-parasite system represents a point in current evolution or a vestige of a completed stage of evolution. We only know that hypersensitivity is not commonplace in epicenters. Bananas in Indochina, *Aegilops* in the Near East, *Tripsacum* and teosinte in Mexico, and *S. demissum* in Mexico probably would attest to this. Hypersensitivity may well exist in low frequency among some of the population of plants in epicenters. The dynamics of evolution virtually assure it.

Certain factors make it difficult to interpret the literature on late blight of potatoes in a manner that would materially negate or substantiate the

contention that vertical and horizontal resistance are controlled by the same genes. Clones of *S. demissum* carry differing numbers of R-genes. Apparently no clone has been determined to possess all nine R-genes. If R-genes function collectively to confer a horizontal resistance, it is not known how many are needed to give some measure of horizontal resistance. Perhaps an opposite analysis would contribute evidence. Graham, Niederhauser and Servin (1959) collected race 0 from *S. demissum* (accession number S-434) in the Toluca Valley, Mexico. Clone S-434 thus had no R-genes by current criteria. When evaluated for its field resistance, clone S-434 was rated as severely blighted as early as July 27; it exhibited no field resistance. In the same studies, only race 1.2.3.4 was recovered from another clone of *S. demissum* (accession number S-449). Apparently S-449 possessed R-genes. When S-449 was evaluated for its field resistance, it was rated as having no blight on July 27 and rated as "slight" on September 18. Apparently S-449 had field resistance. It is suggested from these two comparisons that the R-genes contributed to the field resistance of S-449.

Attempts to equate initial plant reaction in the greenhouse to predicted field resistance indicate that plant reaction to initial inoculum does not consistently represent the plant reaction in the field over time (Graham, Niederhauser and Servin 1959, Main and Gallegly 1964). Perhaps a partial answer to the inconsistencies may be found in the concept of hypersensitivity. It has been widely assumed that a hypersensitive response implies death of the host cells and the pathogen. Apparently that assumption is not universally valid. Browning and Frey (1969) state that for three cereal rusts the pathogen is not dead but quiescent and can resume colonization after long periods of time under proper environmental conditions. The author has recovered *H. carbonum* and *H. turcicum* from a hypersensitive reaction several weeks after the response was elicited (unpublished research). The fact that the presumed events associated with hypersensitivity may not actually occur is not important. What is important is the fact that a pathogen may persist for long periods of time under the condition of hypersensitivity. At least from the literature I have examined, I cannot determine whether a strain of *P. infestans* can be quiescent and subsequently active in a hypersensitive reaction. It may be reasonable to assume that it could be viable for a prolonged period. Many reasons could account for the fact that wild species of *Solanum* exhibit a field resistance characterized by few and small lesions. One of these reasons could be that the pathogen has a long incubation period in wild species. The time from infection (what may be the hypersensitive response) to lesion formation may be influenced by changes in the environment or in the host substrate. The hypersensitive reaction to *H. carbonum* induced in resistant genotypes remains as such until the resistant plants approached maturity, at which time the necrotic flecks began to enlarge to form small lesions. The physiological phenomena associated with senescence apparently lessened the resistance of the host. The lessening of resistance at senescence probably accounts for Niederhauser's observations

(1961) that the few lesions formed on field resistance clones of *S. demissum* tend to be on older, lower leaves. A long latent period of infection is associated with physiological activity of the host. The anthracnose pathogens of banana and tomato infect green, immature fruits, but symptoms of the disease are not evident until the fruits are ripe.

In the greenhouse, the hypersensitive response of potato to *P. infestans* is usually evaluated 5 to 7 days after inoculation. The literature does not reveal whether plants exhibiting hypersensitivity have been maintained for periods after the evaluations (from seedling plants to mature plants, as example) or under changing environmental conditions to determine if blight lesions might develop subsequently. Niederhauser and Mills (1953) observed late blight on *S. demissum* on the slopes of the volcano Popocatepetl from July through September. Little blight was found in July and much was observed in mid-September. It is not known whether hypersensitive responses were more prevalent than blight lesions early in the blight season. A hypersensitive response observed late in the season could mean that the plant is hypersensitive resistant or that incipient infections occurred only recently before observation.

Precisely what specific terms were meant to convey often is unclear in the literature on potato late blight. The term "highly resistant" is particularly confusing. When not clarified, highly resistant could mean exclusively a hypersensitive reaction, a preponderance of hypersensitive reactions vs. blight lesions, few lesions, small lesions, etc. Black and Gallegly (1957) screened plants of different accessions of *S. demissum* and other *Solanum* species for resistance to four races of *P. infestans*. Resistant, susceptible segregations were determined on the basis of disease index classes. Resistant classes included highly resistant (necrotic flecks and small lesions) and moderately resistant (larger lesions often spreading slowly). The plants were inoculated in humidity chambers and evaluated seven days after inoculation. Some confusing segregation ratios were obtained. The ratios may have been confusing because plants with small lesions or larger lesions spreading slowly were grouped as resistant with plants exhibiting necrotic spots typical of hypersensitivity. Since plant reactions seven days after inoculation can only be used accurately to measure resistance to infection after seven days, it would seem more appropriate to have considered only hypersensitive plants as exhibiting resistance at that stage. It is difficult to interpret two different kinds of host resistance with a single disease index. A plant exhibiting one or a few small lesions does not portray the same kind of resistance as a plant reacting exclusively by hypersensitivity.

In Mexico, *Tripsacum* and teosinte, directly or indirectly a part of the early evolution of maize, and the native races of maize, which are among the oldest available representatives of early maize, are neither immune nor hypersensitive to strains of *Helminthosporium turcicum*, the incitant of the northern leaf

blight disease. On the other hand, none of these hosts are severely blighted, but rather resist the build-up of disease. It is difficult to find much blight. Lesions are small, few in number and often exhibit reduced sporulation. Since Mexico is considered the epicenter for maize, the ancestral species and early maize probably co-evolved with *H. turcicum*. The net result appears similar to the *Solanum-Phytophthora* story. Field resistance replaced the hypersensitive reaction, probably by evolutionary sequences analogous to those presented for the evolution of resistance in *Solanum*.

Recent research (Nelson, MacKenzie and Scheifele 1970) with 69 isolates of *H. turcicum* and four inbred lines of maize with differing numbers of chromosome arms carrying genes for vertical resistance present additional factual evidence to support the hypothesis that vertical genes and horizontal genes are the same genes. Maize inbreds with greater numbers of chromosome arms with genes for vertical resistance have a greater horizontal resistance to pathogenic isolates than inbreds with fewer or no chromosome arms with genes for vertical resistance. The greater the number of arms with genes for vertical resistance, the greater the horizontal resistance, as measured by lesion size and number and sporulation. The implication, again, is that genes function vertically when they are separate (such as R-genes) and the same genes function horizontally when working together (such as genes in *S. demissum*).

If the maize inbred with the greatest number of chromosome arms with genes for vertical resistance were the only genotype of maize growing in an area abounding with the aforementioned 69 isolates of *H. turcicum*, the following events would probably take place: (1) the inbred would be immune to certain isolates (36 of 69 according to our results); (2) the 36 non-pathogenic isolates would soon disappear from the population (assuming no alternate hosts); (3) the inbred then would no longer exhibit a vertical resistance, but rather would exhibit a horizontal resistance to all surviving isolates. If we could consider the maize inbred in the context of a "wild" or untampered maize line, the predicted events would be similar to a phase in evolution of the host and parasite. Since our studies were not conducted with host or parasite germplasm found in the epicenter of maize, we were witness to an artifact by concluding the presence of vertical genes in the maize inbred.

From a genetic standpoint, the fact that the same gene can function qualitatively (i.e., in a vertical response) or quantitatively (i.e., as a contributor to a horizontal response) could be explained by the suggestion that a gene will function differently in different genetic backgrounds. There is good evidence that genes have major or minor effects in different backgrounds (Athwal and Watson 1954). An R-gene in a *S. tuberosum* background may well function differently than it does in its native *S. demissum* background. The concept discussed in this paper suggests that such is the case. Horizontal and vertical

resistance are not indications of the action of different genes, but rather are expressions of different actions of the same genes under different circumstances. A future paper will develop the parallel concept that genes for pathogenicity and genes for virulence in the parasite are the same genes, but functioning differently. Recent research (Nelson, Mackenzie and Scheifele 1970) has suggested that isolates of *H. turcicum* with greater numbers of genes for pathogenicity to inbred lines of maize with differing numbers of chromosome arms with genes for vertical resistance are more virulent than isolates with fewer genes for pathogenicity, when the two kinds of isolates are compared on inbreds susceptible to both kinds of isolates. A recent evaluation of the virulence of 72 isolates of *Helminthosporium maydis*, each possessing from 1-13 genes for pathogenicity to nine gramineous hosts, has shown that isolates with a greater number of genes for pathogenicity are more virulent than isolates with fewer genes for pathogenicity, when comparisons are made on host species susceptible to both kinds of isolates (Nelson and MacKenzie 1971).

It seems totally predictable that genes in the parasite that condition the ability to incite disease (pathogenicity) should also contribute to the amount of disease incited (virulence), regardless of whether you are referring to different genes for pathogenicity to different members of one host species or to different genes for pathogenicity to different host species. A successful or unsuccessful host-parasite relationship are relative events of the same interaction, as are the varying degrees of a successful relationship. It is equally predictable that a vertical gene for resistance which is ineffective in vertical way against a certain race could contribute something to the defense of the host when functioning with other "ineffective" vertical genes in a horizontal way.

#### **Some Implications for Breeding :**

If, in reality, genes for vertical resistance and genes for horizontal resistance are the same genes, as the concept presented herein proposes, what implications are apparent to our approach to breeding for disease resistance? Changes in our philosophy and in our technique will be required. Philosophically, we must change our emphasis from the "quality" of genes to numbers of genes. The strength of *S. demissum* is in its number of genes for resistance. If *S. demissum* can survive, so can *S. tuberosum*, but only when the latter has what *S. demissum* has. Changes in our methods of disease evaluation are mandatory before we can ever determine when *S. tuberosum* indeed has what *S. demissum* has. Disease evaluations must change emphasis from resistance to infection to resistance to growth. The failure to detect horizontal resistance in oats to crown rust races is attributed to the use of screening methods designed to

search for vertical resistance (Browning and Frey 1969). A potato clone, for example, must be evaluated at the end of the growing season for the amount of disease and the impact of the disease. The clone must be exposed to a variety of pathogenic strains under environmental conditions suited for disease, just as *S. demissum* was in its evolution. Such approaches are not novel ideas. They are and have been knowingly or inadvertently used in many instances.

It is not surprising that there is little existing data to support or refute this concept. We have simply not given much thought to the possibility that the same gene can condition a qualitative trait and also contribute to a quantitative trait. It is hoped that the possible validity of the proposed concept will prompt a closer analysis of existing data, a re-evaluation of past experiences, and future research to test its merit. Research by Dinoor (1970) and Wahl (1970) on resistance in *Avena sterilis* in its epicenter to crown rust may provide valuable information when evaluated in light of this concept. Wahl's observations on the "abundant occurrence" of horizontal resistance in *A. sterilis* (1970) seem to support the contention that wild species in their epicenters have evolved to horizontal resistance.

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**Horizontal resistance:  
six suggested projects in relation  
to blast disease of rice**

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There are two possible sorts of resistance to disease: vertical resistance and horizontal resistance. Vertical resistance is a differential interaction between varieties (genotypes) of the plants and races of the pathogen. Horizontal resistance is the absence of differential interaction, i.e., resistance is spread equally against all races of the pathogen.

Vertical resistance can be introduced into cultivars relatively easily (if the can attack the host genotype. *Pyricularia oryzae* has shown itself to be plastic and well able to produce the virulence genes that match and make useless the resistance genes which breeders have introduced into rice cultivars. Horizontal resistance, on the other hand, is not affected by the plasticity of the pathogen. The pathogen reaps no benefit from producing new races because horizontal resistance acts against all races. A horizontally resistant cultivar remains a resistant cultivar, however much the pathogen can vary. That is the advantage of horizontal resistance.

Vertical resistance can be introduced into cultivars relatively easily (if the necessary resistance genes are available), and its effects are clearly and immediately obvious. That is why rice breeders and pathologists have chosen it in the past.

Horizontal resistance is relatively difficult to introduce into cultivars, and its effects are often obscure and not immediately apparent. Rice breeders will turn to horizontal resistance, not because it is convenient to use, but because it is necessary. As the years go by and rice becomes more and more vulnerable to blast, so will horizontal resistance become more and more necessary.

The manifestations and inheritance of vertical resistance to *P. oryzae* have been relatively well studied. Horizontal resistance, on the other hand, has been less studied in detail; and much of what we have to say of it must be inferred from what we know about other diseases.

### **Manifestations of Vertical and Horizontal Resistance**

If leaves of a young rice plant are infected artificially with spores of *P. oryzae*, the presence or absence of vertical resistance in the rice plant can be determined within a few days. If the plant responds by forming only reddish flecks or small reddish spots without differentiation into distinct zones, the plant is vertically resistant to the pathogenic race. If the plant responds by forming large spindle-shaped lesions several millimetres broad, and these lesions in time bear spores, the plant is vertically susceptible. The criterion of vertical resistance is the type of lesion, not the number of lesions. (Horizontal resistance affects both the number and the type of lesion, the type of lesion reflecting the abundance of sporulation.)

Horizontal resistance manifests itself in three ways. First, the number of lesions formed in a horizontally resistant variety is less than a susceptible variety, in the same conditions and inoculated with the same number of spores. Second, the time taken by a newly formed lesion for itself to form spores (i.e. the period between inoculation and subsequent sporulation) is longer in a resistant variety. Third, sporulation is less abundant in lesions on a resistant variety. (This third manifestation should be subdivided to allow for the duration as well as the abundance of sporulation, but we shall ignore the distinction here.)

Horizontal resistance is determined by quantitative characters (the number of lesions produced by a given number of spores, the period needed for lesions to sporulate and the amount of sporulation). Research projects must therefore be based on quantitative measurements in natural conditions.

## **Suggested Projects:**

### **1. Determining horizontal resistance as field resistance**

In the absence of vertical resistance, resistance is horizontal. Therefore, if one can exclude all vertical resistance, one can simply compare cultivars or lines in the field, and the comparison will measure horizontal resistance alone. This is the simplest and most direct method.

When is vertical resistance absent? On available knowledge, the answer is: when the lesions are of a vertically susceptible type, i.e., when the lesions are large and normal and classed as reaction type 4 or 5 in the U.S. classification.

The method, then, is to expose lines (or cultivars) to infection in the field by virulent races to which the lines are (vertically) susceptible. The resistance that remains is horizontal resistance.

The difficulty arises here. The lines must be exposed to a race virulent on all of them or to several races, each of which is virulent on all of them. False results are given whenever lines are exposed to a mixture of races, some of which are virulent on some races but avirulent on other races. (Then vertical resistance enters and confuses the results.) The easiest comparisons can be made when all the lines are susceptible to all the local races. Otherwise special precautions are needed.

### **2. The selection of lines and cultivars that are more difficult to infect**

The problem here is to select the rice lines and cultivars that are the most resistant to infection. That is, if one uniformly inoculates several lines or cultivars, one wishes to select those that develop the fewest lesions per plant, per leaf or per square centimetre of leaf. The principle is easy; the practice may be difficult.

First, one needs an inoculator that gives reproducible results so that lines or cultivars can be accurately compared.

Second, one must avoid artifacts. Conditions must be natural. Plants should be of an age at which blast epidemics normally occur; one cannot assume a priori that comparisons made with young seedling plants will hold for older

plants. Plants must be grown under natural conditions; one cannot assume a priori that plants raised under cover behave like plants in a rice field. Therefore, one must devise a method of growing plants in the field, bringing them to the inoculator and returning them immediately to the field without significant disturbance, or alternatively, one must devise an inoculator that can be used in the field.

Third, the ratio of the number of lesions to the number of spores used as inoculum varies with the concentration of spores, if the concentration is high. Therefore one should aim at spore concentrations that give no more than an average of one lesion per square centimetre of leaf surface.

Fourth, it is worth investigating the possibility that resistance to infection as a manifestation of horizontal resistance can be measured even in the presence of vertical resistance (i.e., in the presence of hypersensitivity). It is possible that one could count the average number of infections per square centimetre of leaf surface, irrespective of whether the infections are hypersensitive flecks (indicating vertical resistance) or normal lesions. This would greatly expedite investigations. To test the possibility one could compare two or more rice lines or cultivars by inoculating them with a race of *P. oryzae* virulent on all of the lines (i.e., giving normal lesions on all); repeating the experiment with a race avirulent on all the lines (i.e., giving only hypersensitive flecks on all the lines); repeating it again with a race virulent on one line but not the others; and so on. If one then ranks the rice lines in order of decreasing resistance starting with the line that gives the fewest infections (lesions or flecks) per square centimetre of leaf, the order of ranking should be independent of the race used. In other words all the different races of *P. oryzae* should indicate the same rice line as being the most resistant.

Thus it is evident that a great amount of preliminary research is needed before one can begin to measure resistance to infection quantitatively.

### **3. The selections of lines and cultivars in which the period from inoculation to sporulation is greater**

A long period between inoculation and sporulation, i.e., a long period needed for newly formed lesions to start releasing spores, is a manifestation of horizontal resistance that can be selected for. If plants of different rice lines or cultivars are inoculated and examined regularly, the period needed before sporulation can begin is easily measured.

The isolate of *P. oryzae* used to compare the lines must be virulent on all the lines (i.e., there must be no complications from vertical resistance).

Artifacts must be avoided. (Avoid using detached leaves in laboratory.) Plants must be of an appropriate age and must be grown under natural conditions.

#### **4. The selections of lines and cultivars on which sporulation is less abundant**

The lines to be selected are those that produce fewest spores per lesion. Again, use virulent isolates of *P. oryzae* and avoid artifacts.

It is possible that these second, third and fourth projects will select much the same lines. That is, it is possible that lines which are the most difficult to infect (second project) are on the average also the lines in which the period needed for sporulation is longest (third project) and also those in which sporulation is less abundant (fourth project). If this is so, it will be a great help.

#### **5. The accumulation of resistance by breeding**

Horizontal resistance is almost certainly polygenic in inheritance and should be accumulated by a program of breeding. Selected lines or cultivars could be paired and appropriate segregates isolated in the  $F_3$  or later generations. The parent lines and the segregates would of course be selected both for agronomic characters and for horizontal resistance.

A long-term project suitable for an institution to undertake would be to prepare a composite. Selected parents would be paired in all possible combinations, the  $F_1$ 's bulked, and the bulked composite grown for several generations to remove most of the heterozygotes. Plants could then be selected on agronomic characters to start new lines, and the lines then tested for horizontal resistance.

The benefit of horizontal resistance does not show fully at the start. Horizontal resistance is a slowing-down in the rate of infection. This slowing-down is less evident in single rows or small plots of the host plants because inoculum moves in from outside. Only when the horizontally resistant plants cover whole fields or the whole countryside do the full effects of the slowing-down become apparent. Only if resistance to infection (the subject of the second suggested project) is very high, will the benefit of the resistance be immediately apparent.

One must take care therefore not to ignore amounts of resistance which could ultimately be effective but which are not very impressive while the new line is confined to small experimental plots.

#### **6. The combination of horizontal with vertical resistance**

Vertical resistance is immediately apparent even in small plots. Indeed, in relation to the number of hectares on which a cultivar is cultivated, vertical resistance and horizontal resistance show opposite trends. Vertical resistance is best in small plots; by the time the vertically resistant cultivar is grown over a large area (e.g., by the time it becomes the dominant cultivar in a country) new virulent races of the pathogen are likely to have developed and so "destroyed" the resistance. Horizontal resistance on the other hand keeps on gaining effectiveness in a cultivar when that cultivar is grown over a larger and larger area.

The ideal solution is to combine the two sorts of resistance. The vertical resistance will keep the cultivar protected in its early years. By the time the cultivar is widely grown horizontal resistance can take over.

The two forms of resistance can be combined by using a horizontally resistant line as the recurrent parent in a program of backcrossing.

The sixth suggested project is really a suggestion for continuity with the past plus horizontal resistance. In the past, vertical resistance has been used without proper attention to the horizontal resistance or susceptibility of the cultivar in which it is used. The change suggested for the future is that genes for vertical resistance should be incorporated by backcrossing only into lines which have been selected for horizontal resistance. This would apply both to new genes for vertical resistance and to old genes that have been used and are judged to be still worth using in new cultivars.

The sixth suggested project would still require that the other suggested projects be carried out in order to supply the horizontally resistant lines needed as recurrent parents in backcrossing.

#### **Organization of Research**

It would be proper for this symposium to consider what research is needed and how to organize it.

The suggested projects indicate what research is needed: research on the quantitative relations involved in horizontal resistance. Apparatus must be devised and built. It may be unnecessary to repeat this research in different countries; it may be possible to concentrate it at an international center. That is also a proper subject for the symposium to consider.

**Techniques and philosophies  
on the development and use  
of perfect stages to understand  
pathogen variation and host resistance  
to plant diseases**

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Plant pathogenic fungi are notorious for their ability to vary. Their capacities to generate increased or novel parasitic abilities are the principal reasons for man's continuing efforts to control plant diseases by means of host resistance. Stable resistance would be commonplace in an era of stable pathogens.

Plant pathogenic fungi effectively exercise the conventional mechanisms of genetic variation and, in addition, employ other means of variation which may be unique to certain of their members. Fungi change rapidly and dramatically by mutation and by genetic recombination through "conventional" sexual processes. Genetic reassortment via parasexuality or mitotic recombination is an acknowledged means of variation within some fungal species, as is heterocaryosis, the coordinated capacities of genetically dissimilar nuclei in vegetative or asexual systems. It is not the purpose of this paper to discuss how these mechanisms function or to document them as functional phenomena for any given fungal species.

Although mutations constitute the ultimate source of genetic diversity, the reassortment of genetic material during the sexual process probably is the most important mechanism contributing to pathogen variation within many fungal pathogens. Variation in certain parasitic attributes may be dependent upon the combined or additive effects of two or more genes. In such cases, single mutations within a population would not improve a particular attribute. Another population may incur a different mutation which again would not significantly enhance a given trait. The recombination of these two mutant genes into a single genotype during the sexual process would provide the appropriate gene combination to create a new pathogenic variant. It seems safe to conclude that species with a perfect stage should possess greater opportunities to generate new, important genotypes than species lacking a system that assures genetic recombination.

*Pyricularia oryzae*, the incitant of the blast disease of rice, is recognized widely for its extreme variability. Many pathogenic variants can be obtained from a single monoconidial culture. The mechanism(s) contributing to such variation are not well understood. The perfect stage of the fungus is not known, although a *Ceratospheeria* ascigerous stage of *Pyricularia grisea*, a species which is considered to be morphologically identical to *P. oryzae*, has been produced *in vitro*. To date, isolates of *P. grisea* from crabgrass have not been crossed with isolates of *P. oryzae* from rice. The ability to detect the perfect stage of *P. oryzae* and to readily induce its formation *in vitro* would provide the opportunity to analyze the genetic control of pathogenicity and virulence. An understanding of the factors influencing variation within and among populations of the fungus could be derived from studies with its perfect stage. The detection of stable resistance to any parasite is a more likely prospect when pathogen variation and pathogen potential is at least reasonably well understood. The first portion of this paper discusses some techniques, knowledge, and theories that may be helpful in discovering perfect stages of fungal pathogens. The latter portion briefly outlines and discusses the value of research with perfect stages to better understand pathogen variation and host resistance.

#### **Some Generalities on Perfect Stages**

Certain basic generalities regarding the nature and production of fungal perfect stages probably are germane to most species, although notable and obvious exceptions are readily evident. The following remarks acknowledge these exceptions.

It is reasonable to assume that fungal species that fail to produce their perfect stages readily *in vitro* are heterothallic. Most homothallic species produce their sexual stages abundantly *in vitro* on natural or synthetic substrates when relatively few basic requirements are evaluated. Light and temperature regimes are readily monitored by the investigator. Nutritional requirements usually are less exacting for homothallic species. Exogenous applications of specific compounds enhance reproduction of homothallic species in a quantitative manner more often than they serve as definitive, qualitative nutrient sources.

Assuming that a particular species such as *P. oryzae* is heterothallic, certain generalities are more or less applicable to a search for perfect stages. The vast majority of species which reside in the Fungi Imperfecti and for which no perfect stage is yet known will ultimately be shown to have perfect stages in the class Ascomycetes. There are relatively few examples in which imperfects have been associated with a Basidiomycete stage and no example of an association with a Phycmycete stage, since species in the latter class largely are identified originally on vegetative and/or asexual characteristics.

All known heterothallic species of Ascomycetes exhibit a basic pattern of bipolar sexuality, a mechanism whereby compatibility or incompatibility between strains is conditioned by a single major gene locus. While some species are reported to have a multiple allelic system at the compatibility locus, most heterothallic species of Ascomycetes possess only two alternate states which often are cited in allelic form as + or — or A or a. Such species consist of only two kinds of potentially cross-compatible populations. From the standpoint of probabilities, there is but a 50 percent chance that any two strains of a species when paired will be of opposite compatibility types and potentially capable of producing a perfect stage.

Thus far in our theoretical approach to discover the perfect stage of an imperfect species, we can assume with a high degree of confidence that we are seeking a heterothallic Ascomycete with but two alternate compatibility types. The two principal tasks that remain are finding compatible strains and determining the environmental and nutritional regimes that are conducive for the *in vitro* production of the ascigerous stage. Some of the author's experience with the detection and development of perfect stages of *Helminthosporium* species will be used to illustrate certain basic points.

The failure to detect the perfect stage of a heterothallic plant pathogenic fungal species in nature may be due to the presence of but one compatibility type in a given area, the relative infrequency of its occurrence and/or the failure of the investigator to detect its presence. The author's research with *Cochliobolus heterostrophus* Drechs. (*Helminthosporium maydis* Nisik. and Miyake), the incitant of Southern corn leaf blight, pertains in part to these

possible reasons, at least from the standpoint of probability. The fungus is a heterothallic Ascomycete with two distinct compatibility groups designated in allelic form as A — a. Its perfect stage is readily induced in vitro when compatible strains are paired on an appropriate substrate and under an appropriate environmental regime. The disease is incited exclusively by the asexual or conidial stage, from its onset through initial and subsequent generations. On only rare occasions have strains of both compatibility types been isolated from a single corn field. Whether this phenomenon can be accounted for by concluding that the perfect stage plays a relatively minor role in the life history of the species is a moot point. The perfect stage of *H. maydis* has been detected in nature only on rare occasions and exclusively on dead or senescent plant parts. With notable exceptions, heterothallic species with perfect and imperfect stages fit this pattern, i.e., the perfect stage is not an active part of the parasitic phase, but rather serves as a means of survival during a non-parasitic stage. Perfect stages of some parasites provide inoculum for initial infection, e.g., *Venturia inaequalis*, the incitant of apple scab. When living host material is available for active parasitism, the impetus of the parasite tends towards continued vegetative growth or asexual reproduction. When known compatible strains of a heterothallic species are available for mating studies, a search for its perfect stage would be most profitable in an area where both mating types have been determined to exist in vivo. Furthermore, a search for perfect stages in nature should be confined largely, if not exclusively, to senescent plant material, unless the perfect stage is known to be active during the disease cycle.

The author has sought and successfully detected perfect stages of several *Helminthosporium* species for almost 20 years. A number of lessons have been learned; some of them were learned the hard way. One lesson has virtually become a principle to this investigator. That lesson is to obtain a wide geographic distribution of isolates before attempting to detect the perfect stage. Precisely what constitutes a wide geographic distribution is impossible to comment on. The general principle of diversity is related to probabilities. The probability of securing isolates of both compatibility types among 50 isolates from 50 different areas is far greater than it would be if 50 isolates were collected, for example, from an area of one square mile. Both mating types may well be present in a one square mile area or even in a single field, but the probabilities are less. The chances are further diminished if the perfect stage does not readily occur in nature. In summary, and in a sense a word of sound advice, obtain a collection of isolates from a wide geographic area.

Compatible isolates of *P. grisea* from crabgrass have been reported to be incompatible with isolates of *P. oryzae* from rice. It was suggested, and appropriately so, that the failure of isolates of *P. grisea* to cross with isolates of *P. oryzae* probably was genetically based rather than being due to different

environmental or nutritional requirements. The author has reported previously on the effects of geographic origin and host association on cross-fertility between isolates of *Helminthosporium* species. In one study, for example, a total of 79 isolates obtained from 37 species of 31 genera of Gramineae were studied for mating behavior and degree of reproduction isolation (incompatibility). Viable ascospore progeny were produced in 464 of a possible 1155 pairings between isolates of opposite compatibility, a frequency of 40 percent. Eleven isolates exhibited complete reproductive isolation in all paired cultures. The frequency of fertile crosses between isolates from cultivated hosts was 43 percent of such crosses, while the frequency between isolates from wild hosts was 14 percent. The frequency between isolates from cultivated and wild hosts was intermediate. Ten of the eleven reproductively-isolated strains were obtained from wild species. These results suggest that host association may be an important factor conditioning the evolution and complexity of sexual mechanisms. The intermediate frequency obtained with crosses between isolates from wild and cultivated hosts suggests that the reduced fertility between isolates from wild hosts is due in part to genetic deficiencies and inhibitors rather than to entirely opposing compatibility mechanisms.

The author has reported in several publications on the presence of genetic factors that block the sexual process in *Helminthosporium* species. Genetic blocks are known to inhibit perithecial, ascus, and ascospore formation in pairings of isolates of opposite mating type. Such genetic blocks are detected more frequently in isolates obtained from wild hosts. This thought, as well as the foregoing discussion of host association and cross fertility, may well serve as advice to obtain a majority of isolates that form a wide geographic source for a collection from cultivated hosts. For example, a search for the perfect stage of *P. oryzae* may be more profitable if many of the collected isolates were obtained from rice.

#### **Some Guidelines on Inducing Perfect Stages**

Certain general guidelines useful in studies designed to induce perfect stages of heterothallic species can be derived from a review of Kleb's postulates. His postulates, formulated in 1900, are concerned with growth and reproduction and briefly stated are: 1) growth and reproduction are life processes which all organisms depend upon under different conditions. Lower organisms are influenced to a greater extent by their external environment; 2) reproduction does not set in as long as the external conditions necessary for growth are present. Conditions favoring reproduction in general do not favor growth; 3) working conditions are narrower for reproduction. Growth may occur even

though certain factors inhibit reproduction; and 4) growth appears primarily as a preliminary for the initiation of reproduction and therefore as an inner condition for it.

Other food for thought can be obtained from the reflections of Sachs, the German physiologist, who a century ago advanced the hypothesis that proper development and functioning of sexual organs in plants depend upon specific chemical compounds. Through the ensuing years, research on the physiology of sexual development in the fungi has accepted and supported this hypothesis. Research has repeatedly demonstrated that the initiation and differentiation of sexual reproduction structures are controlled by the manipulation of the chemical and physical factors of the fungus' environment, particularly its nutritional regime. Numerous attempts have been made to understand the physiological processes by which these effects are achieved but it is not clear as yet how such changes occur or what their role is in the processes that regulate sexuality. It has been concluded, nevertheless, that positive qualitative and quantitative responses resulting from exogeneous applications of specific compounds are due to the compounds functioning directly as regulators either of the sexual process or of biosynthetic pathways controlling sexual processes. However, it may be assumed with equal validity that stimulatory compounds trigger metabolic pathways which serve as precursors to subsequent processes which may be initiated by other unrelated biosynthetic pathways.

The perfect stage of *F. grisea* has been induced in culture by pairing compatible strains on Sach's agar with barley grains and rice straw. Perfect stages of several heterothallic species of *Helminthosporium* have been induced *in vitro* between compatible strains by essentially the same technique. The specific substrate or the particular plant parts are probably not as important as some of the general principles that underlie their success. Sach's agar is basically a salts medium with relatively little nutritional value. Most fungal species that the author has studied exhibit sparse vegetative growth on the substrate. Conversely, most species exhibit prolific vegetative growth on potato-dextrose-agar (PDA), an example of a nutritionally rich substrate. Sexual reproduction of heterothallic *Helminthosporium* species is significantly more abundant in matings on Sach's agar as compared with matings of the same strains on PDA. The general principle seems to be that reproduction is more likely to occur and will occur more abundantly on substrates that are not optimum for vegetative growth. That principle probably should be respected when considering possible substrates to use in inducing sexual reproduction.

There are several possible reasons to account for the need of some plant part as a requisite to sexual development in many heterothallic species. The requirement of a plant part on which the sexual structures develop probably satisfies both a physical and a nutritional need. It has been said that competition for oxygen is the fundamental reason for the absence of reproduction under

conditions which allow abundant growth. Some stimulus apparently starts with the utilization of a stored food supply by oxygen and leads to reproduction when an organism is in a hunger state. While some of the particulars of these statements may not be directly pertinent to the present consideration, the general idea of competition for oxygen may relate to enhanced reproduction of heterothallic species when plant parts or other solid materials are used. It is possible that the lack of a direct contact with the agar substrate creates a more aerobic environment which triggers reproduction. Agar substrates are moist and moisture can contribute to an anaerobic state. In this connection, the author has found that increased perithecial production by heterothallic species of **Helminthosporium** is directly correlated with increased concentrations of agar. A 25-fold and greater increase in perithecial formation has occurred consistently on a 5 percent agar substrate as opposed to a 1 percent agar concentration. Substrates with 5 percent agar are considerably more dry than substrates with less agar. The increased dryness of a more concentrated agar substrate may permit a greater availability of oxygen. Other evidence that a physical requirement must be satisfied will be discussed later in the text.

That plant parts satisfy a nutritional need for sexual reproduction is well documented for heterothallic species of **Helminthosporium** and probably have a comparable pertinence to all species which require similar mating techniques. Several general principles appear to have emerged from studies by the author and several of his students and associates. Many of these studies have utilized strains of **Cochliobolus carbonum** Nelson (**Helminthosporium carbonum** Ullstrup), a heterothallic Ascomycete parasitic to corn and other gramineous species. The sexual process is initiated and completed in a precise and consistent fashion when compatible strains are paired under an appropriate chemical and physical environment. Certain paired strains are highly cross-fertile and consistently produce a dense ridge of perithecia in the zone of mycelial contact, permitting a reliable evaluation of qualitative and quantitative responses to altered nutritional environments.

Sexual reproduction in **C. carbonum** occurs readily and abundantly when compatible strains are paired on opposite sides of a small disc of sterile senescent corn leaf (**Zea mays** L.) placed on the surface of Sach's nutrient agar in petri dishes. However, pairings of the same compatible strains on green corn leaves, agar substrates, filter paper, cellophane, or membranes fail to initiate the sexual stage. These observations suggested that paired strains of **C. carbonum** are not able to synthesize all of the biochemical requirements for sexual initiation and development, the biochemical requirements for sexual reproduction are present in senescent corn leaves, and chemical changes occurring in the transition from green to senescent tissue provide the required metabolites. Consequently, a systemic screening of compounds was initiated and included those known to be present in senescent tissues or in differential

amounts between green and senescent corn tissues and those tested previously by other workers in studies on sexual reproduction.

One early phase of the screening studies was concerned specifically with inorganic compounds. Incorporation of any zinc salt in Sach's nutrient agar enabled compatible strains to produce the sexual stage when paired on filter paper. Further studies demonstrated that perithecial production depended upon the concentration of zinc used and that zinc effects in turn were influenced by the concentration of agar utilized in the preparation of the basal medium.

Maximum perithecial development consistently occurred when crosses were made on a Sach's nutrient substrate containing 5 percent agar supplemented with 30 ppm zinc. However, the use of a 5 percent agar substrate posed considerable difficulty in keeping the filter paper sections adhered to the substrate, presumably due to a lesser amount of available free moisture. When 40 ppm or more zinc/liter were used, vegetative growth was retarded and perithecial initiation required several additional days. Thus a 4 percent agar substrate and 30 ppm zinc in the form of  $ZnSO_4$  were used as part of a second basal medium.

Because of the important role of zinc in many physiological processes in fungi and higher plants, the study was expanded to include those related to zinc. Compounds were tested for their effects on reproduction in the presence and absence of zinc. The number of perithecia formed on Sach's nutrient agar with zinc was a quantitative measure of a compound's activity.

A total of 173 organic and inorganic compounds were tested at various concentrations on Sach's nutrient agar supplemented with 30 ppm zinc and without zinc for their effects on sexual reproduction in *C. carbonum*. Of these, 37 organic and inorganic compounds increased perithecial production in the presence of zinc, as compared to crosses paired on Sach's nutrient agar plus zinc. However, sexual reproduction did not occur when these compounds were used in the absence of zinc. The compounds included several carbohydrates, vitamins, sterols, amino and fatty acids.

Eight compounds: betaine, choline, homocysteine, homocystine, lecithin, DL-methionine, D-methionine and L-methionine increased perithecial production at most concentrations in the presence of zinc and supported perithecial development to varying extents in crosses paired on Sach's nutrient agar without zinc. Sexual reproduction in crosses on Sach's nutrient agar without zinc was sparse when betaine, choline or lecithin was applied to the filter paper sections, while application of homocysteine, and D-, L- or DL-methionine resulted in perithecial development numerically similar to that occurring in check crosses paired on Sach's nutrient agar plus zinc.

The remaining 128 compounds, including all inorganic salts other than zinc compounds, were either non-stimulatory or inhibitory in the presence of zinc and totally ineffective in the absence of zinc. With all salts of zinc tested, perithecial production occurred consistently on Sach's nutrient agar, provided the concentration of agar was at least 2 percent.

Vegetative growth was not stimulated markedly by compounds increasing perithecial production, although some increases were observed. Some inhibition of vegetative growth occurred in the presence of compounds inhibiting perithecial production, although no general correlation existed between vegetative and sexual inhibition. In general, no stimulation of ascus or ascospore development was evident when perithecial formation was increased, suggesting that the stimulatory compounds function in the early stages of reproductive ontogeny. Similarly, ascus and ascospore formation were not markedly reduced in crosses in which perithecial development was inhibited.

These results suggest that the diverse kinds of compounds that initiated or stimulated sexual reproduction in *C. carbonum* may have served as common sources of specific nutrilites in different biosynthetic pathways that lead to the ultimate synthesis of further compound(s) directly responsible for the regulation of the sexual process. If so, no one specific precursor biosynthetic pathway would be categorically required to initiate the sexual process.

One specific characteristic of most of the compounds which initiate or increase sexual reproduction is the presence of methyl groups. Further analysis of the molecular configurations of these compounds showed that the C- and O-methylated compounds and C- and O- compounds bearing methyl groups were effective stimulators of reproduction only in the presence of zinc. Conversely, the N- and S-methylated compounds were active in initiating reproduction in the absence of zinc and stimulatory in the presence of zinc. The relationship of methyl groups and zinc involvement may explain the role of zinc in the demethylation of compounds and/or in the enzymatic breakdown of the stable binding of the methyl group linked to C- and O-methylated compounds. The active effect on N- and S-methylated compounds in the absence of zinc may be based on their known alkylating properties. Increased activity in the presence of zinc may be due to the effect of the ion on this reaction.

One phase of our studies has shown a complete dependency of sterol activity on sexual reproduction on the presence of zinc ions. This apparent association of zinc in the conversion of sterols may offer an opportunity to investigate the biochemical nature of sterol activity in sexual reproduction in the fungi. Preliminary studies with SK & F compound 3301-A, a known inhibitor of cholesterol biosynthesis, suggest that sterol metabolism per se is not an essential requisite for sexual reproduction. Perithecial production in *C. carbonum* was totally inhibited by applications of 0.03 mg/mating of the

inhibitor compound. Sexual reproduction in similar crosses treated with the inhibitor was partially restored by applying .4 mg/mating of methionine. Gas chromatographic studies have shown that products of cholesterol degradation by *C. carbonum* have retention times similar to steroids without the side chain. These results tend to substantiate our contention that the diverse group of stimulatory compounds, including sterols, may serve primarily as sources of nutrillites.

The stimulatory effect of several concentrations of sterols was obtained consistently and without difficulty. The activity of sterols appears to be dependent upon the presence and/or length of the chain at the C<sub>17</sub> position, since none of the steroids, including estrone, estriol, progesterone or testosterone, was active even in the presence of zinc.

A few compounds not bearing methyl groups were active stimulators in the presence of zinc. Some of the compounds may be involved in a process serving a regulatory function. Xylose, ribose, and some amino acids are involved in nucleic acid synthesis. Sugars and fatty acids are energy sources. Reducing agents such as the sulfites are active in the reductive fission of the S-S link in biological systems and iodoacetic acid is a well-known alkylating agent. It is possible also that these non-methylated compounds may form part of a biological methylation by fission so as to eliminate a molecule being involved in an as yet unknown one carbon fragment metabolism.

The components of agar have been studied. Components of particular interest here are agarose and agarpectin, which are products of the hydrolysis of agar. Agarose is comprised of several O-methylated sugars and may, in part, account for the increasing perithecial production with increasing agar concentrations in the presence of zinc. It is possible that methylation of the sulfur atom occurs readily, involving hydrolysis, reduction, and further methylation. Preliminary studies with different lengths of time of sterilization of Sach's nutrient agar have shown that perithecial production is increased proportionately with increased sterilization time and thus increased hydrolysis, at least up to and including 60 minutes.

The effect of zinc and S- and N- methylated compounds, such as methionine, on sexual reproduction in *C. carbonum* led us to investigate the role of such activity at a metabolic level. Briefly summarized, a selective inhibition of perithecial production was obtained by certain concentrations of several azaderivatives of RNA bases, but not of DNA bases. Reproduction was partially or completely restored when various concentrations of their normal bases were applied simultaneously with inhibitory concentrations of the analogues. Significantly, sensitive applications of DL-methionine also restored sexual reproduction when applied with inhibitory concentrations of the analogues. RNA content per unit dry weight ranked from least to greatest in crosses on

Sach's agar, Sach's agar plus zinc, Sach's agar plus methionine, and Sach's agar plus zinc plus methionine. RNA content increased with increasing sexual reproduction stimulated by increasing amounts of methionine. Incorporation of  $^{14}\text{C}$  of the methyl group of L-methionine in extractable RNA was detected in crosses on Sach's agar plus methionine and in increased amounts in the presence of zinc. The dependency of the sexual process on RNA metabolism is indicated by zinc and methionine stimulation of sexuality through a primary effect on RNA synthesis.

The biochemical requirements for the formation of perithecia, asci, and ascospores in *C. carbonum* are present in senescent corn leaves. A crude chloroform-methanol extract from senescent corn leaves, an "ether extract" and a "methanol extract" from the original crude extract were shown to stimulate perithecial production when aliquots were applied to filter paper. No concentration of the extracts stimulated ascus and ascospore formation. Perithecial production increased only when extracts were applied prior to the physiological time of perithecial formation, which, under the conditions of our studies, was six to seven days after pairing.

Gas chromatographic analyses of sterol and fatty acid fractions of each of the three extracts revealed no qualitative and no quantitative differences among the extracts. The bulk of the fatty acid fractions was made up of palmitic acid and linolenic acid. Injections of the sterol fractions produced peaks corresponding to ergosterol, beta-sitosterol and stigmasterol.

Chemically pure palmitic and linolenic acid failed to stimulate perithecial formation or reduce ascus and ascospore production when applied singly or in combination at several concentrations. Similarly, no response was observed when ergosterol, beta-sitosterol, and stigmasterol were applied to filter paper.

Relatively large and easily measurable quantities of sterols and free fatty acids were obtained from 500 mg samples of crude extract by the procedure used during preparation of sterols and fatty acids for gas chromatography. Each 500 mg sample yielded about 40 mg of sterols and 25 mg of free fatty acids, an 8 percent and 5 percent yield respectively. These two fractions were then tested for activity at known concentrations.

The 0.1-0.2 mg/pairing applications of the sterol fraction significantly increased perithecial numbers over solvent checks. The fatty acid fraction failed to stimulate perithecial formation. Combinations of the two fractions were no more active than the sterol fractions. No concentration of the sterol fraction was as effective as the 0.1 mg/pairing application of the crude extract.

Perithecial numbers increased to 6-7 fold over solvent-treated checks when the sterol fraction and a solution of water soluble materials from senescent corn leaves were applied together. At certain concentrations of the mixture perithecial development was greater than when 0.1 mg of crude extract was applied.

These studies demonstrate that the metabolites required for initiation of the sexual process in *Cochliobolus carbonum* can be extracted from senescent corn leaves with chloroform-methanol. Suitable concentrations applied to filter paper stimulated perithecial formation comparable to that in crosses made on the corn leaf. Materials required by the fungus during perithecial formation apparently were present in the crude extract.

Other compounds active in stimulating perithecial production may be present in the corn leaf or in the chloroform-methanol (crude) extract and were not identified during this investigation.

Time of application studies suggests that the mode of action of perithecial-stimulating chemicals extracted from senescent corn leaves is nutritional. The extracts stimulated perithecial development only when they were applied prior to the physiological time at which perithecia form. The similar stimulation observed from applications made from the beginning up to six days suggests that the materials become part of a nutritional reserve pool available to the strains as they reach the physiological time for sexual differentiation.

These studies further suggest that the requirements for perithecial formation in *C. carbonum* are different from the requirements for ascus and ascospore formation. None of the materials which stimulated perithecial production increased the numbers of asci or ascospores formed per perithecium at any of the concentrations tested.

Concentration of materials and concentration balance between materials appear to be important considerations. Active materials had an optimum stimulatory concentration and a limited range of concentrations that resulted in increased perithecial numbers.

The nutritional studies summarized to this point have been treated in some detail to offer possible techniques and approaches that may be useful to others who seek to culture perfect stages of different species. The specific materials and methods that have proven useful in our studies with *Helminthosporium* species may not be similarly useful to others. The general principles which we have learned, however, should be pertinent to other species.

Our studies on the biochemical requirements for the initiation and successful completion of sexual reproduction provide an enhanced understanding of

certain other phenomena. It was suggested earlier in the text that plant parts or other solid materials may satisfy a physical, as well as a nutritional, requirement for reproduction. We may now conclude that such is the case. Sexual reproduction can be induced when compatible strains are paired on opposite sides of a section of sterile filter paper to which one of a number of chemical compounds or extracts from senescent corn leaves have been applied. No sexual development occurs when the same compounds or extracts are incorporated in or applied to agar substrates.

It was stated earlier that the perfect stage of *H. maydis* has never been found in nature on actively growing corn plants. We have learned that sexual reproduction *in vitro* does not occur when sections of green corn leaf tissue are used instead of senescent corn tissue. The reason for this quite probably is one of nutrition. Actively growing corn leaves either do not possess the necessary nutrients or not enough of them to enhance fungal reproduction or they contain metabolites that are inhibitory to the sexual process. Many biochemical changes occur as active plant growth ceases and maturation and/or senescence prevail. Sterols, for example, increase significantly with maturation. Seeds or kernels have stored compounds not present in green leaves or which are present in markedly lower quantities. From these observations, it seems likely that the perfect stage of *H. maydis* cannot occur during the active growth phase of its host. The same likelihood may well exist for other heterothallic species, even if compatible strains are present in the same localized area. This prospect should give an added impetus to concentrating a search for perfect stages in nature on dead or senescent plant parts. There is always a possibility, however, that diseased tissue may be comprised of breakdown products that may support reproduction.

If the need to use plant parts to induce sexual reproduction in paired cultures is at least theoretically acknowledged, the choice of plant parts comes under consideration. *H. maydis* is parasitic to a variety of gramineous hosts other than corn. We have demonstrated that the perfect stage of the fungus is induced readily on senescent leaf material of a number of gramineous species. Leaf tissue of some species induces reproduction to a greater extent than others. The point seems to be that many species of the Gramineae probably possess a variety of common biochemical constituents. Their evolutionary relationships make it understandable that they would. If a choice of host species were to be made, it may be logical to select senescent leaf tissue from plant species which serve as hosts for the fungal parasite in question. Related plant species not serving as hosts to the parasite would be a sound alternative choice.

Light requirements should be considered in any attempt to induce perfect stages of heterothallic species. Sexual reproduction in heterothallic species of *Helminthosporium* and other genera of Ascomycetes is retarded and can be

totally inhibited when paired strains are cultured under continuous light. While this phenomenon may not exist for all species, the potential influence of light is sufficiently important to culture pairings of potentially compatible strains in light and darkness.

Temperature requirements usually are not overly exacting. To be sure, maximum sexual reproduction often occurs within a relatively narrow range, e.g., + or - 5°C. However, deviations above or below the more optimum temperature range usually restrict the abundance of reproduction rather than totally inhibit it. Temperatures which support satisfactory vegetative growth probably will also support sexual reproduction.

### **Summary of Techniques and Philosophies**

A brief summary of the techniques and philosophies that may be useful in studies designed to induce the perfect stage of a plant pathogen in artificial culture may be helpful to the reader. The assumption will be made that the search is for the perfect stage of a plant pathogen and that our potential guidelines are based on probabilities.

1. The fungus will be a heterothallic Ascomycete.
2. A basic pattern of bipolar sexuality will be present, whereby compatibility between isolates is governed by a single gene with two alternate alleles.
3. The perfect stage does not play an active role during the parasitic phase, but rather serves as a means of survival during a non-parasitic stage, if and when it does occur.
4. Isolates of both compatibility types seldom will be present in a confined localized area, e.g., a field.
5. The perfect stage will be induced most often between isolates collected from different geographic areas and will occur more often in pairings of isolates obtained from cultivated species serving as hosts to the parasite.
6. The production of the perfect stage *in vitro* will be dependent on certain physical and nutritional requirements which include:
  - a. The need for some plant tissue or solid material on which the sexual structure will be produced.

- d. The agar substrate will be a minimal medium which fails to support vigorous vegetative growth.
- e. Senescent plant tissue may contain the biochemical requirements for sexual initiation and development; green plant tissue will not support reproduction.
- f. Certain nutrients applied exogenously to senescent plant tissue may enhance reproduction and will induce reproduction when applied to inert materials such as filter paper.
- g. Certain compounds will trigger different biosynthetic pathways that will lead to the ultimate synthesis of further compounds which regulate sexuality.
- h. The selection of compounds to induce perfect stages can take advantage of available knowledge concerning their potential activity and usefulness in other fungal systems.
- i. Light and temperature requirements should be monitored.

The techniques and conceptual approaches pertinent to inducing fungal perfect stages *in vitro* may not be familiar to many of the readers who have not had the need to pursue such a venture. The potential benefits of being able to work with the sexual stage to study several genetic aspects of a parasite no doubt are well known to most readers. They are briefly cited herein and discussed only in a cryptic fashion.

Normally, different strains or populations of a fungal pathogen will vary in their ability to attack different host genotypes and/or in the extent to which they can do so. A considerable understanding as to the number of genes conditioning these qualitative and quantitative parasitic abilities can be gained by evaluating hybrid progeny obtained from crosses of isolates exhibiting different parasitic aptitudes. At least theoretically, it should be possible to develop a more effective and stable resistance in plants with some knowledge of the genetic capacities of a parasite.

One of the more significant benefits that accrue from working with the perfect stage of a plant pathogen is a genetic study designed to evaluate and/or predict the genetic potential of future races. Races of plant pathogens arise totally independent of the relative resistance or susceptibility of their hosts to existing races. To assume any degree of dependency upon their hosts would dictate an acceptance of the idea of "directed" origin for which there is no evidence. Host genotypes will influence the ultimate frequency or the sustained presence of new races, but not their origin.

The continued appearance of strains of fungus pathogens that are virulent to supposedly resistant varieties of plants has reduced many programs of breeding for disease resistance to a stop-gap basis. Knowledge of the number of genes conditioning pathogenicity, the mechanisms controlling their inheritance, and the pathogenic response of genes in the fungus to genes for resistance in the host should permit a partial evaluation of the potential pathogenicity of the species. With such knowledge, it might be possible to develop plants with resistance to current and potential genotypes, particularly in instances where increased resistance is due to greater numbers of genes rather than to more favorable gene combinations. The term "potential" is used herein in an operational sense, in that there probably is no theoretical limit to potential resistance or pathogenicity.

The increased frequency of virulent races and the concurrent "loss" of resistance of a host variety usually is associated with cases in which varieties have been developed with resistance to a specific race. Resistance of this type commonly is referred to as race-specific resistance or vertical resistance (VR). While VR is effective against a certain race or races it is equally ineffective against other races; plants lacking VR to a race usually are highly susceptible. Genes for VR function against epidemic development of plant diseases by reducing the initial amount of inoculum available for disease onset. Races lacking virulence genes to match VR genes are essentially disqualified from epidemic involvement. Genes for VR are considered to have no influence on epidemic increase of disease by races with patching genes for virulence. From a genetic standpoint, race-specific VR is usually conditioned by a single gene.

A race which increases in distribution and frequency among populations of a plant pathogen must exhibit two fundamental assets. The race must, of course, possess the necessary gene(s) for virulence which negates a particular gene for VR. It must also possess the ability to become a more dominant member of the species. These abilities can be characterized as "fitness attributes" and include the ability to attack its host under the different environmental regimes in which the host is grown, the ability to become disseminated over wide areas, and the ability to persist or survive in the absence of its primary host.

Other attributes may not directly affect the ultimate frequency of a race but influence the rate at which that frequency is attained. Races able to cause a greater amount of disease in less time and able to produce greater amounts of inoculum would assume a more dominant position among populations in a shorter period.

In contrast with race-specific or vertical resistance, some resistance mechanisms are effective to some extent against all races. Resistance of this

type often is called non-specific. Non-specific resistance functions by reducing the amount of disease and the rate of disease development. Such resistance mechanisms may retard penetration, increase the incubation period, restrict lesion size and reduce the amount of sporulation and the period in which sporulation occurs. Some or all of the effects are imposed upon all races of a pathogen in a similar although not equal manner.

From a genetic standpoint, non-specific resistance is usually polygenic in inheritance. More often than not, non-specific resistance has remained stable and effective for long periods of time. It is likely that a race would have to acquire several new genetic abilities to overcome resistance conditioned by several genes. Each genetic improvement in the pathogen occurs independently of other improvements needed to overcome polygenic resistance. The stability of polygenic resistance, then, seems based on probabilities of sequential events occurring in the pathogen.

The need to detect the occurrence of new races before they cause serious problems is imperative, whether the accent is on race-specific or non-specific resistance. Anticipating genetic potentials need not be a theoretical exercise in wishful thinking. The author has demonstrated that genetic studies with several *Helminthosporium* species can reveal new and potent genetic capacities among strains obtained by crossing compatible strains. If new genotypes can be "manufactured" *in vitro* they most certainly can occur in nature by similar means. Whether new strains can arise to overcome non-specific resistance is only a matter of probabilities. Investigating these probabilities by genetic analysis of recombinant strains may provide considerable insight into this matter.

Some recent studies by the author on Southern corn leaf blight suggest that virulent races can acquire epidemiological attributes over time. The 1970 epidemic of Southern corn leaf blight in the United States was incited by race T of the pathogen, with an unprecedented virulence to corn hybrids in male sterile cytoplasm. Studies with isolates of the pathogen collected in previous years demonstrated that race T has been in existence in the United States at least since 1955. Comparative studies with an isolate of race T collected in 1955 and preserved in limbo in leaf tissue since that time and an isolate of race T collected in 1970 revealed that the 1970 isolate possesses up to 15 times greater sporulation capacities and colonizes susceptible tissue more rapidly than the 1955 isolate. Either of these attributes could contribute significantly to the increased frequency of the race. Studies are currently in progress to evaluate the genetic control of such improved fitness and serve as another example of the value of working with the perfect stage of a plant pathogen.

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## **Variability of *Pyricularia oryzae* Cav. and its relation to varietal resistance**

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It has been shown that disease resistance in plants which depends upon one or a few major genes is usually unstable, i.e. the resistance breaks down as a new virulent race appears. This has been referred to as "vertical resistance," "specific resistance" or "major gene resistance." Another type of resistance is stable; it is unaffected by variation in pathogenicity of races. This type of resistance has been called "horizontal resistance," "field resistance," "general resistance," "race non-specific resistance," and many other terms. Many genes are usually involved in controlling this type of resistance (van der Plank 1963, Caldwell 1968, Robinson 1969).

Vertical resistance against a specific race will break down because when a new virulent race multiplies, the population increases and all individuals are pathogenic to the variety, i.e. breed true to the new race. If, however, the new race does not breed true and produces other races in its progeny and if the variety has a strong gene or genes for resistance or a broad-spectrum of resistance against most races developed, severe outbreak will not occur because few of the original pathogenic races occur in the progeny. This seems to happen for the blast fungus, *Pyricularia oryzae*, against varieties that have a broad spectrum of resistance. The fungus seems to be extremely variable in pathogenicity. Even with pathogenic races, the resistance of the varieties does

not seem to break down because the fungus has changed. The resistance seems to be stable (Ou et al. 1971). It could be called horizontal resistance but it does not agree with the definition of the term by Robinson (1969).

#### **Variability in Nature and Identification of Broad-spectrum Resistance through the International Blast Nurseries**

During the half century, numerous tests have been made in several countries to identify blast resistant varieties and use them for breeding. The success in these breeding programs has been limited. The new varieties were resistant only for a few years, possibly because the variability of the fungus was underestimated. Varietal reaction varies from locality to locality as well as from season to season in the same locality while the work of testing varietal resistance in the past was limited to a relatively small number of varieties, a few seasons, and a limited number of geographic areas. The resistant varieties selected as donors of resistance have not been exposed to many pathogenic races and consequently they did not have a very broad base of resistance.

Some of the work done in the Philippines illustrates the change of varietal reaction by localities and by seasons. From 1962 to 1964, 8,214 varieties of the world collection of the International Rice Research Institute (IRRI) were tested in a blast nursery. Of these, 1,457 were found highly resistant in the first test. These resistant varieties were tested seven additional times in the same blast nursery. Only 450 remained resistant. The 450 varieties were tested in seven stations in different regions of the Philippines and after a few repeated tests, only 75 remained that showed resistant reaction in all tests at all stations.

In closer examination of changes of races in a blast nursery during a 21-month period (Quamaruzzaman and Ou 1970) (Fig. 1), we found that both the composition (different races) and frequency (population of each race) are different each month. Of the 363 samples tested, 60 races were identified. Although the number of samples is small in comparison with actual number of conidia and races that might have been present in the nursery, it nevertheless illustrates the changes of races in the same blast nursery. It is conceivable that such changes also occur in the field and this may explain the observations that certain varieties, while resistant in the seedling stage, are susceptible to neck blast.

To identify material that has a broad spectrum of resistance, blast resistance must be tested repeatedly in many geographic regions. Thus, an international program is essential. The International Uniform Blast Nurseries (IBN) was

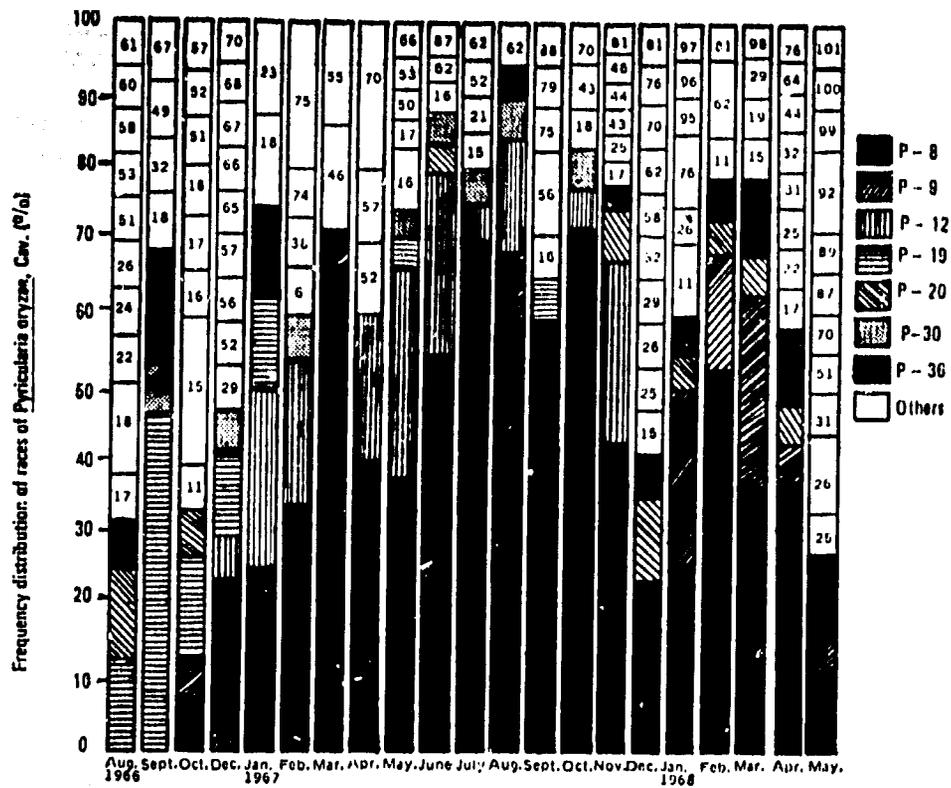


Figure 1. Population of the Philippine race groups of *Pyricularia oryzae* Cav., sampled monthly at the blast nursey, Los Baños, Laguna, The Philippines, from August 1966 to May 1968. (Quamaruzzaman and Ou, 1970).

started in 1963. Testing materials included 258 leading commercial varieties and the varieties used by three countries for differentiating races. In 1966 another 321 resistant varieties selected from the IRRI blast nursery were added to constitute group II of testing varieties. In 1969 most of the susceptible varieties were deleted, a few other varieties were added and the two groups were consolidated to form one group of 356 varieties. Up to 1970 more than 200 test results were obtained from 50 stations in 26 countries, mostly in Asia, some in Latin America and Africa. Detailed data are reported biannually in International Rice Commission Newsletters: 1964, 1966, 1968, and 1970.

The results of the IBN showed that many rice varieties which are resistant in one station, one country or in a region of several countries are susceptible in other stations, countries, or regions because of the existence of different prevailing races. Many of the varieties tested in a new region are resistant, at least initially, i.e. many japonicas are resistant in tropical Asia while many indicas are resistant in Japan and Korea. The blast fungus apparently is capable of producing new races all the time. But the new races can only survive when there are susceptible host varieties. Thus, after a long period of time, prevailing races in Japan or Korea are virulent to japonicas while the races in the tropics are virulent to indicas.

The most valuable information obtained from the IBN is the identification of many varieties that have a broad spectrum of resistance, although no variety was resistant in all tests (Ou et al. 1970). Some of the most resistant varieties are shown in Table 1. Varieties such as Tetep are consistently more resistant than others. It is resistant in 97.5 percent of the tests made. Fanny, a susceptible variety, was resistant in only 19 percent of the tests.

#### **Variability among Conidia from Single Lesions and from Single Conidial Cultures**

Strains of *P. oryzae* differing in pathogenicity were first noticed by Sasaki (1922). Latterell et al. (1954) first identified physiologic races in the United States. During the last decade, such identification of races was carried out in many countries using different sets of differential varieties. Many races were identified in each country (Ou and Jennings, 1969). Atkins et al. (1967) recommended an international set of differentials and Ling and Ou (1969) suggested the standardization of the international race numbers.

In all the above studies, a pure culture was obtained by a single conidium from a sample. The inoculum was prepared from the pure culture for testing pathogenicity. Ou and Ayad (1968) tested 56 monoconidial cultures from one leaf lesion on the Philippine set of differentials and found 14 races. The

Table 1. The most resistant varieties selected from the International Uniform Blast Nurseries.

Variety	1964-65		1966-67		1968-69		1970		Total		% Resistant
	No. Test	No. Suscept.									
<b>From Group I varieties</b>											
Tetep	22	2	59	0	63	2	23	1	189	5	97.5
Nang sbet cuc	39	2	49	2	63	3	23	0	176	7	96.0
Tadukan	56	3	57	2	63	5	23	0	201	10	95.0
R 67	51	4	51	2	63	1	21	3	188	10	94.7
C46-15	56	2	60	3	63	4	22	4	203	13	93.6
CI 7787	50	4	59	2	63	4	20	2	194	12	93.9
Pah Leuad 29-8-11	47	4	59	4	59	4	20	2	187	14	92.5
D25-4	31	3	60	6	64	3	23	2	180	14	92.3
Trang Cut L. 11	27	4	50	5	64	2	23	0	166	11	94.0
Pah Leuad 111	18	2	55	5	63	3	22	2	160	13	91.9
Fanny (susceptible)	54	51	49	34	47	32	23	23	173	140	18.0
<b>From Group II varieties</b>											
Mamoriaka			32	0	62	0	22	1	117	1	99.1
Huan-sen-goo			32	1	63	1	21	0	116	2	96.4
Dissi Hatif (DH-2)			32	0	63	1	22	2	117	3	97.5
Carreon			31	0	62	3	22	0	115	3	97.4
Pah Leuad 29-8-11			31	1	61	0	22	2	114	3	97.3
Ram Tulasi			32	0	60	1	22	2	114	3	97.3
C46-15			30	1	61	1	23	1	112	3	97.3
Ram Tulasi (sel)			33	0	60	2	19	1	112	3	97.3
Ca 435/6/5/1			31	1	62	2	22	1	115	4	97.4
DNJ-60			29	1	62	1	22	2	113	4	96.5



44 monoconidial cultures from another lesion were differentiated into eight races. They also found that 25 monoconidial subcultures each from two single conidial pure cultures were differentiated into 9 and 10 races (Table 2).

Giatgong and Frederiksen (1969) found that 20 monoconidial lines were separated into four to seven races by testing on four varieties. In three consecutive generations, the monoconidial lines continued to change into new races in each generation.

These studies indicated that the conventional method of race identification by use of only one conidium presents only a partial and transitory picture of pathogenicity.

It is known that plant pathogenic fungi do change, but once changed, the new races are generally stable. *P. oryzae*, as shown in the above study, changes in each generation, and if more varieties were used as differentials, each of the conidia would have different pathogenicity. This was shown early in our study on races of *P. oryzae* (Bandong and Ou 1966). Of the 50 monoconidial isolates none had the same pathogenicity on the 110 varieties selected as candidates for differentials. *P. oryzae* seems to have a new dimension of variability.

The cause of such great variability is still uncertain. Suzuki (1965) reported that the conidia, appresoria and mycelial cells are in a "persistent" heterokaryotic state, that anastomosis is common, and that each of these cells contain three to seven nuclei. Yamasaki and Nilzeki (1965) however, reported the contrary, that most of the cells are uninucleate, though in certain strains 13 to 20 percent of the cells were multinucleate, containing from two to six nuclei. Anastomosis and migration of the nucleus were observed, and nuclei had apparently fused to form diploids. Other studies also showed that most cells are uninucleate (Wu 1967, Giatgong and Frederiksen, 1969), Kiyosawa (1967) reported that the frequency of spore mutation from avirulent to virulent to a variety may be as high as 26.3 percent in certain strains. A cytological study of Giatgong and Frederiksen (1969) concluded that variation could have derived from mutation, sexual hybridization, the parasexual cycle or heterokaryosis. The perfect stage of *P. oryzae* is not known nor is any evidence of any of these genetic changes known.

#### **Variability in Relation to Varietal Resistance**

As a result of constant change in pathogenicity, numerous races are present in the field and the blast nursery. This was substantiated by isolating 363 single conidia from a blast nursery from which 60 races were identified as mentioned above (Quamaruzzaman and Ou 1970).

**Table 3. Lesion development on varieties Tetep, Carreon, and Khao-teh-haeng 17 inoculated at the same time with isolates and reisolates of *P. oryzae* from Tetep.**

Isolates and reisolates from Tetep	Average number of lesions per seedling <sup>a</sup>		
	Carreon	Tetep	KHT
FR-1	0	0.0	63.4
FR-4A10	0	14.1	53.3
FR-13-141	0	0.1	67.3
FR-13-1a	0	0.3	42.5
FR-28	0	0.0	39.2
FR-30A2	0	0.4	20.3
-30A3	0	2.5	26.0
-30A5	0	5.6	44.5
-30A6	0	2.6	43.0
-30A7	0	2.1	61.4
-30A8	0	0.2	62.8
-30A42	0	0.4	15.2
-30A43	0	0.0	15.7
-30A44	0	0.9	17.0
-30A45	0	0.5	14.6
-30-1a	0	0.1	39.4
-30B1	0	0.8	14.1
-30B2	0	0.6	29.7
-30B3	0	0.0	14.6
FR-31	0	0.1	58.3
FR-35-1b	0	0.7	38.6
FR-50-1b	0	0.3	30.3
FR-52-1b	0	0.0	24.1
FR-54-1b	0	0.4	17.7
FR-56	0	4.8	55.6
-56A2	0	2.5	15.6
-56A9	0	5.0	16.3
FR-57	0	0.3	35.5
-57-1b	0	0.2	17.2
FR-59 A1	0	8.1	34.5
-59-1b	0	0.2	20.3
FR-78	0	0.8	22.4
-78A4(1)	0	3.7	44.0
-78A4(2)	0	3.8	19.9
-78-1a	0	3.1	21.5
-78-1b	0	1.3	9.7
-78-16	-	16.1	44.6
Average	0	2.2	32.7

<sup>a</sup> Counted from 20 plants.

The variability of *P. oryzae* also extends the range of the host varieties. The group of varieties with a broad spectrum of resistance identified by the International blast nurseries (Ou et al. 1970), while usually free from infection, occasionally showed susceptible reaction (Table 1). According to conventional thought new races have developed and the resistant varieties will break down when the population of the new races increases.

These varieties have also been tested in our blast nurseries over 40 times during the last 8 years. Under epiphytotic conditions a few large susceptible type lesions occasionally appeared. This gives us the opportunity to study the fungus races and to find whether these varieties will break down or maintain their level of resistance by producing only a few lesions.

The possible reasons these varieties produce few lesions in the blast nurseries are that conidia population of the pathogenic races specific to these varieties are low, and that there is a genetically controlled interaction between the fungus and the host variety. To determine this, the pathogenic races on Tetep, one of the most resistant varieties, were isolated, cultured and inoculated back to Tetep and another resistant variety, Carreon. The races were also re-isolated and inoculated. A very susceptible variety, Khao-teh Haeng 17 (KTH), was used as control.

The results of 37 such inoculations show that Tetep consistently produced a few susceptible type lesions while there were many on KTH (Table 3). The average number of lesions per seedling on Tetep was 2.2 and on KTH, 32.7. One inoculation produced 14.1 lesions on Tetep and another produced 16.1 lesions. Several isolates produced no lesions on Tetep. These results indicated that the few lesions produced on Tetep are not due to the low conidial population of the pathogenic races inoculated.

The small number of lesions on Tetep and the large number on KTH in the same inoculations suggest that many of the conidia failed to infect Tetep even though the fungus was isolated from Tetep. To determine this, many single-conidium subcultures were made from six of the pathogenic isolates from Tetep: 160 from isolate FR-1, 48 from FR-1-138 (single-conidial reisolate from FR-1), 45 from FR-78, 100 from FR-78-16 (the most pathogenic single-conidial reisolate from FR-78; it produced 16.1 lesions on Tetep), 52 from FR-79 and 45 from FR-80. All these subcultures were inoculated in Tetep, Carreon, the 12 Philippine differential varieties (Bandong and Ou 1966) and eight International differentials (Atkins et al. 1967). The number of susceptible and intermediate types of lesions on Tetep, Carreon and KTH were counted in each inoculation. Lesions of intermediate type were not included in the data as they produce relatively small numbers of conidia and are unimportant epidemiologically.

**Table 4. Pathogenic races of *P. oryzae* from monoconidial subcultures of six isolates from Tetep; based on the reaction of Philippine differential varieties.**

Parental isolates and race	Philippine races (no. isolates)	Total no. of races	Total no. of monoconidial subcultures
FR-1 (P8)	P 8(61) P18 (1)P32 (3) P64 (2) P118(1) P149(1) P12(29) P19 (2)P38 (4) P70 (1) P125(1) P150(2) P15 (1) P21 (1)P50(14) P80 (1) P141(4) P153(1) P16 (1) P25 (1)P52 (4) P81(13) P142(2) P17 (3) P30 (2)P62 (1) P87 (2) P143(1)	28	160
FR-1-138 (P81)	P 8(19) P17 (6)P52 (1) P118(1) P 9 (1) P36 (2)P62 (1) P141(1) P12(10) P50 (4)P98 (1) P175(1)	12	48
FR-78	P87 (2) P92(33)P120(1) P131(2) P89 (1) P112(4)P123(1) P166(1)	8	45
FR-78-16 (P-92)	P 8 (1) P 26(1)P77(1) P148(1) P179(1) P189(1) P15 (1) P 28(3)P87(11) P152(1) P180(1) P190(1) P16 (1) P 33(1)P89(2) P153(1) P182(1) P191(1) P17 (1) P 35(2)P90(1) P166(2) P183(1) P192(1) P18 (2) P 46(1)P92(14) P167(1) P184(1) P193(1) P19 (3) P 48(1)P100(1) P172(1) P185(1) P194(1) P20 (1) P 52(8)P102(4) P173(3) P186(1) P21 (1) P 66(5)P114(1) P174(1) P187(1) P25 (1) P 70(1)P120(2) P178(2) P188(1)	51	100
FR-79	P 8(10) P 35(4)P102(5) P168(4) P12 (1) P 81(4)P120(2) P169(1) P16 (1) P 90(2)P165(1) P170(1) P17 (5) P 92(4)P166(1) P177(1) P18 (2) P100(2)P167(1)	19	52
FR-80	P 8(37) P 17(2)P 52(1) P117(1) P12 (2) P 50(1)P 62(1)	7	45

By the Philippine differentials, the 160 single-conidial subcultures of FR-1 were separated into 28 pathogenic races; 48 of FR-1-138 into 12 races, 45 of FR-78 into 8 races, 100 of FR-78-16 into 51 races, 52 of FR-79 into 19 races, and 45 of FR-80 into 7 races (Table 4). Altogether, 78 different races were identified among the 450 single-conidial subcultures of the six isolates. These races differ greatly in their pathogenicity. Some infect only one or two varieties, others infect 11 or all the 12 differential varieties. Based upon the number of the Philippine differential varieties infected by these races, they were grouped as shown in Table 5. The distribution of subcultures among the races developed vary. Usually a few races have a larger number of subcultures.

The numbers of races separated by the international differentials and combination of the two sets are shown in Table 6. When more differentials are used, more races are differentiated.

The number of races and number of subcultures that infect Tetep, Carreon and KTH, and the number of susceptible type of lesions on these three

Table 5. Pathogenic races derived from isolates FR-1, FR-1-138, FR-78, FR-78-16, FR-79, and FR-80 grouped by the number of the Philippine differential varieties infected.

No. of differential varieties infected	FR-1		FR-1-138		FR-78		FR-78-16		FR-79		FR-80	
	No. Races	No. Subc.										
1							1	1				
2	1	1					1	1				
3	1	1										
4	4	4					6	6				
5	7	15	2	2			7	9	5	12		
6	5	22	5	11			5	7	1	1		
7	1	61	1	19			7	10	5	17	2	3
8	3	34	3	12	2	3	6	12	2	11	1	27
9	3	17	1	4	3	6	6	13	2	2	2	3
10					1	33	7	11	3	5	2	2
11	2	3			2	3	3	17	1	4		
12	1	2			2	3	2	13				
Total no. of races	28		12		8		51		19		7	
Total no. of subcultures		100		48		45		100		52		45

varieties are shown in Table 7. Many of the races and many of the subcultures originally isolated from Tetep failed to infect Tetep. The numbers of lesions on Tetep and Carreon were consistently and significantly smaller than that on KTH. Even if the races or the subcultures are considered pathogenic, the numbers of lesions produced on Tetep and Carreon are small.

Table 6. Number of pathogenic races differentiated from the single conidial subcultures of seven single conidial parental isolates of *Pyricularia oryzae* by three different sets of differential varieties.

Isolate and total no. of subcultures	By 8 international differential varieties	By 12 Philippine differential varieties	By combination of two sets and Tetep & Carreon (20 varieties)
FR-1 (160)	20	28	59
FR-1-138 (48)	6	12	22
FR-78 (45)	3	8	11
FR-78-16 (100)	23	51	63
FR-79 (52)	25	19	37
FR-80 (45)	3	7	12

Table 7. Qualitative (pathogenic races) and quantitative (no. susceptible lesions) pathogenicity of monoconidial subcultures of isolates FR-1, FR-1-138, FR-78; FR-78-16, FR-79, FR-80, isolated from Tetep when inoculated on Tetep, Carreon, and Khao-teh-haeng 17 (KTH).

Isolate	Variety	No. of races		No. subcultures		Ave. no. lesion per plant by all subcultures	Ave. no. lesion per plant by pathogenic subcultures
		Total	Pathogenic	Total	Pathogenic		
FR-1	Carreon	28	11	160	60	0.3	1.1
	Tetep	28	5	160	19	0.1	1.4
	KTH	28	28	160	160	33.9	33.9
FR-1-138	Carreon	12	6	48	15	0.8	2.8
	Tetep	12	1	48	3	0.1	6.2
	KTH	12	12	48	48	56.6	56.6
FR-78	Tetep	8	7	45	44	5.2	6.1
	KTH	8	8	45	45	22.6	22.6
FR-78-16	Carreon	51	1	100	1	0.1	8.7
	Tetep	51	17	100	43	3.6	8.9
	KTH	51	48	100	97	17.4	18.0
FR-79	Carreon	19	1	52	1	0.01	0.5
	Tetep	19	11	52	17	0.7	2.5
	KTH	19	19	52	52	46.3	46.3
FR-80	Carreon	7	3	45	7	0.6	4.1
	Tetep	7	1	45	1	0.2	7.9
	KTH	7	7	45	45	47.9	47.9
All isolates	Carreon					0.3	1.7
	Tetep					1.5	6.6
	KTH					34.6	34.8

Tetep and Carreon were planted in several blocks each of 10 rows in our blast nursery and susceptible variety, Tjeremas, was planted as control after every two rows to either Tetep or Carreon. Before the appearance of any lesion on young seedlings they were inoculated with an isolate from Tetep, FR-78-16. The number of lesions on 100 seedlings were counted every other day, about a week after inoculation. Tetep and Carreon showed only a small number of lesions in comparison with Tjeremas (Fig. 2). The results agree very well with greenhouse inoculations.

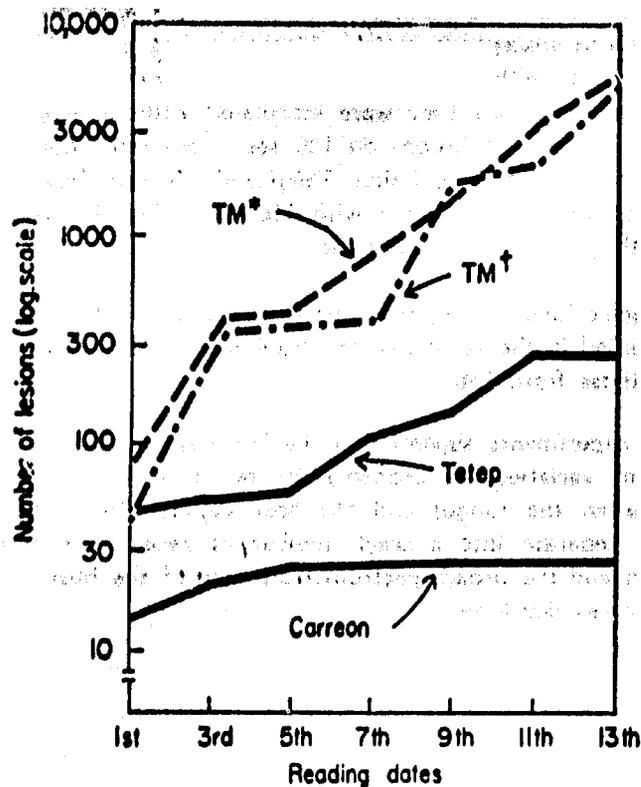
The pathogenic fungus races from the few lesions on other resistant varieties are being studied in the same manner. Preliminary results show they behaved similarly to those from Tetep.

The above experiments suggest that the few lesions produced on Tetep and other resistant varieties are probably the result of a genetically controlled reaction between the fungus and the host variety. The original pathogenic fungus races separate into a great number of races in each generation of multiplication and the broad-spectrum resistance of the host operates against most of the races developed.

### Discussion

The above studies showed the extreme variability in pathogenicity of *Pyricularia oryzae*. Many races are present in nature and are produced from single lesions and single conidial cultures. They vary greatly in pathogenicity. This phenomenon is unusual, but not unique. In studying the variability in *Fusarium*, Snyder (1933), said, "All evidence from studies upon variation in fungi illustrate the hazard of using single-spore culture in the study of a species exhibiting variation, unless large numbers of monoconidial cultures are employed". Moreover, "...within a given monoconidial line it was possible to assemble, through the phenomenon of dissociation, a group of cultures almost representative of the range in colony types and virulence exhibited by the entire group of strains. Thus a monoconidial parent has been shown in certain instances by its dissociates to possess the potentialities of most of the type of colony character and virulence of the 15 strains studied." Snyder and Hansen (1954) stated, "Although the principle (variability of fungi) is recognized and accepted, the significance of variability is not yet fully appreciated, nor is it widely utilized." It is well illustrated by the pathogenic variability of *P. oryzae*. Such variability may also exist in some other fungus pathogens.

Stakman (1954), after the outbreak of race 15B of *Puccinia graminis tritici* wrote, "Concepts regarding the dynamics of rust must be broadened and



**Figure 2.** Number of lesions per 100 seedlings on resistant varieties (Tetep, Carreon) and on susceptible variety Tjeremas adjacent to Tetep (TM\*) and adjacent to Carreon (TM†) inoculated with isolate (FR-78-16) from Tetep in blast nursery. (Ou et al 1971)

deepened by extensive and intensive investigation." In addition, "The number of biotypes of *P. graminis tritici* appears to be comparable to *Ustilago zaeae* and *Helminthosporium sativum*. At least 15,000 biotypes of *U. zaeae* and at least 1,000 of *H. sativum* are present in Minnesota and there is no visible limit to numbers."

The great variability of *P. oryzae* enables the fungus to extend the range of host varieties. On the other hand, a particular pathogenic race cannot build up rapidly, it becomes separated into many races. The population of original races present in the progeny is small or nil, as indicated by some isolates (Table 3). Since the varieties possess a broad spectrum of resistance, most of the races developed cannot infect the varieties. Thus at most a few lesions developed. These varieties therefore not broken down by the presence of new pathogenic races.

Tetep and other varieties seem to be stable in resistance to blast but they are not "race non-specific" nor "horizontal" as defined by Robinson (1969). They react differently to different races. They are resistant to most races, but susceptible to a few, at least in a qualitative sense, though they have few lesions. Such a pathogen-host relationship resulting in a stable resistance is seemingly new.

The level of resistance in varieties such as Tetep depends on how broad the spectrum of resistance is. The more races the varieties are able to resist, the fewer lesions that develop. As shown in Table 3 Carreon is resistant to the isolates from Tetep. It may be possible to combine the resistance of such varieties to further broaden the spectrum of resistance. The degree of resistance to blast may be measured by the percentage of all races, potential or in existence, to which a variety is resistant.

Sakuri and Toriyama (1967) and Yunoku et al. (1970) reported varieties St 1 and Chugoku 31 have "field resistance." By greenhouse and blast nursery tests, both varieties produced a small number of lesions. It may be speculated that a similar genetic mechanism, as described above, is involved, though they did not study the fungus in detail.

The genetics of resistance in Tetep and other varieties are not known. It would be most interesting to learn whether a few strong genes or many genes are involved. Because of the lack of genetic information extensive and intensive tests must be used to select genotype with broad-spectrum resistance in breeding programs.

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**Recent progress of studies  
on horizontal resistance in rice breeding  
for blast resistance in Japan**

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Since the establishment of scientific rice breeding in Japan, great efforts have been made to develop the varieties possessing resistance to blast disease caused by *Pyricularia oryzae* Cav. As a result, some outstanding blast-resistant varieties have been developed. These blast resistant varieties have greatly contributed to the stabilization of rice production by controlling an epidemic of blast in Japan.

The blast resistant paddy rice varieties which were developed hitherto in Japan were classified into four groups: (1) varieties developed from crosses among Japanese domestic varieties, (2) varieties derived from the cross with the upland rice variety, (3) varieties possessing the resistance gene incorporated from Chinese japonicas and (4) varieties possessing the resistance gene or genes in the indica varieties. Of these, the varieties belonging to the third and fourth groups had been generally considered to be highly resistant to blast until they were unexpectedly affected by blast more severely than Japanese domestic varieties. Breakdown of high resistance from alien varieties occurred within three to five years after the release of the varieties possessing resistance of this kind. The damage on the highly resistant varieties was recognized to be due to the selective propagation of newly developed pathotypes to the resistance

gene or genes from alien varieties. Therefore, utilization of horizontal resistance to blast has been emphasized in the rice breeding program in Japan.

Horizontal resistance has been called either field resistance or generalized resistance. In the present report the term "field resistance" will be employed in place of the term "horizontal resistance" because resistance showing horizontal reaction in the strict sense as defined by van der Plank (1963) was not observed in any rice varieties by Japanese investigators to date. Rice breeders in Japan, therefore, classified blast resistance into two categories: "true resistance" and "field resistance". In this sense, true resistance is specific and qualitative resistance characterized by hypersensitivity to a pathogen. On the other hand, field resistance is recognized to be remainders of resistance other than true resistance. In order to clarify the essential nature of field resistance, the main efforts of investigation in Japan have been made on the basis of information on true resistance using a process of elimination.

The present report will cover recent progress of studies on field resistance to blast together with some important investigations on true resistance in rice breeding in Japan.

#### **Existence of Field Resistance to Blast**

Varieties possessing the true resistance gene or genes exhibit a high resistance due to a hypersensitive reaction to the pathogen when the varieties are exposed to the fungus races without virulence to true resistance. In this case, field resistance of the varieties cannot be exhibited because true resistance plays epistatically over field resistance. Field resistance, therefore, is distinguished under such conditions where the fungus races virulent to all the true resistance gene or genes of given varieties are prevalent.

Information on the true resistance genes and on the virulence of fungus races were materially necessary to investigate field resistance to blast, because varietal difference on field resistance can be discriminated by the virulent fungus races only on the same bases of the true resistance genotype. In Japan, genotypes for true resistance to blast were estimated by the reaction pattern to an injection testing method using the seven standard fungus isolates selected by Ymasaki and Kiyosawa (1966). By the injection method and some additional means, eleven genes for true resistance to blast were found by Kiyosawa and his co-workers (1971). Those are  $Pi-a$ ,  $Pi-l$ ,  $Pi-ta$ ,  $Pi-ta^2$ ,  $Pi-z$ ,  $Pi-zt$ ,  $Pi-k$ ,  $Pi-k^*$ ,  $Pi-kp$ ,  $Pi-kh$  and  $Pi-m$ . By the reaction pattern to the seven standard isolates in the injection test, Japanese rice varieties, including domestic and newly bred varieties with alien blast resistance genes, are classified into

twelve reaction types: Shin 2, Aichi-asahi, Kanto 51, Ishikari-shiroke, Yashiro-mochi, Pi 4, Fuku-nishiki, Toride 1, To-to, Shinsetsu, Shimokita and Zenith (Kiyosawa 1967, Yokoo and Kiyosawa 1970).

For dividing reaction types in more detail than the above system, some other fungus strains can be used as shown in Table 1. Thereby, 24 varietal groups can be discriminated according to the reaction pattern (Table 1).

In the testing field of the blast nurseries it has been observed that resistance to blast is different among the varieties possessing even the same genotype for true resistance. The varietal difference within the same genotype for true resistance is considered to be caused by the difference in field resistance of given varieties (Hirano et al. 1967, Hirano and Matsumoto 1971, Asaga and Yoshimura 1969).

#### Evaluation of Degree of Field Resistance

If the varieties lack field resistance, they are severely affected by the virulent fungus races against the true resistance gene or genes. Field resistance of the varieties is estimated by the degree of damage in the field where the virulent fungus races are prevalent. In general, constitution of the fungus races varied with year, location and season (Goto et al. 1964, Yamada and Iwano 1970). For example in Fukuyama, Hiroshima Prefecture, strains belonging to the N race group propagated in the early season of the rice growing period, then the strains belonging to the C race group followed (Matsumoto and Okamoto 1963, Okamoto and Matsumoto 1964, Ezuka et al. 1969b). This phenomenon of race change was repeated every year. Major fungus strains of the N race group collected in the Fukuyama field probably belonged to the N-2 race, because they showed virulence to the  $PI-a$  gene of the Aichi-asahi type but did not attack the gene  $PI-l$  of Ishikari-shiroke type. The strains of the C race group which followed the N race group were estimated to belong to the C-8 race, because they were virulent to the  $PI-k$  and  $PI-a$  genes of To-to type and avirulent to  $PI-l$  gene (Ezuka et al. 1969b).

In Soma, Fukushima Prefecture, however, the composition of fungus races was distinctly different from that in Fukuyama. The gene  $PI-k$  did not show any resistant reaction from the early season of rice growth. The gene  $PI-a$  exhibited moderate resistance, because the major fungus strains in Soma were virulent to the gene  $PI-k$  but were avirulent to the gene  $PI-a$ .

Resistance in the field, therefore, did not directly indicate field resistance itself because of the complex reaction against races. If field resistance of

varieties is evaluated only by the observed value in the testing field, field resistance of the varieties possessing **PI—l** gene may be ranked at a high level of resistance and field resistance of the varieties possessing **PI—k** gene may be classified as high grade when observed in the early season of the rice growth in Fukuyama. For the same reason, field resistance of the varieties with **PI—a** gene may be graded at higher classes than those without the **PI—a** gene in Soma. Of course, there were a few pathogens virulent to **PI—l** and **PI—a** genes in Fukuyama and Soma, respectively. These facts indicate that a comparison of field resistance is worthwhile only when within the varieties of the same genotype for true resistance.

Some attempts were made to develop a testing method to evaluate field resistance of rice varieties under different conditions: paddy field, upland nursery and the greenhouse.

#### **Evaluation of Field Resistance in the Paddy Field**

Ezuka et. al (1969b) made an attempt to evaluate field resistance of rice varieties in the paddy field at two locations. Some varieties which were representative of Shin 2 (**PI—l**), Aichi-asahi (**PI—a**), Kanto 51 (**PI—k**), To-to (**PI—a**, **PI—k**) and Ishikari-shiroke (**PI—l**) types were grown in the paddy fields of different localities, and their levels of field resistance were evaluated by the number of susceptible lesions per hill. Although the outbreak of blast in the varieties with the **PI—k** gene was delayed as compared with that in the varieties without the **PI—k** gene, marked differences among varieties within the same genotypic group were observed as shown in Fig. 1. In this experiment it was pointed out that the true resistance gene **PI—a** had no influence on the degree of damage probably due to prevalence of the fungus races virulent to the **PI—a** gene. For comparison of field resistance of given varieties, therefore, the varieties belonging to Shin 2 type and Aichi-asahi type, and those belonging to Kanto 51 type and To-to type were lumped together as the same group, respectively.

The results indicated that Moko-ine, Norin 8, Norin 17, Norin 18 and Jukkoku showed a low level of field resistance, especially Moko-ine which showed the lowest. Ginga, Norin 22, Homare-nishiki, Fujiminori and Shuho showed a fairly high level, and St 1 showed the highest of all. Among the varieties with the **PI—k** gene, Kusabue and Yuukara showed a low level of field resistance, but Chugoku 31 showed a much higher level than the others. Since the varieties of Ishikari-shiroke type showed only a small number of susceptible lesions because of minor races possessing virulence to the **PI—l** gene, the varietal difference within this type was not recognized.

Table 1. Reaction of varietal group to fungus strains

Inoculation method	Injection method								Spray method		Genotype estimated
Fungus strain	P-2b	Ken 53-33	Ina 72	Hoku 1	Ken 54-20	Ken 54-04	Ina 168	Ina 168	Ina 168	Ken 65-20	
Fungus race											
Reaction type	T-2	T-1	C-3	N-1	N-2	N-3	N-4	$\frac{+k^*}{-k^*}$	$\frac{+k^*m}{-k^*m}$	$-z^1$	C-8 C-6
Shin 2 type	S	S	S	S	S	Ms	S			S	* *, (?)
	S	S	S	S	S	Ms	S			R	
Aichi-asahi type	S	S	R	S	S	S	R			S	Pi-a Pi-a (?)
	S	S	R	S	S	S	R			R	
Ishikari-shiroke type	M	S	M	S	MS	MR	M			S	Pi-i Pi-i (?)
	M	S	M	S	MS	MR	M			R	
Shinetsu type	M	S	R	S	MS	MR	R			S	Pi-a, Pi-i Pi-a, Pi-i (?)
	M	S	R	S	MS	MR	R			R	
Kanto 51 type	MR	S	S	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	S	S	S	Pi-k
	MR	S	S	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>			R	Pi-k, Pi-i
	MR	S	S	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	M	S	S	Pi-k, Pi-m
	MR	S	S	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>			R	Pi-k, Pi-i, Pi-m
To-to type	MR	S	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	S	S	S	Pi-a, Pi-k
	MR	S	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>			R	Pi-a, Pi-k, Pi-i
	MR	S	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	M	S	S	Pi-a, Pi-k, Pi-m
	MR	S	R	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>			R	Pi-a, Pi-k, Pi-i, Pi-m
Yashiro-mochi type	S	S	M	MR	M	MR	S				Pi-ta
Shimokita type	S	S	R	MR	M	MR	R				Pi-a, Pi-ta
Pi 4 type	S	M	R <sup>h</sup>	R	R	R	MR				Pi-ta <sup>2</sup>
	S	M	R <sup>h</sup>	R	R	R	R				Pi-a, Pi-ta <sup>2</sup>
Fukunishiki type	M	M	M	MR	M	MR	M				Pi-z
Zenith type	M	M	R	MR	M	MR	R				Pi-a, Pi-z
Toride 1 type	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>		S		Pi-z <sup>1</sup>
	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>	R <sup>h</sup>		R		Pi-a, Pi-z <sup>1</sup>

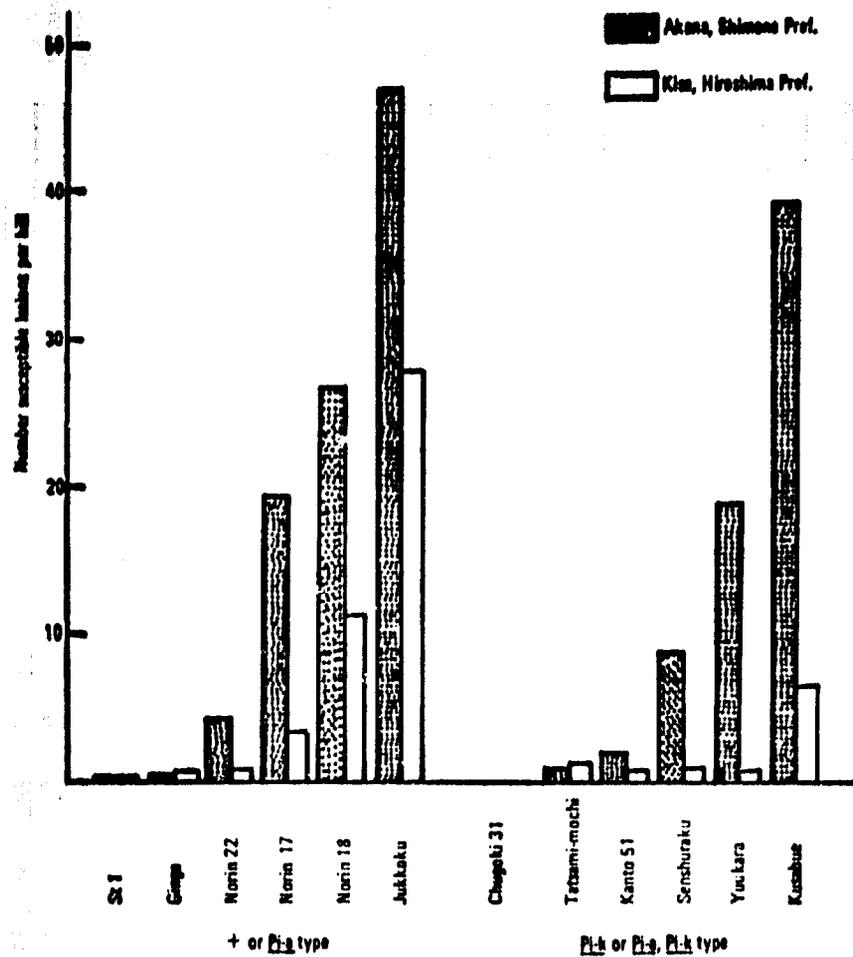


Figure 1. Varietal difference of field resistance to rice blast disease evaluated by the paddy field test.

It was apparent that there was a different level of field resistance among the varieties possessing the same true resistance, and that the severe damage of the so-called "highly resistant variety" such as Kusabue and Yuukara possessing the  $Pi-k$  gene from Chinese varieties was caused by the lack of field resistance and by the rapid propagation of virulent pathogens.

### Evaluation of Field Resistance in the Blast Nursery

Ezuka et. al. (1969b) tried to evaluate the level of field resistance and to determine a fluctuation of field resistance throughout the year in blast nursery beds. The tests were repeated six times from June to August. A progressive status of the severity of the disease in representative varieties is shown in Fig. 2. Since the Pi—a gene appeared to have no effect in this experiment, the varieties were placed in three groups: Shin 2 ( ) and Aichi-asahi (Pi—a) group, Kanto 51 (Pi-k) and To-to (Pi—a, Pi—k) group and Ishikari-shiroke (Pi—l) group. Records were taken on the amount of diseased leaf area as a percentage of total leaf area. Daily percentages of the diseased leaf area in given varieties were summed up in order to determine the approximate quantity of the integral calculus of the curved line in Fig. 3.

Each of the representative varieties was then given a disease rating index according to the formula devised by Sukurai and Toriyama (1967). The index is calculated from the ratio of susceptibility of a given variety to that of a standard variety which is the most susceptible variety chosen from the same true resistance genotype, or

$$\text{Disease rating index} = \frac{\text{Summed up value of given variety}}{\text{Maximum summed up value in the group}} \times 100.$$

The disease rating indices of the representative varieties are shown in Fig. 4.

The tendency of the varietal difference in the blast nurseries resembled considerably well those in the paddy field as shown in Fig. 5. However, the difference among the varieties possessing a lower level of field resistance were more evident in the paddy field than in the blast nurseries, but the differences among the varieties possessing a higher level of field resistance were recognized more readily in the blast nurseries than in the paddy fields. Blast nurseries, therefore, have been used by many rice breeders for evaluating field resistance of their breeding materials.

### Evaluation of Field Resistance by the Seedling Inoculation Method

Niizeki (1967), Sakurai and Toriyama (1967) and Yunoki et al. (1970a) tried to evaluate field resistance of rice varieties by a spray method for seedlings, and Kiyosawa (1966a, b, 1970b) used an injection method. When field resistance is evaluated in the paddy field or in the blast nursery, the degree of damage is influenced by weather conditions such as temperature and

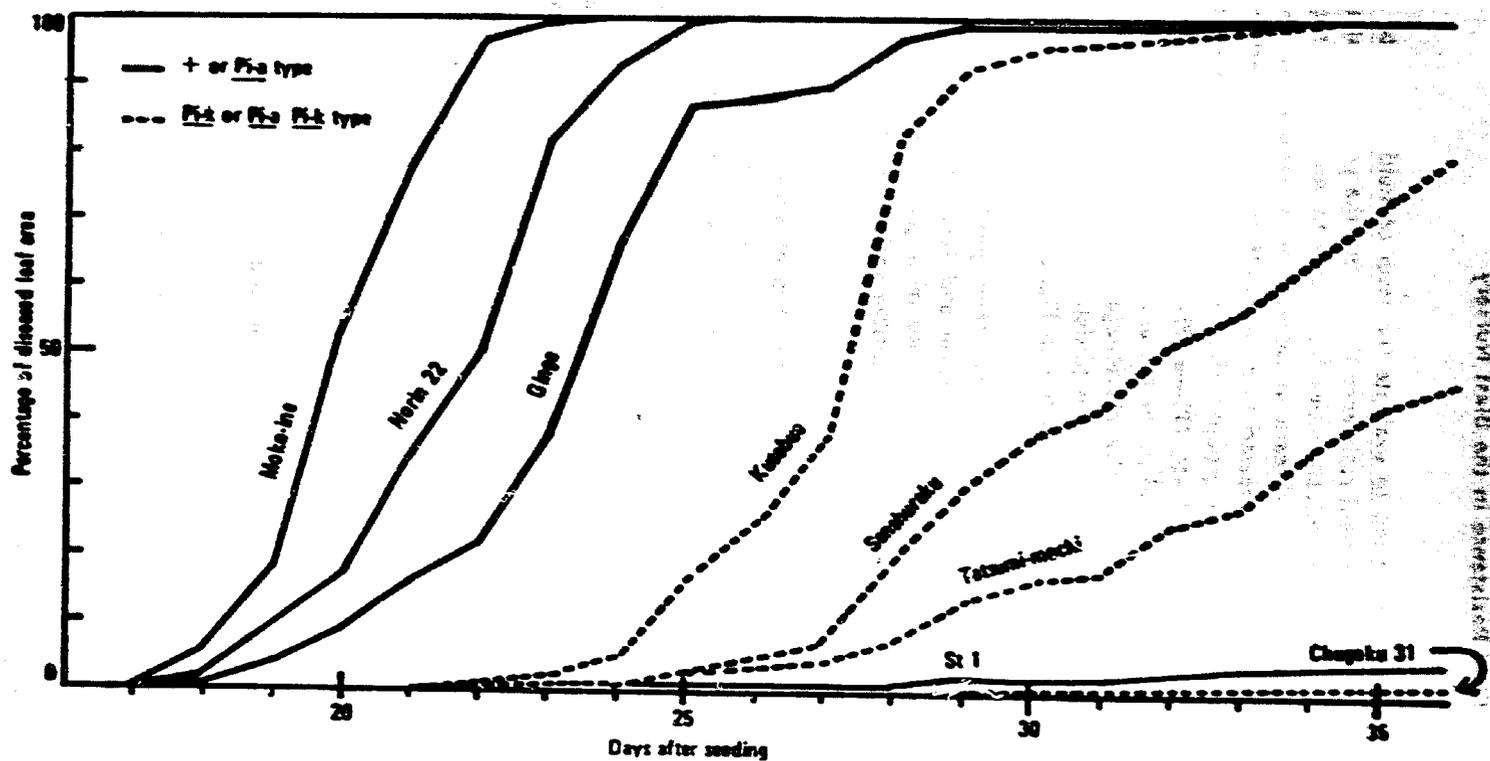


Figure 3. Progressive status of rice blast disease severity in the blast nursery test.

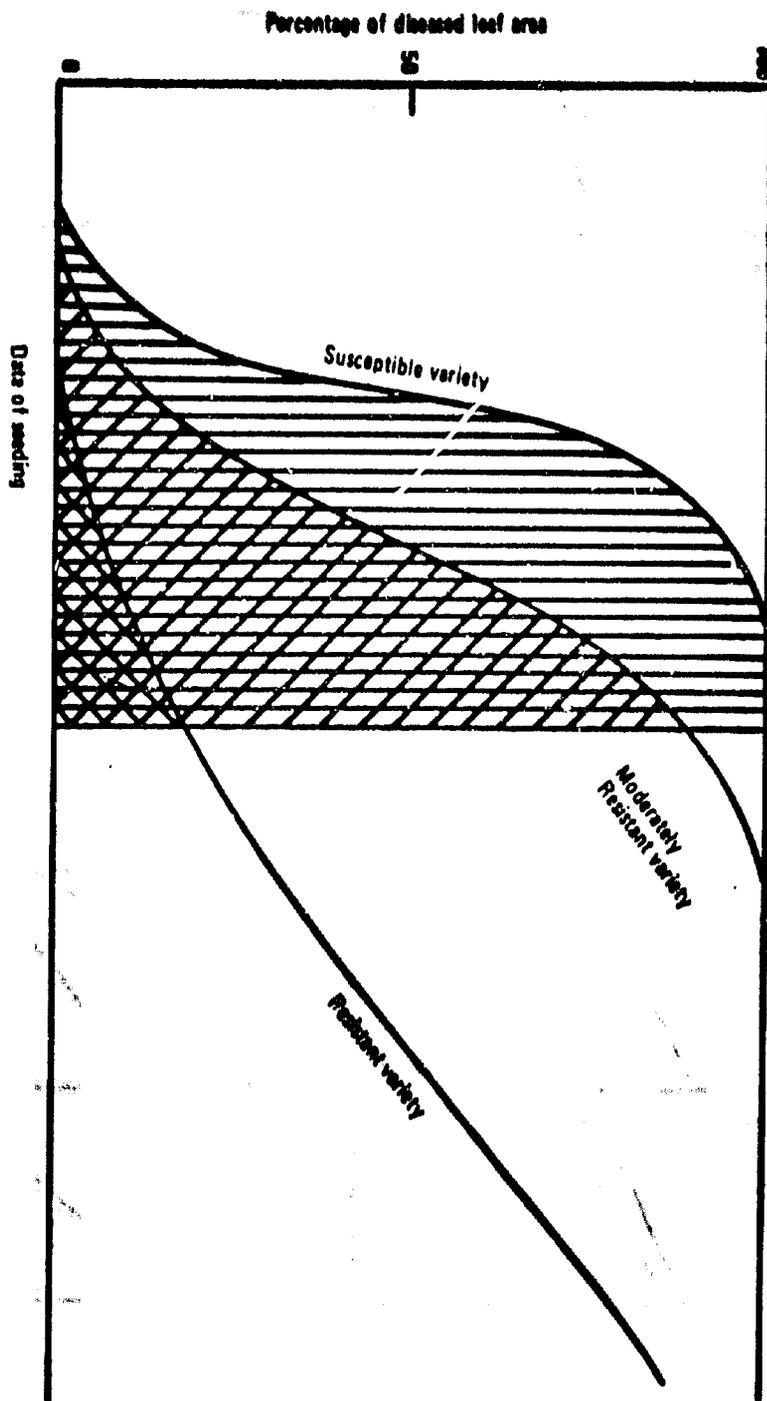


Figure 2. Model figure of daily progress of percentage of diseased leaf area on rice seedlings in the blast nursery bed.

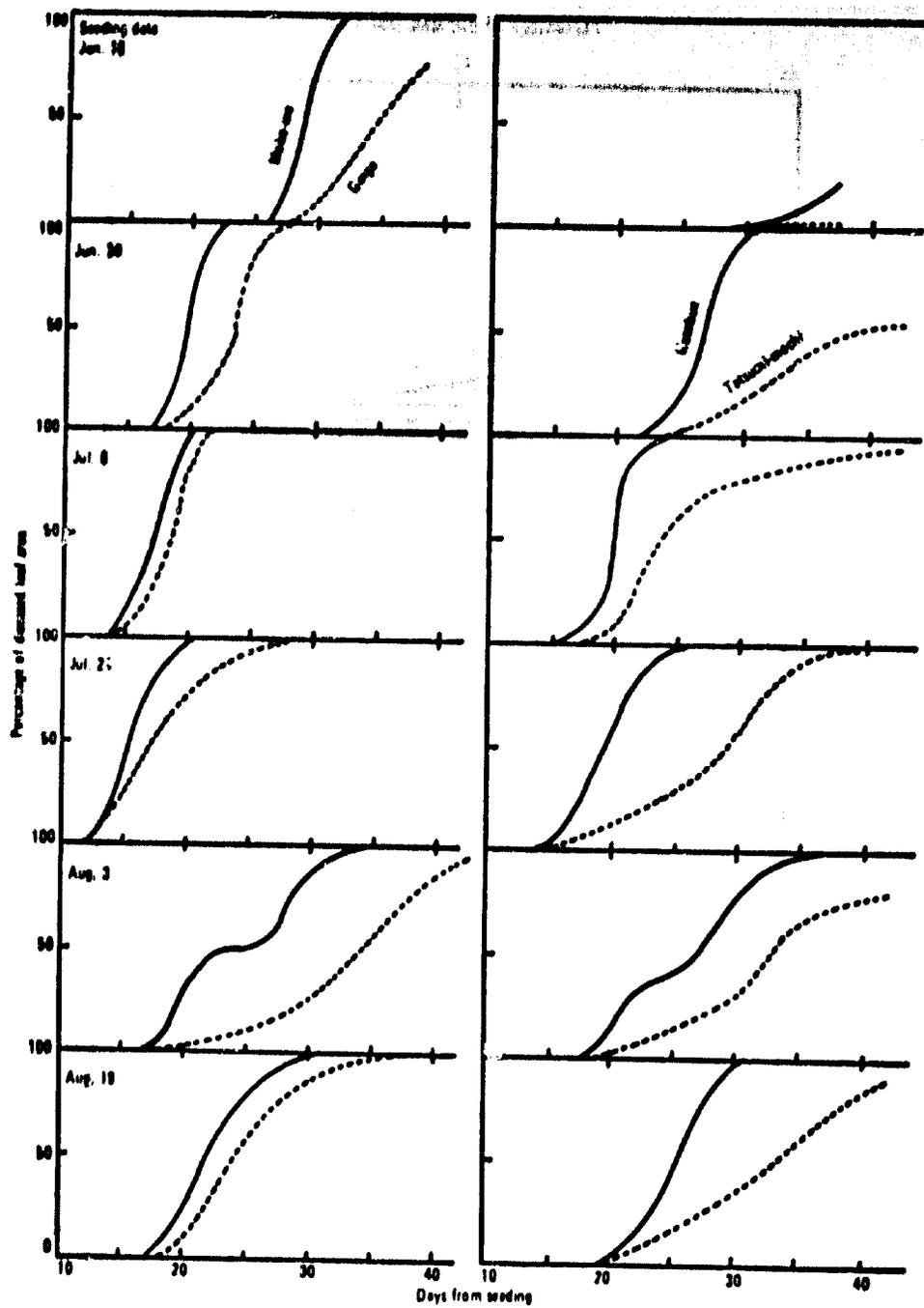


Figure 4. Progress of disease severity of representative rice varieties in the blast nursery bed at different sowing times.

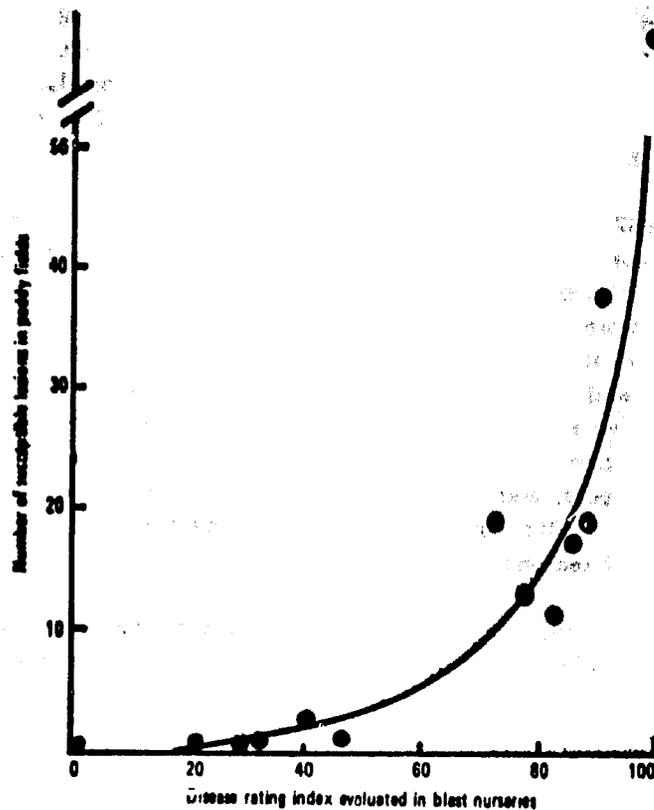


Figure 5. Relationship between field resistance to rice blast disease evaluated by paddy field test and by nursery test.

rainfall and by the constitution of fungus races existing in the testing field. Conversely, the seedling inoculation method can use the virulent fungus strains to the testing rice varieties and can be conducted under the artificially controlled condition. The testing result, therefore, is expected to be evaluated at the same level in every test. At the same time, it may be observed whether the evaluation of field resistance is influenced by the fungus strains or environmental factors.

Nitzeke (1967) conducted the seedling inoculation test in a greenhouse and the field test in a paddy field for three years repeatedly with the same varieties at the same place. He chose the testing paddy field where the most of the fungus strains belonged to C-1 race group. The testing varieties were transplanted late with much nitrogen fertilizer in order to bring out a natural epidemic of blast. The susceptible variety, Kusabue, in the testing field was badly stunted. For the seedling inoculation test, rice seedlings were grown in a nursery box in a greenhouse. The seedlings were inoculated by the spray method with suspension of the C-1 race fungus spore when the seedlings attained the fourth, fifth and sixth leaf-age.

The relationship between the grade of field resistance evaluated in the paddy field and by the seedling inoculation method is shown in Fig. 6 and 7. Field resistance evaluated at the sixth leaf-age showed a high correlation coefficient of 0.864, but that at the fourth leaf-age showed a low of 0.389, and that at the fifth leaf-age showed halfway between them.

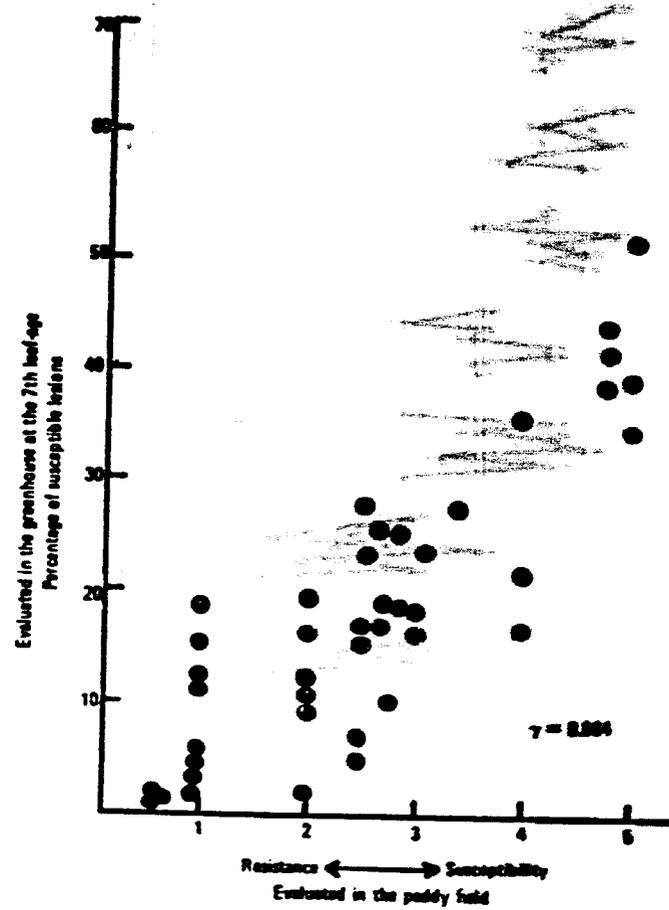
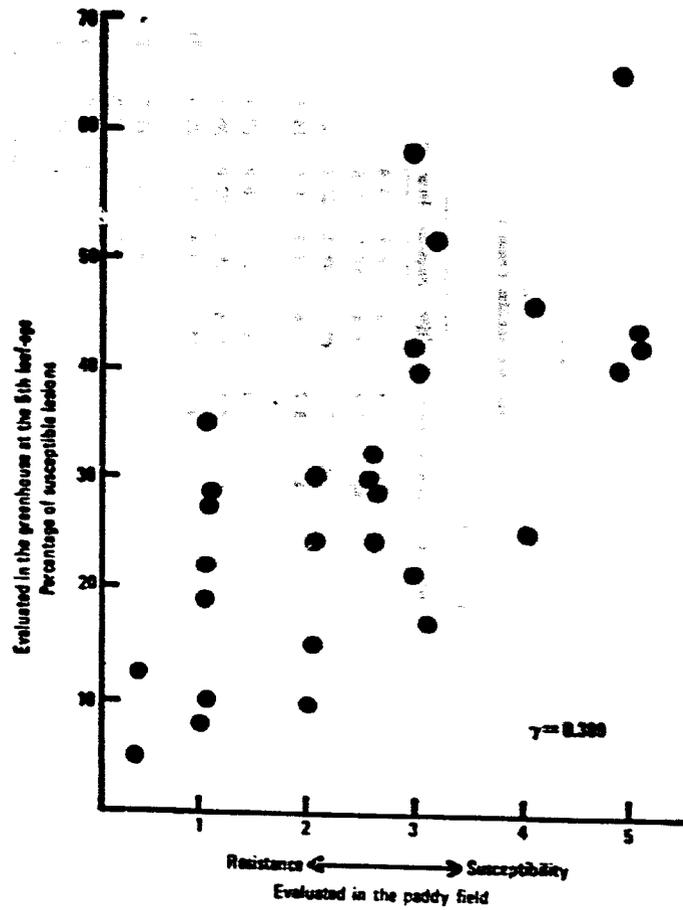
Nilizeki (1967) evaluated the degree of field resistance of some varieties which were representative of the most susceptible to the most resistant ones graded by the rice breeder's experiences for a long time. At the sixth leaf-age the seedlings were inoculated with eight fungus strains belonging to different races. As shown in Fig. 8, the resistant varieties such as Homare-nishiki and Yamabiko showed resistance, and the susceptible varieties such as Jukkoku and Asahi showed a high susceptibility in this test. The grade evaluated by the seedling inoculation method coincided well with the grade estimated by the breeder's experience, and the order of field resistance of the varieties was influenced little by the fungus strains, the amount of fertilizer application, temperature and seasons.

Nilizeki (1967) concluded that field resistance was effective for a number of fungus strains, and only one fungus strain virulent to all the testing materials was enough to evaluate the grade of field resistance when inoculated by spore suspension spray at the sixth leaf-age of varieties.

Sakurai and Toriyama (1967) and Yunoki et al. (1970a) also examined inoculating conditions such as the amount of nitrogen fertilizer, leaf-age of seedlings and the concentration of spore suspension. Three fungus strains belonging to the N-1, C-1 and T-1 races were employed to inoculate the seedlings of varieties which were representative of the different levels of field resistance. Field resistance was evaluated by the number of susceptible lesions per seedling.

The relationships between field resistance and amount of the nitrogen fertilizer application is shown in Table 2. A large amount of nitrogen application (10 g of ammonium sulphate per 35 x 27 x 12 cm pot) markedly increased the number of susceptible lesions on the seedlings inoculated, and the differences of the number of susceptible lesions among the varieties increased with the amount of nitrogen application.

As to the leaf-age, field resistance of the seedlings appeared to become higher with the increase of age as shown in Table 3, and the varietal difference seemed to increase with the age as shown in Fig. 9. For example, the ratio of the resistant variety, Homare-nishiki, to susceptible variety, Jukkoku, was about one and one-half in the number of susceptible lesions at the fifth leaf-age, but it became about three times at the seventh leaf-age.



77 Figures 6 and 7. Correlation between the degrees of field resistance evaluated at two leaf-age stages and those evaluated by paddy field test.

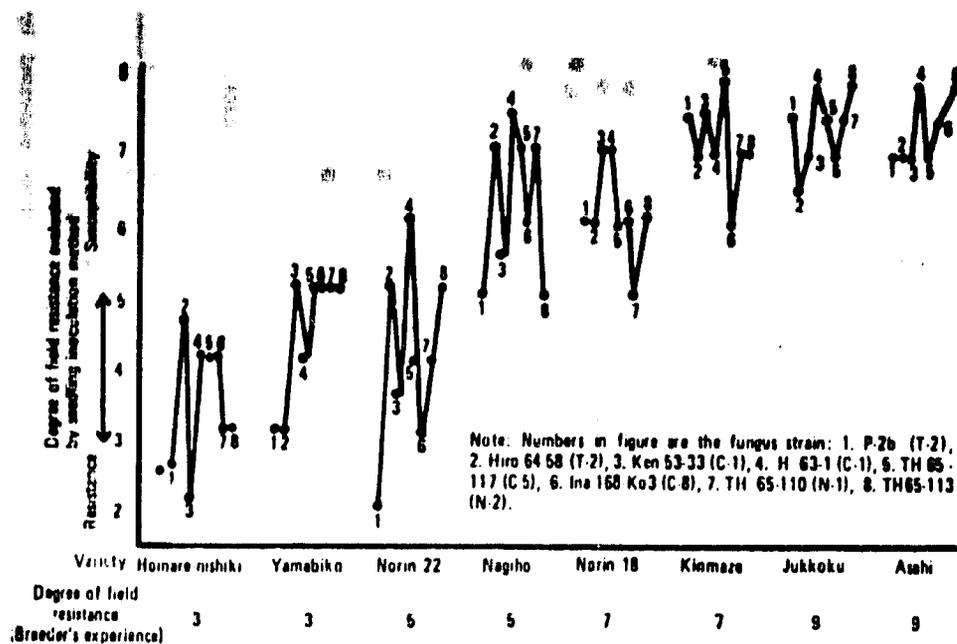


Figure 8. Field resistance to rice blast disease of eight rice varieties evaluated by spraying with eight fungus strains.

Table 2. Field resistance and amount of nitrogen fertilizer

Variety	Genotype for true resistance	Fungus strain (race)								
		Hoku 373 (N-1)			Ken 60-19 (C-1)			Ken 53-33 (T-1)		
		low	middle	excess	low	middle	excess	low	middle	excess
St 1	PI-f	2.8	5.8	9.2	3.9	5.3	6.4	4.4	6.8	10.7
Norin 8	+	19.6	25.6	35.4	16.2	21.8	30.7	17.5	25.6	40.5
Jukkoku	PI-a	22.0	28.7	33.0	17.5	20.2	38.4	23.4	29.0	39.7
Fujiminori	PI-a	7.9	10.6	24.2	8.7	17.6	18.9	6.9	14.8	21.2
Ishikari-shiroke	PI-l	3.8	14.7	23.0	10.4	18.2	21.3	5.6	17.3	19.5
Kusabue	PI-k	0	0	0	22.1	30.4	45.2	22.0	30.5	38.0
Senshuraku	PI-k	0	0	0	14.8	24.6	35.4	10.7	22.3	31.5
Mangetsu-mochi	PI-k	0	0	0	15.0	23.8	26.1	13.5	17.8	22.8
Chugoku 31	PI-f, PI-k	0	0	0	2.3	3.6	6.7	1.8	4.9	8.6
Yuukara	PI-a, PI-k	0	0	0	20.8	32.6	40.8	18.9	21.3	41.4
Kongo	PI-a, PI-k, PI-m	0	0	0	4.5	10.6	14.5	9.6	11.0	18.4

Note. Figures in the table mean the number of susceptible lesions.

Table 3. Leaf age and field resistance

Variety	Genotype for true resistance	Leaf age								
		5th			6th			7th		
		low	middle	excess	low	middle	excess	low	middle	excess
St 1	PI-f	9.8	14.3	14.8	6.3	5.5	10.7	3.9	3.5	7.4
Norin 8		36.6	47.5	55.1	19.6	24.8	35.4	18.9	29.6	33.4
Jukkoku	PI-a	48.4	60.9	63.4	18.7	33.3	46.8	16.1	34.7	45.0
Shuho	PI-a	32.4	33.8	49.8	15.4	20.5	28.3	13.7	18.1	23.7
Homare-nishiki	PI-a	29.6	40.3	42.4	10.6	17.4	24.5	11.4	20.4	15.0
Kasabue	PI-k	34.7	58.7	70.9	24.8	29.8	43.6	24.0	27.9	41.0
Senshuraku	PI-k	30.5	50.4	58.3	28.3	21.4	29.1	14.7	13.4	27.5
Mangetsu-mochi	PI-k	28.7	45.6	54.2	13.6	17.3	31.6	15.1	15.4	20.8
Chugoku 31	PI-f, PI-k	10.8	20.8	19.7	4.4	3.8	7.4	1.8	1.5	3.1
Kongo	PI-a, PI-k, PI-m	19.5	29.4	35.8	8.7	14.2	20.6	9.7	16.7	14.6

Note. Figures in the table mean the number of susceptible lesions.

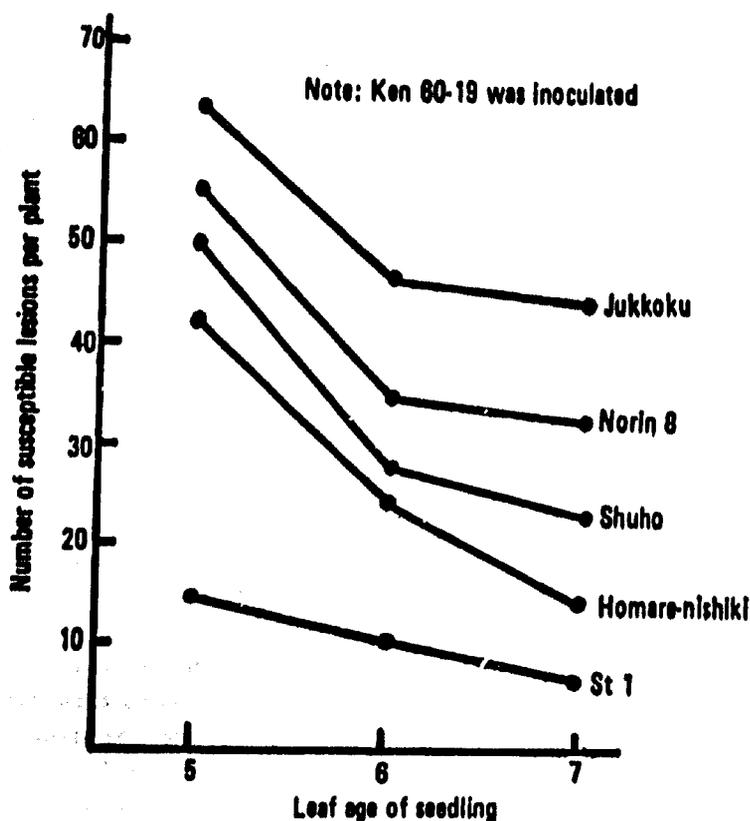


Figure 9. Relationship between the number of susceptible disease lesions and the leaf-age inoculated in five rice varieties.

The number of susceptible lesions per plant was also influenced by the concentration of spore suspension. When too dense or too thin a concentration of inoculum was used for seedling inoculation, it became difficult to determine the varietal difference of field resistance because the number of susceptible lesions was either too much or too little as shown in Fig. 10. The optimum concentration of spore suspension was determined to be 100,000 to 250,000 spores per 1 ml of water.

The order of field resistance of varieties in each test determined by the seedling inoculation method coincided well with that in the paddy field and the blast nursery bed. Accordingly, Sakurai and Toriyama (1967) proposed a testing method for seedling inoculation as follows: (1) inoculum is selected from the fungus strains possessing a pathogenicity to the varieties tested, (2) a large amount of nitrogen fertilizer is applied, (3) seedlings of the sixth to seventh leaf-age are inoculated, (4) the spore suspension for inoculum is concentrated at 100,000 to 250,000 spores per ml of water.

By this method, Yunoki et al. (1970a) evaluated field resistance of a number of varieties grown in Japan.

Kiyosawa (1966a) considered that field resistance would be evaluated by the injection method in a greenhouse when a weakly aggressive fungus strain was used for inoculation, and he examined his proposal by using the fungus strain Ken 54-04, which was chosen as an unspecifically and a weakly aggressive strain. At the 5.3 leaf-age of seedlings, two fungus strains including Ken 54-04 and the check strain Ken 54-20 were inoculated by the injection method with a suspension of 200,000 spores per 1 ml and the number of lesions were counted according to their symptom types. As shown in Table 4, there were no varietal differences when the fungus strain Ken 54-20 possessing ordinary aggressiveness was injected. On the other hand, when the weakly aggressive strain Ken 54-04 was inoculated, the percentage of lesions showing the pg symptom type (gray center with purple margin, the most susceptible type) in total lesions was found to be significantly different among the varieties tested. The degree of field resistance evaluated by this injection method showed considerably high correlation coefficients from 0.63 to 0.84 with the value observed in the paddy field and the blast nursery bed.

Kiyosawa (1966a) also examined a symptom type by the injection method in a total of 909 varieties, and tabled the results obtained by these two fungus strains to each of the true resistance genotype of varieties. As shown in Table 5, about one-third of the varieties belonging to Shin 2 type (-+) showed the S reaction type (pg symptom type excelled) when Ken 54-04 was injected, and the remainder of this varietal group showed more or less a resistant reaction, but the reaction to Ken 54-20 was almost the S type. In Aichi-asahi

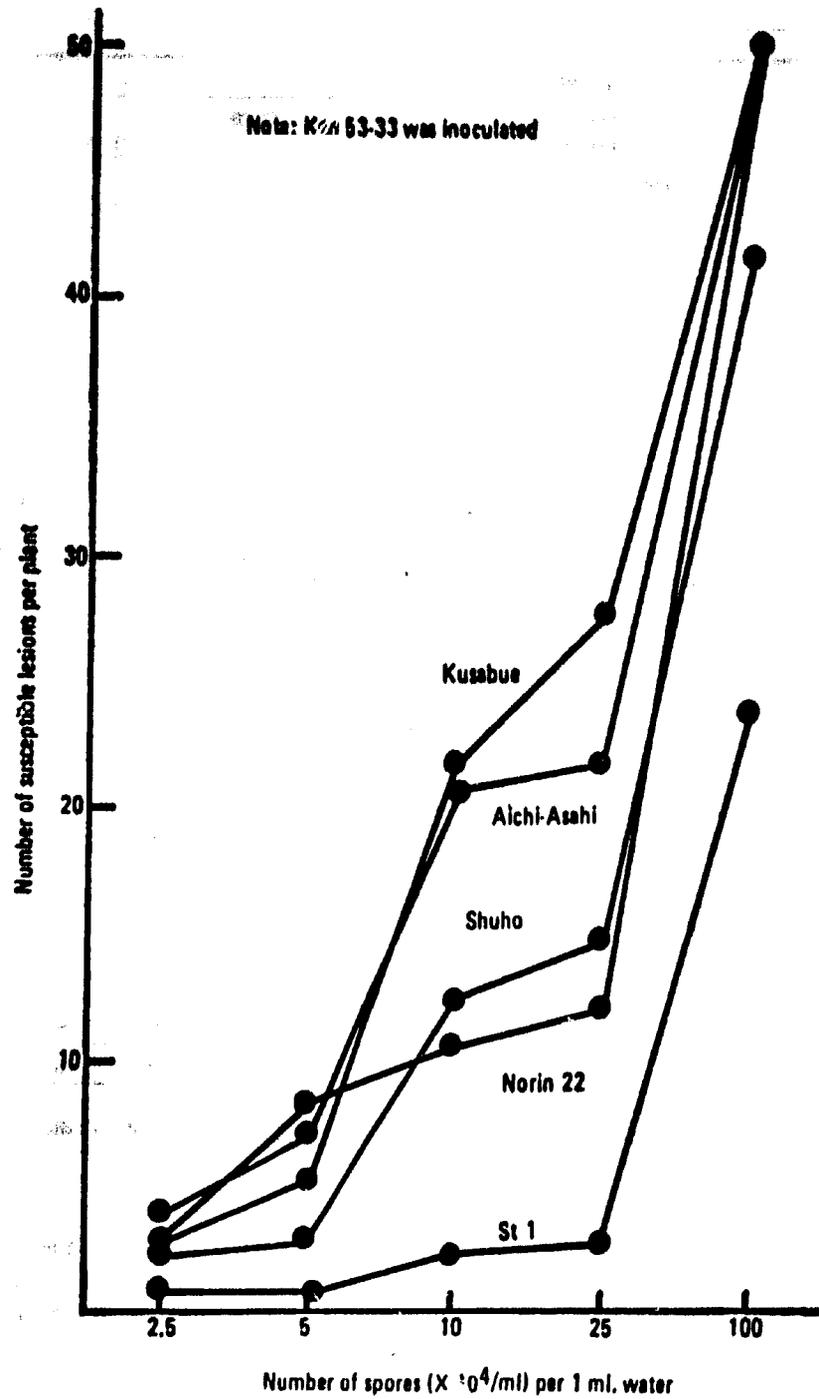


Figure 10. Relationship between the number of susceptible disease lesions and the concentration of inoculum spores, in five rice varieties.

**Table 4. Reaction to fungus strains by injection method**

Variety	Percentage of susceptible lesions showing pg symptom						Evaluation by exceeding symptom type	
	Ken 54-04 (weakly aggressive strain)			Ken 54-20 (ordinary aggressive strain)			Ken 54-04	Ken 54-20
	Mean value	Order	Significance	Mean value	Order	Significance		
Aichi-asahi	67.3	1	**	88.1	1	not	S	S
Moko-ine	44.7	2		63.2	4		MS	S
Kameno o	31.1	3	c	72.1	7		MS	S
Shin 2	26.1	4	c	87.8	2		MS	S
Ta-sensho	13.8	5	b	87.2	3		M	S
Putaba	7.6	6	a b	70.6	8		M	S
Norin 8	5.4	7	a	81.0	6		M	S
Shinju	2.4	8	a	81.7	5		M	S
Norin 22	2.2	9	a	69.8	9		M	S
Oinga	0.6	10	a	62.6	10		MR	S

Note. Item of significance is measured by Duncan's multiple range test (1% level)  
MR: moderate resistance, M: medium, MS: moderate susceptibility, S: susceptibility.

**Table 5. Resistance of 'Shin 2' type evaluated by an predominant symptom type with the injection of weakly aggressive strain Ken 54-04 and ordinary aggressive strain Ken 54-20**

Fungus strain		Rh	R	MR	M	MS	S	Total
Ken 54-04	Ken 54-20							
Rh								0
R								0
MR								0
M								0
MS					5	2	1	8
S		1	4	18	74	24	52	173
Total		1	4	18	79	26	53	181

Note. Rh: High resistance, R: Resistance, MR: Moderate resistance, M: Medium, MS: Moderate susceptibility, S: Susceptibility.

type varieties possessing the  $Pi-a$  gene, almost all the varieties showed the S reaction type by Ken 54-20, but there was wide variation by Ken 54-04 as shown in Table 6. The same tendency was observed in the varieties of the Ishikari-shiroke type possessing the  $Pi-i$  gene and of the Shinsetsu type possessing  $Pi-i$  and  $Pi-a$  genes. The reaction of the foreign varieties is shown in Table 7. Though the deviation of frequency in the table showed little difference from that of the Japanese varieties, a weak aggressiveness of Ken 54-04 was also observed in the foreign varietal group.

**Table 6. Resistance of 'Zichi-asahi' type evaluated by an predominant symptom type with the injection of weakly aggressive strain Ken 54-04 and ordinary aggressive strain Ken 54-20**

Fungus strain Ken 54-04		Rh	R	MR	M	MS	S	Total
Fungus strain Ken 54-20	Rh							0
	R							0
	MR							0
	M							0
	MS				3	1		4
	S	1		11	47	25	60	144
Total		1		11	50	26	60	148

Note. See Table 5.

**Table 7. Resistance of foreign varieties evaluated by an predominant symptom type with the injection of weakly aggressive strain Ken 54-04 and ordinary aggressive strain Ken 54-20**

Fungus strain Ken 54-04		Rh	R	MR	M	MS	S	Total
Fungus strain Ken 54-20	Rh	15	2					17
	R	2	19	2				23
	MR	4	6	18	3		1	32
	M		6	21	67	2	1	101
	MS				23	7		30
	S			2	8	6	11	27
Total		21	33	47	101	15	13	230

Note. See Table 5.

Kiyosawa (1969) concluded that a weak aggressiveness of Ken 54-04 would be non-specific, and the resistance gene or genes to Ken 54-04 would also be non-specific. Therefore, he suggested that the resistance gene or genes of this kind would exhibit resistance more or less in a paddy field, because field resistance was generally considered to be non-specific.

#### Evaluation of Field Resistance by the Sheath Inoculation Method

The sheath inoculation method was proposed by Takahashi (1951) for evaluating resistance to blast. In this method, it was recommended by

Takahashi (1967) that the highest degree of hyphal growth in the host cell be used as the criterion of susceptibility or resistance. The value of resistance evaluated by this method was more complex than that of the spray or injection methods, because the hyphal growth of pathogens in host cells was affected by both true resistance and field resistance of the varieties tested. The degree of field resistance evaluated by this method considerably coincided with the disease rating index which was proposed by Sakurai and Toriyama (1967) within the same varietal group of the true resistance genotype in blast nurseries.

### **Field Resistance and Fungus Strain**

As the definition of field resistance of rice among the Japanese rice breeders is the remaining resistance except for true resistance, exhibition of field resistance was generally considered to be non-specific to fungus strains. Early experiments by Niizeki (1967) and Sakurai and Toriyama (1967) showed the possibility of existence of non-specific resistance. In these experiments, the degree of field resistance was evaluated by the number or percentage of susceptible lesions and the varieties showing susceptible lesions were classified into the same group against the pathotype.

When St 1 and Chugoku 31 were inoculated by the spray method with the virulent strains which showed pathogenicity by the injection and the spray method, only a few susceptible lesions were usually observed, and a small number of the susceptible lesions of these varieties were also observed in blast nursery beds. Therefore, it was considered that both of St 1 and Chugoku 31 were the highest grade of field resistance. However, it was reported that St 1 was severely diseased in Fukushima Prefecture when these varieties were widely tested to ascertain their high field resistance by the blast nursery method all over Japan. Therefore, Yunoki et al. (1970b) tried to ascertain whether field resistance varied with the fungus strains or not.

In general, when the rice varieties possessing true resistance were inoculated with avirulent fungus strains by the spray method, no lesions usually formed on the seedlings but in a few cases susceptible lesions were occasionally observed on the resistant varieties. In such cases, fungus strains isolated from these susceptible lesions on resistant varieties showed virulence to the varietal group from which fungus strains were isolated. Appearance of a new pathotype of this kind was considered to be due to mutation of pathogenicity, and the different mutation ratios for different resistance genes were observed by Niizeki (1967).

Some fungus strains were isolated from the susceptible lesions of St 1 and Chugoku 31 in the blast nursery bed in Fukuyama, Hiroshima Prefecture, and were inoculated by the spray method in the seedlings of St 1 and Chugoku 31, respectively. As shown in Table 8 only a few susceptible lesions were found on the seedlings. Then, the fungus strains were re-isolated from the susceptible lesions of inoculated seedlings, and the re-isolated fungus strains were again used to inoculate each variety. These re-isolation tests were repeated three times, and the results were similar to the first isolation test. In this respect, it was considered that the high resistance of St 1 and Chugoku 31 belonged to a different category from true or vertical resistance in rice.

Yunoki et al. (1970b) collected fungus strains from different locations and from different rice varieties, and tested their aggressiveness to St 1 and Chugoku 31. Of these collections, some fungus strains isolated from Kisa, Hiroshima Prefecture, showed strong aggressiveness to St 1 and Chugoku 31, and the number of susceptible lesions on both varieties was about equal to those on the usual varieties possessing a low level of field resistance as shown in Table 9. Erosion of high resistance of these varieties was also observed when the fungus strains from Fukushima Prefecture and some other strains were used for inoculation. Furthermore, it was observed that the resistance of these varieties became low when they were grown under an unfavorable condition in a greenhouse during the winter season.

Such breakdown of high resistance of St 1 and Chugoku 31 was apparently due to the specific reaction to the fungus strains, and this phenomenon is similar to the breakdown of vertical resistance.

High field resistance alike to St 1 and Chugoku 31 was found in Zenith and its derivatives by Ezuka et al. (1969b) and Yunoki et al. (1970b). By the injection method, Zenith and its derivatives were classified into two varietal groups according to the reaction pattern against the seven standard fungus strains; Zenith, Fukei 67 and Fukei 73 were classified into the Zenith type possessing  $PI-a$  and  $PI-z$  genes; 54 BC-68, Fuku-nishiki, Ou 243 and Ou 244 were classified into the Fuku-nishiki type possessing the  $PI-z$  gene. Nevertheless, these varieties were clearly divided into two groups of a different category from the above grouping when tested in the blast nursery bed in Fukuyama and inoculated by the spray method with virulent fungus strains such as FS 66-59, Chu 66-45 and TH 65-105. One is the high level of field resistance, to which Zenith, 54 BC-68 and Ou 244 belonged, and another is the low level of field resistance, to which Ou 243, Fukei 67, Fukei 73 and Fuku-nishiki belonged. Though the varieties belonging to the high level of field resistance showed a typical susceptible symptom, they developed only a small number of susceptible lesions. Breakdown of high resistance in Zenith and Ou 244 has not been observed in Japan, but the possibility of breakdown has yet remained because the susceptible reaction of Zenith was reported at some

Table 8. Resistance of "St 1" and "Chugoku 31" to fungus strains isolated repeatedly from "St 1" and "Chugoku 31"

Fungus strain	Variety from which isolated	race	Number of susceptible lesions											
			St 1			Chugoku 31			Norin 22			Moko-inc		
			1*	2*	3*	1	2	3	1	2	3	1	2	3
Chu 66-10	St 1	C-8	2.2	5.2	5.4	0	0	0	12.0	13.8	35.8	42.0	39.7	223.7
Chu 66-11	St 1	C-8	0.9	0	1.0	0.5	0.6	0.8	12.9	2.1	8.1	55.0	12.2	70.6
Chu 66-12	St 1	C-8	1.9	1.3	2.2	0.3	0.9	0.8	15.5	2.8	7.9	82.0	14.4	82.4
Chu 66-13	St 1	?	2.9	0.2	2.7	1.8	0	3.3	16.3	0.3	33.5	50.6	1.4	201.9
Chu 66-14	St 1	N-1	8.8	1.3	10.4	0	0	0	15.9	2.3	49.2	71.4	3.6	357.8
Chu 66-15	St 1	N-1	0.2	3.5	3.7	0.3	8.9	0.3	23.0	6.5	28.5	134.9	48.8	97.9
Chu 66-22	Chugoku 31	C-8	2.4	8.6	1.0	2.6	10.3	1.7	23.9	7.2	15.5	134.7	32.4	108.1
Chu 66-23	Chugoku 31	?	0.8	1.2	1.6	1.6	0.2	0.4	18.8	2.2	21.2	89.7	8.8	179.5
Chu 66-24	Chugoku 31	?	0.6	9.6	0.1	0.2	4.9	0.3	50.9	9.7	4.1	224.0	37.0	56.2
Chu 66-25	Chugoku 31	?	1.4	1.1	0.2	0.2	0.5	0.1	21.4	0.4	24.0	81.4	6.2	213.3
Chu 66-26	Chugoku 31	?	0.1	3.9	0.4	0.1	4.5	0.3	3.1	1.2	4.9	12.6	5.6	29.0
Chu 66-27	Chugoku 31	?	1.3	2.3	0.1	0.2	1.2	0.1	19.7	2.0	9.9	112.6	17.6	103.3
Ken 60-19	Kanto 52	C-1	1.9	4.3	1.6	1.0	2.4	1.2	3.3	7.2	6.2	86.3	22.7	115.0

\* Means times of isolating.

**Table 9. Field resistance and the fungus strains collected from different locations**

Fungus strain	Location collected	Variety isolated	Race	Number of susceptible lesions					
				Si 1	Chugoku 31	Norin 22	Alchi-asaahi	Homare-nishiki	Kanto 51
Chu 66-1	Akana	Koshi-hikari	C-8	0.6	0	4.3	10.6	0	6.0
Chu 66-3	do	do	C-1	0.2	1.6	4.8	12.2	2.0	14.8
Chu 66-32	Fukuyama	Nakei 212	T-2	1.0	0	11.4	42.6	12.7	0
Chu 66-38	do	do	N-6	3.2	0	15.8	28.2	0	0
Chu 66-35	do	do	C-1	0.8	0.4	10.5	22.3	2.5	30.4
Chu 66-46	do	Fuku-nishiki	N-1	0	0	12.0	33.5	1.2	0
Chu 66-51	Kisa	Norin 18	N-2	1.0	0	21.4	34.2	4.4	0
Chu 66-58	do	Senshu-raku	N-2	45.0	0	18.2	33.8	24.0	0
Chu 66-59	do	do	C-3	21.6	0	13.5	0	0	41.0
Chu 66-64	do	Kanto 51	C-1	7.5	31.0	22.0	57.0	22.4	107.0
Chu 66-65	do	do	C-1	64.4	16.3	40.3	44.0	9.0	35.9

locations in the world during 1964-1965 in the international uniform blast nurseries (FAO 1966).

Some Japanese upland rice varieties such as Kuroka and Fukuton have also been found to have an extremely high field resistance like Zenith. By the injection method, Kuroka was found to have the true resistance gene *PI-a* only, and Fukuton to have no true resistance gene (Ezuka et al. 1969a). Nevertheless, when tested in blast nursery bed and when inoculated by the spray method with virulent fungus strains to the *PI-a* gene, these varieties developed only a few lesions of moderately resistant symptom type, and were recognized to be of a high level of field resistance (Ezuka et al. 1969b, Yunoki et al. 1970a). Recently, it was found that high field resistance in these upland rice varieties was specific to the fungus strains, because some fungus isolates could develop a number of susceptible lesions on these upland varieties (Sekiguchi, personal communication). This was quite unexpected evidence for the Japanese rice breeders. Up until this finding, they believed that the Japanese upland rice varieties should be a favorable gene source for high field resistance, because the Japanese upland rice had been planted for many years in Japan and had exhibited stable resistance to blast. However, breakdown of high level of field resistance in the upland rice varieties was only observed in a laboratory test, and it has not yet been proved under field conditions.

The other type of high field resistance may be due to a simultaneous effect of a true resistance gene to some fungus strains. Some varieties descending from the upland rice variety Sensho were found to have true resistance when

inoculated by the spray method with the fungus strains belonging to the N-6 or C-6 races. These varieties were observed to have a considerably high level of field resistance in the blast nursery beds and in the paddy fields where fungus strains virulent to these varieties were prevalent, as compared with the ordinary varieties susceptible to the N-6 and C-6 races (Nakanishi and Nishioka 1967).

Conversely, it has been considered that an existence of the true resistance gene *Pi-k* brought decreasing field resistance against fungus strains virulent to *Pi-k* (Suzuki and Yoshimura 1966, Iwano, Yamada and Yoshimura 1969). However, it was found that the gene *Pi-k* was independent with the degree of field resistance (Asaga and Yoshimura 1970). Decreasing field resistance in the varieties possessing *Pi-k* gene might be due to the Vertifolia effect pointed out by van der Plank (1963).

The effect of fungus strains on field resistance of a middle or lower level was also investigated. According to early investigations by Hirano et al. (1967) and Niizeki (1967), almost the same reaction to different fungus strains was observed on the respective varieties for different levels of field resistance. Hirano and Matsumoto (1971) repeated the seedling inoculation test with six fungus strains belonging to the C-1 race and with two other fungus strains. As shown in Table 10, highly significant correlations were obtained between fungus strains employed. As far as these results are concerned, middle to lower level of field resistance in rice had the same meaning of horizontal resistance as defined by Robinson (1969). However, the variations of field resistance of a middle to lower level were observed by Yunoki et al. (1970b) and Ito (personal communication).

Yunoki et al. (1970b) investigated the variability of field resistance in numerous varieties with fourteen fungus strains of six races which were collected from northern to southern Japan. The degree of field resistance was evaluated by the ratio of a susceptible lesion number on the given variety to that on the standard variety and was graded according to following criteria: rr is less than 20, r 21 to 40, m 41 to 60, s 61 to 80 and ss is more than 81 percent. Some

Table 10. Correlation coefficient between field resistance evaluated by different fungus strains

Fungus strain	A C-1	E C-1	F C-1	H C-8
E C-1	0.939 ***			
F C-1	0.971 ***	0.954 ***		
H C-8	0.953 ***	0.972 ***	0.973 ***	
I N-1	0.935 ***	0.970 ***	0.898 ***	0.965 ***

\*\*\* 0.1 percent level.

examples of results were shown in Table 11. Some varieties such as Hatsu-nishiki showed a wide range of variation from rr to ss; on the other hand some varieties such as Akibare showed a stable degree of resistance between rr to r, and some varieties such as Norin 29 and Aichi-asahi showed a constant susceptibility of ss. The varieties were grouped into a variable or stable one for field resistance, and it was found that the varieties in the variable group by the seedling inoculation method were also variable when tested in the blast nurseries. On the contrary, the varieties showing stability by the seedling inoculation method were also stable in the blast nurseries.

If field resistance of varieties possessing the same true resistance gene or genes is not influenced by the fungus strains, the order of field resistance in the varietal group with the same true resistance gene or genes is expected to be constant even in different environments, such as locations and growing season. Interaction between variety, fungus strain and location was investigated by Ito in co-operation with five national agricultural experiment stations (personal communication, briefly reported in the Year Book Cent. Agr. Exp. Sta. 1969, 1970). Fifteen varieties, of which ten belonging to Kanto 51 type and five to Shin 2 type, were inoculated by spraying with six fungus strains under the isolated nursery condition. Of the six fungus strains, five belonged to the C-1 race and two to the N-2 race. The experiments were conducted at five locations with two replications each. The degree of field resistance was graded from 0 (rr) to 11 (ss) at interval of 0.5, and the results of analysis of variance on field resistance are shown in Table 12. Significant interaction was indicated between the main factors; variety X fungus strain,

Table 11. Range of variation of field resistance of varieties possessing middle to low level of resistance

Fungus strain	Race	Variety Genotype	Hatsu-	Kansai	Shin-	Kogane-	Yama-	Reimei	Fujioka	
			nishiki	6	ei	nishiki	biko		Akibare	5
			+	+	+	+	PI-a	PI-a	PI-a	PI-l
Ken 53-33	T-1		ss	rr	r	r	m	rr	rr	r
Hoku 63-27	T-1		rr	rr	rr	r	r	rr	rr	ss
Ken 60-19	C-1		s	rr	s	s	ss	r	r	r
Ina 72	C-3		m	rr	rr	r	-	-	-	-
Hoku 1	N-1		r	r	r	r	r	r	r	r
Hoku 373	N-1		r	rr	r	rr	r	r	r	rr
P - 12	N-1		ss	rr	m	rr	m	r	r	m
Ken 54-20	N-2		rr	rr	m	ss	rr	rr	r	-
P - 2b	N-2		s	r	s	s	m	r	r	-
H 67-1	N-2		ss	r	r	m	m	r	r	-
H 67-4	N-2		ss	r	s	m	s	r	r	-
FS 67-14	N-2		ss	m	r	m	s	s	rr	-
FS 66-59-0	N-2		ss	rr	s	rr	m	rr	rr	-
Ina 168	N-4		r	rr	rr	rr	-	-	-	-

rr: high resistance, r: resistance, m: medium, s: susceptibility, ss: high susceptibility.

**Table 12. Analysis of variance of field resistance by varieties, locations and fungus strains**

Source of Variance	Degree of freedom	Mean square	F
Varieties	14	63.6	43.27 **
Locations	4	302.6	205.82 **
Fungus strains	4	120.2	81.74 **
Varieties X Locations	56	4.6	3.11 **
Varieties X Fungus strains	56	2.3	1.58 *
Locations X Fungus strains	16	44.1	30.03 **
Error	224	1.5	

\*\* and \* : Significance at 1 and 5 percent level, respectively.

variety X location and fungus strain X location. For example, Tatsumi-mochi, which was considered to be of a moderately high level with the order of 3 or 4, within 15 varieties, showed marked variation of field resistance with the fungus strain. When fungus strain Ken 53-11 was inoculated, Tatsumi-mochi was evaluated as having a low level of resistance, and the order of resistance was 11 within 15. Therefore, when the level of field resistance was evaluated according to the order by each fungus strain, the range varied with variety. In the same manner, the order evaluated in each location varied with variety. The variable range by fungus strains and by locations indicated a positive correlation as shown in Fig. 11. As a result, it is concluded that field resistance of rice varieties varied with the fungus strains, so the term field resistance does not coincide with the term horizontal resistance in a strict sense.

#### **Inheritance of Field Resistance**

A high level of field resistance in St 1 and Chugoku 31 descended from the Pakistani variety Modan by successive back-crossing to the Japanese paddy rice variety Norin 8. Inheritance of this high field resistance was investigated with eleven crosses by Toriyama, Yunoki and Shinoda (1968). High field resistance was dominant over susceptibility, and monogenic inheritance was observed through all the crosses investigated. Linkage relation between the high field resistance gene *Pi-f* and the true resistance gene *Pi-k* was also analyzed with the crosses involving Chugoku 31 which possessed both *Pi-f* and *Pi-k* genes. A recombination value at 14.5 percent between *Pi-f* and *Pi-k* was obtained, and the arrangement of genes on Chromosome 9 (1a linkage group; Group 8) was estimated as shown in Fig. 12 (Toriyama et al., 1968).

Inheritance of high field resistance of Ou 244 which was incorporated from Zenith has not been investigated yet, but this trait segregated very clearly into

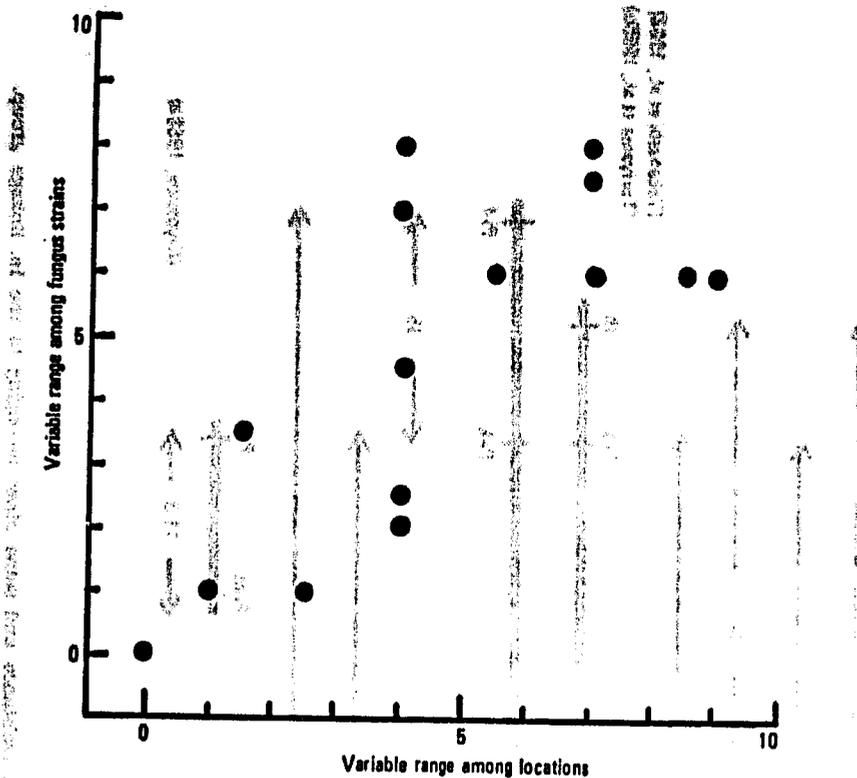


Figure 11. Correlation between variable ranges of varietal resistance determined by rice blast fungus strains and locations.

either high field resistance or susceptibility in the offsprings of the crosses involving Ou 244. Major genetic inheritance, therefore, was expected in this high field resistance of Ou 244, like that of St 1.

The genetic scheme of high resistance in Japanese upland rice was investigated with the crosses between the upland rice Kuroka and the chromosome reciprocal translocation lines by Shinoda et al. (1970). High field resistance was dominant over susceptibility, and major genetic inheritance was observed, but the number of genes concerned was not determined whether to be two or three. By the analysis of linkage relationships between high field resistance and the chromosome reciprocal point, it was found that one of the genes for high field resistance was located on Chromosome 4 (A linkage group, Group 3) and the other on Chromosome 11 (PI linkage group, Group 2).

The other kind of moderately high field resistance was found in the same varieties descending from upland rice variety Sensho as previously mentioned. Exhibition of this moderately high field resistance was estimated to be due to the simultaneous effect of a true resistance gene to the fungus races N-6 and C-6. This true resistance was recognized when the spray method was used

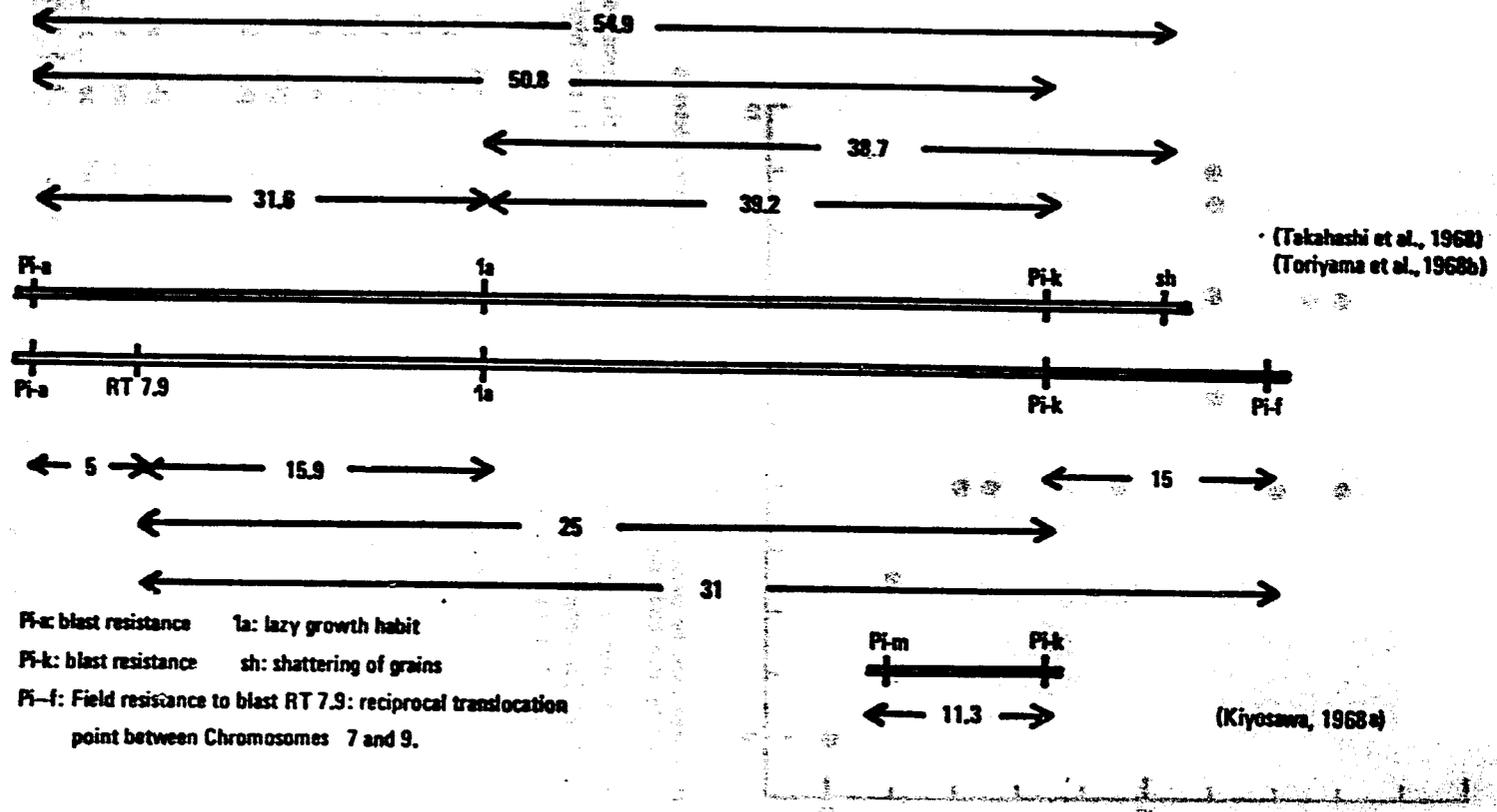


Figure 12. Linkage relationship among genes for rice blast resistance and some other belonging to the 1a. linkage group.

for inoculation but did not manifest itself even when injected with the same fungus strains. Though inheritance of this true resistance gene has not been investigated yet, it was estimated that true resistance was controlled by the major genetic system from the view point of the varietal lineage.

The genetic system of the moderately high field resistance of Homare-nishiki, which was one of the descendants of Sensho, was investigated by  $F_3$  line analysis under blast nursery conditions by Yunoki, Toriyama and Kiyosawa (1970). It was observed that moderately high field resistance was controlled by two pairs of complementary genes which were linked with 20 to 30 percent of recombination value. In order to find out the relationship between field resistance and reaction to the injection with the weakly aggressive fungus strain Ken 54-04,  $F_3$  lines of the crosses involving Homare-nishiki and Ginga were employed for the injection test with Ken 54-04 by Kiyosawa (1970b). One major gene and two minor genes were estimated to control the reaction to the Ken 54-04 injection, and the sensitivity to environmental conditions appeared to be dependent mainly upon the minor genes and partially upon the major gene. Comparison between the data by the injection method and by the blast nursery on the same  $F_3$  lines indicated that exhibition of resistance to two testing methods was controlled by different genetic systems because of a non-significant correlation between them (Yunoki, Toriyama and Kiyosawa 1970).

All the examples of high field resistance mentioned above were estimated to be specific to fungus strains or to be a simultaneous effect of the true resistance gene. The major genetic system of inheritance of field resistance, therefore, might correspond to the specific pattern of resistance to fungus strains.

Inheritance of a middle or lower level of field resistance and the relationship between field resistance and true resistance were analyzed with the  $F_3$  and  $F_4$  sister lines of three crosses by Asaga and Yoshimura (1969, 1970, 1971). Three crosses were made among Kusabue, Yamabiko and Norin 29, of which Kusabue had the  $Pi-k$  gene and extreme susceptibility, Yamabiko had the  $Pi-a$  gene and a middle level of field resistance, and Norin 29 had no true resistance genes and susceptibility.  $F_3$  lines were grouped according to the true resistance genotypes:  $Pi-k$  and  $Pi-a$  group,  $Pi-k$  group,  $Pi-a$  group, + group and heterogenic group. The degree of field resistance was evaluated in the blast nursery where fungus strain Ken 60-19 belonging to the C-1 race was inoculated by spraying in order to eliminate the action of the true resistance genes. The mean and standard deviation of field resistance in each genotype are shown in Table 13. Marked differences of field resistance were found among sister lines with the same resistance genotypes, but the variable range within sister lines was less than the difference between the parental varieties, and the mean values were

Table 13. Mean and standard deviation of field resistance in F<sub>3</sub> lines.

Cross	Genotype	Number of lines	Mean	Max.	Min.	Standard deviation
Yamabiko X Kusabue	<u>PI-h</u> <u>PI-e</u>	23	22.3	34	16	6.3
	<u>PI-h</u>	17	22.1	30	13	4.7
	<u>PI-e</u>	16	21.1	32	12	6.6
	-	10	20.9	33	12	4.9
Kusabue	Hetero	294	21.3	37	10	6.2
Norin 29 X Kusabue	<u>PI-h</u>	85	27.7	39	18	4.8
	-	76	26.0	38	16	6.0
	Hetero	156	26.9	37	12	6.2
Yamabiko X Norin 29	<u>PI-e</u>	60	14.8	27	10	3.8
	-	62	16.4	23	10	3.5
	Hetero	193	16.2	26	10	3.3
Kusabue	<u>PI-h</u> <u>PI-e</u>	1	33.8	39	20	3.6
Norin 29	-	1	18.5	27	9	3.9
Yamabiko	<u>PI-e</u>	1	11.7	18	7	2.1

Note: Figures in the table mean the degree of field resistance; 0 to 100: Susceptibility to resistance.

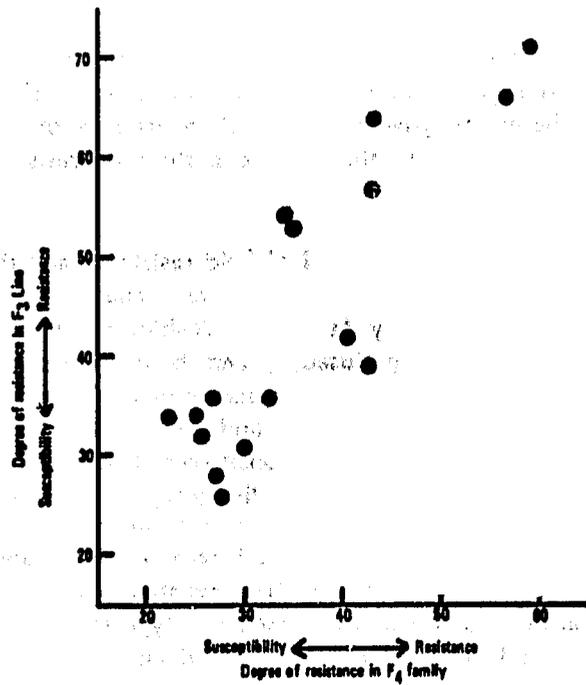


Figure 13. Parent-offspring correlation of field resistance between F<sub>3</sub> and F<sub>4</sub> families.

about the same of mid-parent.  $F_3$  lines of the highest and the lowest degree of field resistance were selected from each true resistance genotype group in each cross. Each  $F_4$  family including 45 lines was tested for its resistance to stem rot in the paddy field. Differences within each  $F_4$  family were observed on field resistance and marked differences between the two families selected from the same genotype were also observed. As shown in Fig. 13, a highly significant correlation was obtained between the  $F_3$  lines and  $F_4$  families. This positive correlation, 0.891 \*\*\*, and the nearly normal distribution of field resistance within the family meant that the middle or lower level of field resistance was inherited and would be controlled by the polygenic system. In this experiment, field resistance evaluated in the blast nursery bed and paddy field showed a high correlation, and the resistance to stem rot also correlated well with the degree of field resistance.

#### Mathematical studies on field resistance

Mathematical study on epidemiology was established by van der Plank (1963). He used the equations

$$\frac{dx}{dt} = \gamma x \quad (1)$$

as a model of increase of infection, where  $X$  is the proportion of disease and  $\gamma$  is infection rate, and

$$\frac{dx}{dt} = \gamma x(1-x) \quad (2)$$

as a model when the amount of the host limited, and the proportion of disease at  $t$  is

$$x = x_0 e^{\gamma t} \quad (3)$$

where  $X_0$  is the initial proportion of disease, and when the logarithmic stage of an epidemic was considered

$$x_t = x_{t-l-p} e^{(l+p)\gamma} \quad (4)$$

where  $p$  is the latent period and  $l$  is the infection period.

Van der Plank (1963) pointed out that horizontal resistance reduces the infectious rate  $\gamma$ , increases the latent period  $p$  and reduces infectious period  $l$ .

Independently of the work of van der Plank, Kiyosawa (1965) proposed the following equations

$$\frac{dl}{dt} = \lambda l \quad (5)$$

$$\frac{dl}{dt} = \lambda l \left(1 - \frac{l}{L}\right) \quad (6)$$

$$l = l_0 e^{\lambda t} \quad (7)$$

$$l = \frac{L}{1 + ke^{-\lambda t}} \quad \left(k = \frac{L - l_0}{l_0}\right) \quad (8)$$

where  $l_0$  is the number of lesions at the initial time,  $l$  is the number of lesions at  $t$ ,  $\lambda$  is the fitness of pathogens and  $K$  is the coefficient related with the number of lesions at the initial infection.

In these equations,  $l_0$  depends upon true resistance of the variety but not upon the degree of field resistance. On the other hand  $\lambda$  depends upon the degree of field resistance but not upon true resistance from the point of view on variety. Namely,  $l_0$  depends upon virulence but not upon aggressiveness or fitness, and depends upon aggressiveness or fitness but not upon virulence from the point of view on fungus strain.

Kiyosawa (1969b) proposed that true resistance and field resistance were defined by the variables of equations. By his definition, field resistance is expressed by  $1/\lambda$  and true resistance corresponds to  $l_0$ . He suggested that the varietal difference of field resistance should compare the value of  $1/\lambda$  in each variety.

The influence of an environmental condition and the increase of resistance with aging of the plant was theoretically given as follows;

$$\frac{dl}{dt} = \lambda l \left(1 - \frac{t}{T}\right) \quad (9)$$

$$l = ke^{\lambda \left(t - \frac{t^2}{2T}\right)} \quad (10)$$

$$l = \frac{L}{1 + ke^{-\lambda \left(t - \frac{t^2}{2T}\right)}} \quad (11)$$

where field resistance of plants linearly increases until a time  $T$  when increase of the number of lesions becomes 0, in other words the disease increase terminates.

Using the equation (11), Chiba et al. (personal communication) investigated the effect of some factors on degree of field resistance. The variable for field resistance,  $\lambda$ , was affected largely by yearly difference of climate factors and the amount of additional fertilizer, and slightly by varietal difference among the factors investigated. A significant negative regression of  $\lambda$  against  $I_0$  was found as a result of a density effect. The value of  $\lambda$  corrected by the regression coefficient showed that the ranges of variation of  $\lambda$  by year, the variety and additional fertilizer were 0.32, 0.20 and 0.31, respectively. Average of  $\lambda$ 's obtained under various conditions for four years was 0.36.

### **Conclusion**

Field resistance to blast disease in rice is now being investigated not only by rice breeders but also by plant pathologists in Japan. Therefore are many problems to be investigated. As was reviewed in this paper, we have too little information on field resistance in rice, especially on a varietal difference of latent period and infectious period.

Through this review, it was emphasized that field resistance of Japanese investigators did not coincide in part with horizontal resistance as defined by van der Plank (1963), and that the difference between true resistance and field resistance was due to the difference of a standpoint for recognition. In fact, descendants of the upland rice variety Sensho, which was recognized to have true resistance by spraying with the C-6 or N-6 races, showed a high field resistance to numerous fungus strains in the paddy fields and blast nurseries. In addition, varieties such as St 1, Ou 244 and Kuroka which were recognized to have an extremely high field resistance according to a few susceptible lesions under spray inoculation were found to show specific reaction to some fungus strains on the number of susceptible lesions. Since, in horizontal resistance, there was no interaction between pathotype and pathodeme, the definition will be applicable in the limited field until the discovery of the new pathotype which shows the pathotype-pathodeme interaction. It is because the possibility of an existence of such new pathotypes cannot be denied.

Rice breeders have to give strong attention to develop promising varieties which possess high and stable field resistance to blast.

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**Recent advances in studies  
on horizontal resistance to blast disease  
of rice in Japan**

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Since a systematic rice breeding program with the cooperation of eight National and forty-seven Prefectural Agricultural Experiment Stations was organized in 1927 in Japan, much effort has been directed to the breeding of blast resistant varieties, using Japanese paddy rice in the first stage, and by the use of resistant varieties derived from Japanese upland rice, then by the use of resistant varieties derived from Chinese and indica varieties.

In these decades, special efforts were made to introduce race-specific resistance genes of foreign varieties into Japanese paddy rice, because most of the foreign varieties were found to be immune to most races of the causal fungus in Japan. Many new varieties were developed in this way. The breeding processes of representative new varieties were reviewed in detail by R. Ito (1965), K. Nagai (1966) and T. Hirano (1967).

These new varieties showed excellent resistance for some years after being discharged as commercial varieties, but lost their high resistance due to the occurrence of a new race or races within three years in the case of the shortest period, and about ten years in most cases. The dynamic changes of predominant races make a breeding program of resistant varieties very complicated, and the

Importance of the use of horizontal resistance has been re-recognized. Further research has started in Japan from the point of distinguishing between vertical or race-specific and horizontal resistance of the rice varieties.

In this paper horizontal resistance is understood as a quantitative resistance which can be measured by either the number or the size of lesions of susceptible reaction type, but not as a qualitative one detected by the reaction type of lesions or by the hypersensitive reaction of host cells. Exactly defining horizontal resistance is another problem.

### Varietal Difference in Horizontal Resistance of Rice

Among the most widely used testing methods is grouping test varieties on the basis of blast resistance genotype or of reaction type to the races. For the test of horizontal resistance, it is of primary importance to use compatible races that are highly virulent to all varieties to be tested. For this purpose, the test varieties are grouped generally on the basis of race-specific resistance genotype or of reaction type to the races found in Japan, then two or three test isolates are selected for each variety group.

Almost all varieties in Japan have been tested for their reaction to the races in Japan and grouped into more than eleven groups of reaction type. Genetic studies have also been conducted for the representative varieties of each reaction type, and eight or more major resistance genes are identified (Kiyosawa, 1967) (Ezuka et al. 1969). Most Japanese varieties are included in three or four reaction types (Table 1). (Yamada et al. 1969).

Standard test isolates for each variety group have also been selected, but as described later there remain some unsolved problems in selecting test isolates.

Table 1. Number of Japanese origin varieties of major reaction types to the races found in Japan

Reaction type	Resist. gene	Reaction to the races							Number of varieties included
		C-3	C-6	N-1	N-2	N-3	N-4	N-5	
Shin 2 type	no	S	S	S	S	S	S	S	910 (67%)
Aichiasahi type	PI-a	R	S	S	S	S	R	R	332 (25)
Ishikari-shiroke type	PI-l	R	S	S	R	R	R	S	47 (3)
Others									57
<b>Total</b>									<b>1349</b>

Another testing method is spray inoculation of seedlings under greenhouse conditions. The seedlings of the varieties to be tested are grown in small seedling boxes under upland conditions as in the case of race identification. They are inoculated by spraying with a spore suspension at the 7 to 8 leaf stage and incubated at 26°C for 18 to 20 hours, then kept in an air conditioned greenhouse at 26°C for about two weeks. Evaluation is made on the first two leaves from the top at the time of inoculation on the basis of lesion area (Fig. 1).

There is also the nursery test by inoculation. In this method the preparation of the nursery is the same as in the case of the International Uniform Blast Nursery Program established by the Food and Agricultural Organization. Border rows consisting of the most susceptible varieties are inoculated with infected leaves by scattering; these are prepared in advance in another nursery bed by spray inoculation with test isolates and cut into small pieces 3-5 cm long at the time of inoculation.

Evaluation is made on the basis of percent of diseased leaf area when the disease covers almost all leaves of the most susceptible varieties.

Special caution is taken in order to allow the disease to develop from the 7 to 9 leaf stage; too early or too late inoculation should be avoided.

In the field test method, test varieties are transplanted by ordinary cultivating methods in rows, three to five rows of two to three meters long per variety. The most susceptible variety is mixed with every two or three test varieties as border rows. Inoculation is often made 10 to 20 days after transplanting by scattering on border rows diseased leaves which are prepared in advance in the same way as in the nursery test. Sometimes they have been naturally infected.

Evaluation for leaf blast is usually made at the young panicle formation stage to booting stage on the basis of percent of diseased leaf area or of percent of diseased leaves. Evaluation for neck blast is made about 25 days after heading on the basis of percent of diseased panicle.

Intensive tests for horizontal resistance of rice were conducted in Japan in several National Agricultural Experiment Stations in 1966-68, in which almost all varieties obtained in Japan (including foreign varieties or inbred varieties with foreign varieties) were tested by nursery or field tests.

The results showed that there is a clear difference in horizontal resistance among the varieties of the same blast resistance genotype or reaction type to the known races in Japan. The resistant varieties have either a fewer number of lesions or smaller size of lesions of susceptible reaction type, or both, in comparison with the susceptible varieties.

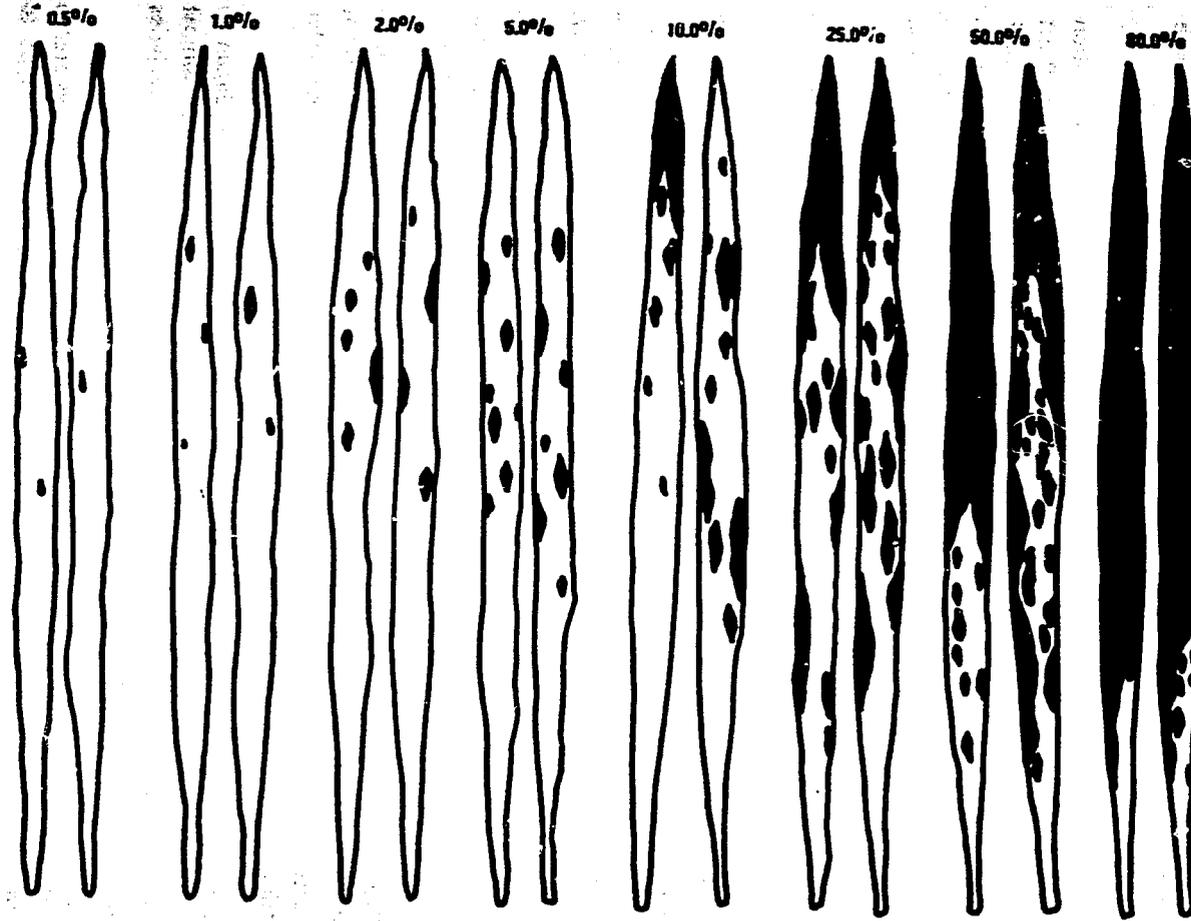


Figure 1. A standard scale for disease rating on the basis of percent of diseased leaf area.

The representative results are shown in Table 2:

The results obtained also indicate that some varieties are very consistent in their horizontal resistance, but some other varieties—more than ten percent of the total—fluctuate considerably among localities and years, in spite of the conditions of the same race constitution (Table 2).

### Major Influential Factors in the Evaluation of Horizontal Resistance of Varieties

A joint work on horizontal resistance of 59 selected varieties of five representative blast resistance genotypes was carried out in 1967-68 in ten localities throughout Japan in order to detect the major factors responsible for the fluctuation of the varieties in resistance. The results are summarized as follows:

Table 2. Number of varieties of different degree of horizontal resistance included in representative variety groups of reaction types to the races in Japan (Ezuka et al. 1969).

Reaction type	Resist. genotype	Horizontal resistance	Number and name of varieties included
Shin 2 type	+	rr	13 — Chiyohkari, Ohu 247 Tokai 26, Sanin 63, Harima, Shito, St 1, Suzuhara-mochi, Norinrikuto 24, Rikuto-Kanto 83, Sensho, Fukuton, Rikuto Norin mochi 26
		r	30
		m	36
		s	34
		ss	22
Aichiasahi type	PI-a	rr	10 — 64A-8, Heirokumochi, Rikuto Norin 1, Shinhakaburi, Hiderishirazu, Kuroka, Kirishima, Kahel, Hirayama, Akamai
		r	27
		m	21
		s	30
		ss	10
Ishikari-shiroke type	PI-l	rr	7 — Yoneshiro, Hokkai 220, Joiko 232, Soraku 9, Sorakai 22, Toyamawese, Kumochi
		r	8
		m	3
		s	1
		ss	2
Kanto 51	PI-k	rr	1 — Reishiko
		r	3
		m	8
		s	9
		ss	12

**Table 3.** The number of lesions of susceptible type on a variety Manryo by spray inoculation with different isolates of the races at  $20 \times 10^4$  / ml spore concentration (Suzuki and Yamada 1969).

Races Identified	Isolates	Number of susceptible lesions per 60 plants
C-1	1	1472
	2	810
	3	1917
	5	540
	6	1080
	7	900
	8	990
	9	2160
	10	2120
	N-1	1
2		240
3		630
4		780
5		1170
6		960
8		2160
9		1170
N-2		1
	2	1250
	3	1619
	5	1258
	6	2130
	7	1730
	8	1120
	9	490

It was found generally that the isolates, compatible to all the test varieties and belonging to the same race, differ from each other in their virulence to the varieties (Table 3).

A highly virulent isolate produces a large number or larger size of susceptible reaction type lesions on the plants in comparison to the isolates of the less virulent. It is, moreover, recognized that the degree of resistance of the varieties is somewhat different to each of the isolates.

Fig. 2 shows one of the tests with different isolates of the same Japanese race C-1, in which the isolate Ken 66-19 was the most virulent and Ken-117 the least virulent, and a variety such as Ugonishiki was variable in a wider range in its resistance to the isolates. Fig. 3 also shows a similar result indicating that Norin 8 and Norin 25 were very variable in resistance to each isolate (Figs. 2 and 3).

Another extreme example is observed in a line St. 1. This variety shows high horizontal resistance to many isolates of all known races in Japan, producing a very small number of lesions of susceptible reaction. Some isolates collected from Fukushima, Ibaragi and Hiroshima Prefectures, however, showed

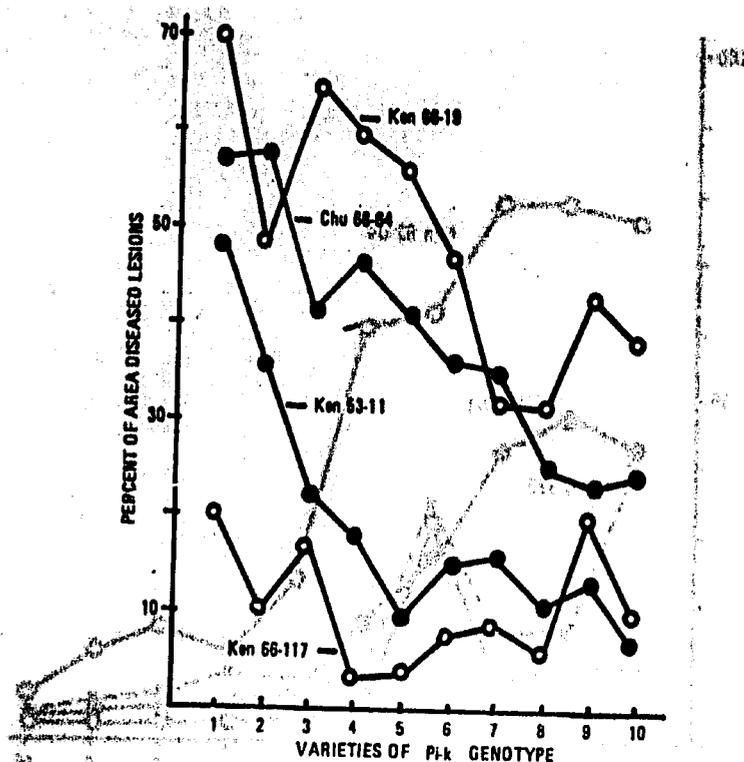


Figure 2. Differences in varietal responses to four isolates of rice blast disease. The listed varieties: (1) Kanto 51, (2) Sanin 68, (3) Kusabue, (4) Fu-69, (5) Tatsumi-mochi, (6) Kanto 59, (7) Ohu 248, (8) Mangetsumochi, (9) Ugonishiki, (10) Senshuraku.

high virulence to the variety, producing as many lesions as in the most susceptible varieties as shown in Fig. 4 (Sakurai 1969). A major gene  $PI-f$  is assumed to control this type of resistance in St. 1 (Toriyama et al. unpublished).

A variety St. 1 was derived from the fifth backcross involving Norin 8 as a recurrent parent and Modan as a donor for the purpose of developing stripe virus-resistant varieties. Norin 8 is a Japanese paddy variety susceptible to stripe, while Modan is a typical indica variety of Pakistani origin showing high resistance to stripe. St. 1 is highly resistant to stripe as well as Modan.

A very similar result was also obtained in a variety Minehikari. Minehikari showed high horizontal resistance to all isolates of Japanese race C-8 until 1968, but became susceptible to the isolates of the same race collected from Aichi Prefecture in 1969 (Aichi Agr. Exp. Sta. 1969). Genetic studies have not yet been made. Minehikari is one of the leading varieties in central Japan derived from multiple crossing between a Chinese variety Hokushi-taimai and a Japanese upland and lowland variety by breeders Drs. Iwatsuki and Ujihara.

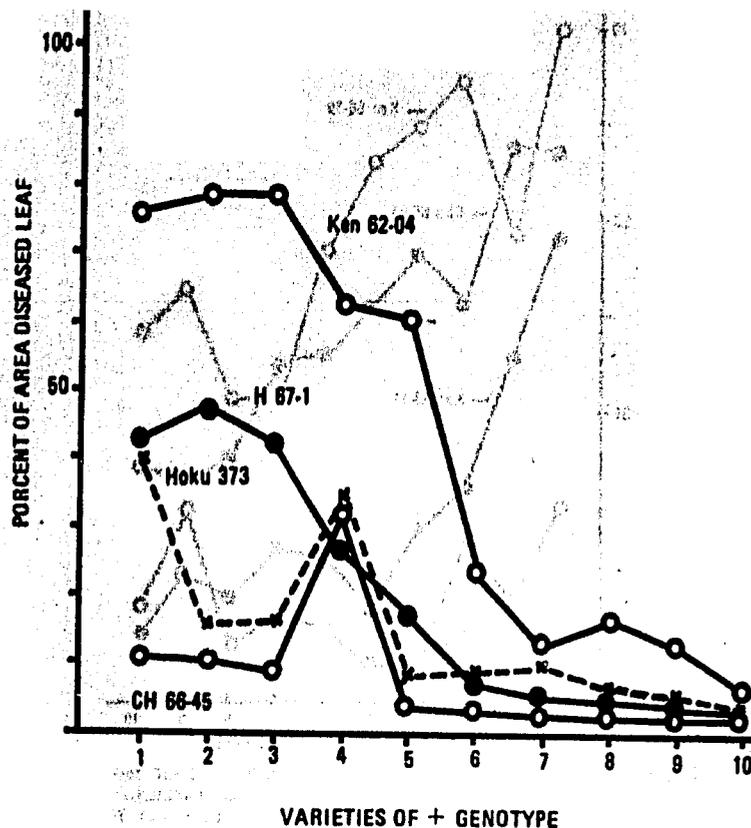


Figure 3. Differences in varietal responses to four isolates of rice blast disease. The listed varieties: (1) Norin 29, (2) Manryo, (3) Kos-hihikari, (4) Norin 8, (5) Norin 25, (6) Norin 22, (7) Nohonbare, (8) Chiyohikari, (9) Kogane-nishikik, (10) Ginga.

It is immune to the Japanese N race group, which includes the most predominant races in Japan, and also highly horizontally resistant for more than ten years to Japanese races C-8 and C-1, the second dominant races.

As mentioned at the beginning, I understand horizontal resistance as a quantitative one which can be measured by either the number or the size of lesion of susceptible reaction type, but not as a qualitative resistance detected by the reaction type of lesion or by the hypersensitive reaction of host cells. In this tentative definition, the resistance observed in St. 1 and Minehikari is, beyond doubt, considered to be horizontal resistance, because they produce a small number of lesions to some isolates and a larger number of lesions to some other isolates, but the resistance of these varieties is found to be an isolate specific, probably controlled by a single major gene. Isolate specific resistance does not differ essentially from race specific resistance, which is known generally to be qualitative.

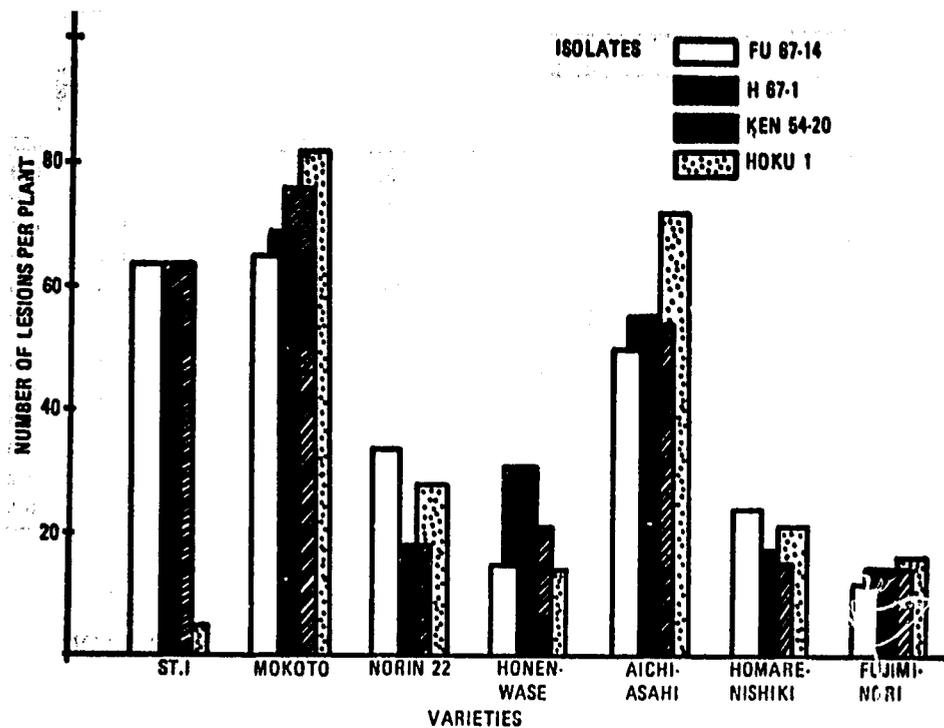


Figure 4. Varietal resistance to four isolates of the same race of the rice blast disease. Note the response of a variety St. 1. (Sakurai, 1969).

As many varieties have been recognized as highly horizontally resistant with the tests using a limited number of isolates, there is a small possibility that some of them may be highly susceptible to some unknown isolates, with a higher possibility in more resistant varieties.

These facts suggest much difficulty in evaluating horizontal resistance of the varieties with a limited number of isolates.

It is well known that plants are more susceptible at the younger stage, 4-5 leaf stage, and become more resistant with aging (Table 4).

The rate of increase of resistance with aging differs among varieties, although it was influenced by other factors such as fertilizer. Some varieties which are of the most susceptible at the younger leaf stage become more resistant at the older stage in comparison with some other susceptible varieties.

Table 4. Change of varietal resistance of rice with aging. Figures indicate the degree of disease of 0 (healthy) to 10 (dead). (Central Agr. Exp. Sta. 1967).

Varieties	Resist. genes	Leaf stages at the time of inoculation				
		4	6	8	10	12
Kongo	PI-k, a	5.0	5.5	2.3	2.2	0.2
Esunan 30	PI-k, a	7.0	7.0	4.3	3.5	1.0
Senshuraku	PI-k, a	-	-	4.8	1.5	0.7
Kanto 59	PI-k, a	10.0	8.5	5.5	4.5	1.8
Kasabus	PI-k, a	10.0	9.0	6.3	5.5	0.8
Shinano-hikari	PI-l	9.0	8.0	3.0	0.7	1.5
Takano-nishiki	PI-l, a	8.5	6.0	4.8	3.7	0.5
Homare-nishiki	PI-a	7.0	7.0	1.8	0.7	0.3
Aichiasahi	PI-a	9.5	8.5	6.3	5.5	2.0
Kogane-nishiki	-	-	-	4.5	2.1	1.0
Norin 22	PI-a	8.5	7.5	4.8	2.0	0.8
Norin 29	PI-a	9.5	8.0	5.2	4.3	0.8

The used isolate: Ken 60-19.

In many cases, a definite varietal difference in horizontal resistance is exhibited well after the 8 to 10-leaf stage.

The results indicated in Fig. 5 show that the susceptible varieties did not become resistant by the time of the 9 to 10-leaf stage, while resistant varieties become more resistant in earlier stages.

In the field under natural conditions, the disease occurs generally soon after transplanting at the 8 to 9-leaf stage in southern Japan, and after the 10-leaf stage in northern Japan. These facts support the importance for the practical purposes of the evaluation of horizontal resistance of older plants after the 8 to 9-leaf stage.

In Japan a good supply of nitrogen fertilizer and a long spell of cloudy days are major factors that favor the disease. Therefore, the difference in the response of the varieties to those two factors is important. The results showed that the order of the varieties in resistance was not so greatly disturbed by these factors. One of the results is shown in Fig. 6.

As little research was made, a definite conclusion has not yet been obtained regarding the influence of air temperature at the time of inoculation. Results indicated in Fig. 7 showed that the optimum temperature for infection is somewhat different among varieties, but generally the susceptible varieties are infected severely in any test temperature.

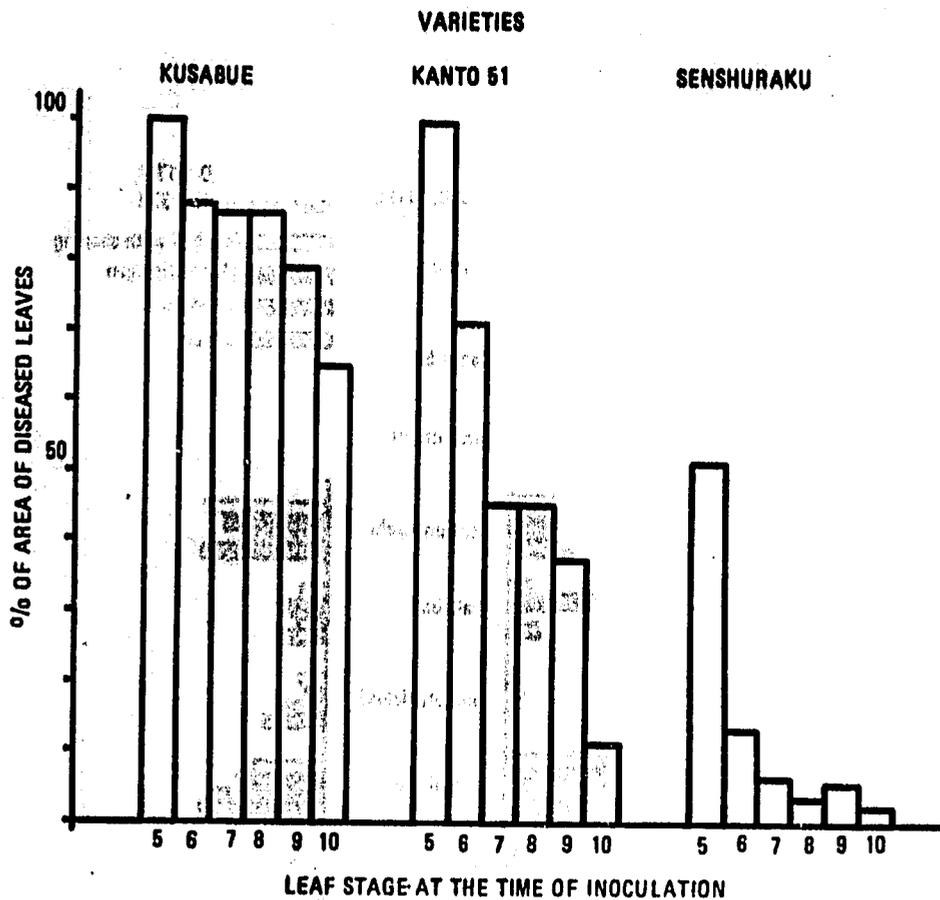


Figure 5. Varietal differences in increased resistance to rice blast disease according to aging of the rice plant. (Central Agr. Exp. Sta. 1968).

A definite conclusion has not yet been obtained regarding the influence of air temperature after inoculation. One of the results is shown in Table 5. Fewer varietal differences in response to the temperature were found.

With the increase of spore concentration, the number of lesions produced increases, but the rate of increase sometimes differs slightly among varieties (Fig. 8) even in the case with the same isolates.

Figure 9 shows a representative example of mixed inoculation. As shown in the figure, if one of the two isolates to be mixed is incompatible with the test varieties and the other is compatible, the number of lesions or size of lesions produced on the compatible varieties decreases proportionally to the mixing ratio of incompatible isolates due to the interaction between isolates, which is recognized to be the effect of production of phytoalexin (Ohata and Kozaka 1967). Mixed inoculation should be made with the isolates which are both virulent to the varieties to be tested.

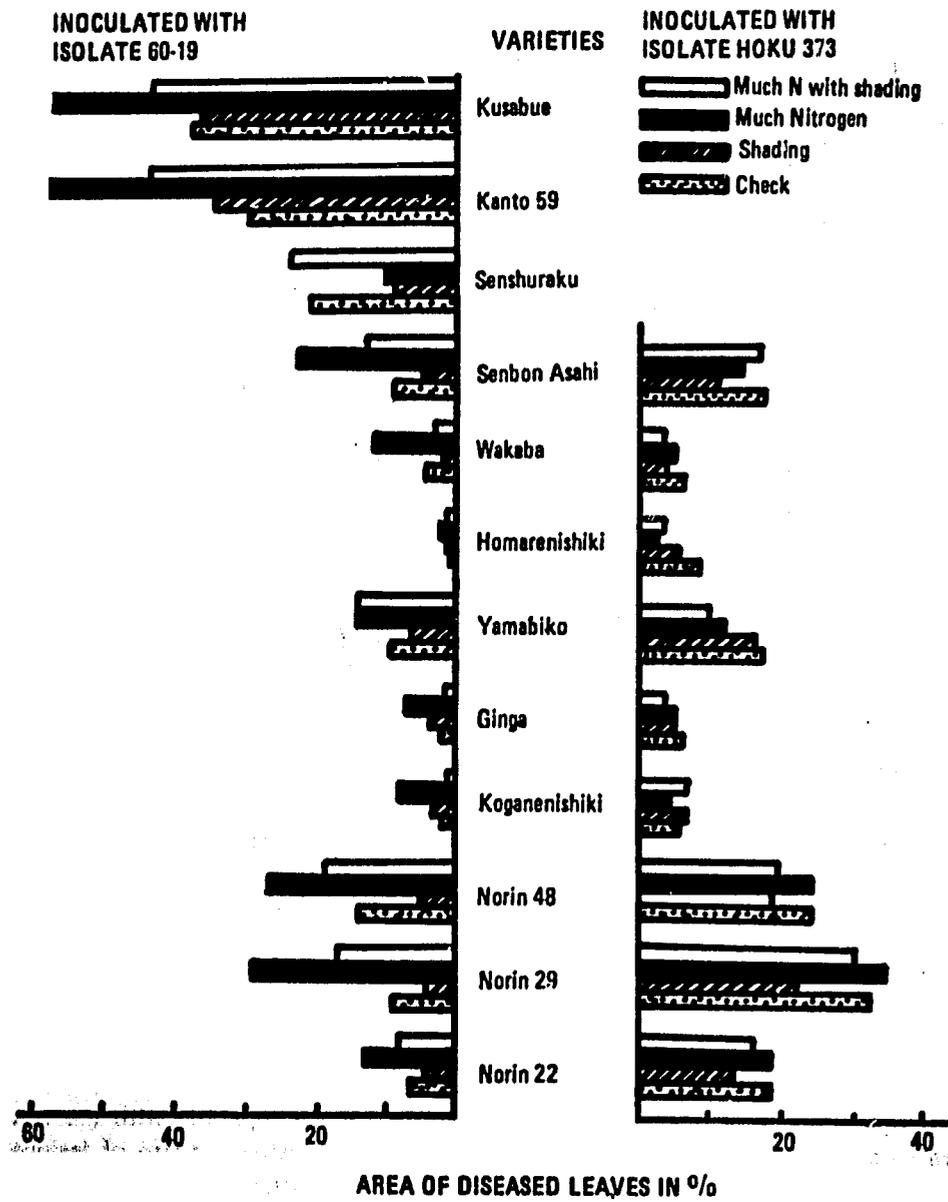


Figure 6. Effects of nitrogen fertilizer and shading from sunshine on resistance to rice blast disease in twelve varieties. (Central Agr. Exp. Sta. 1967).

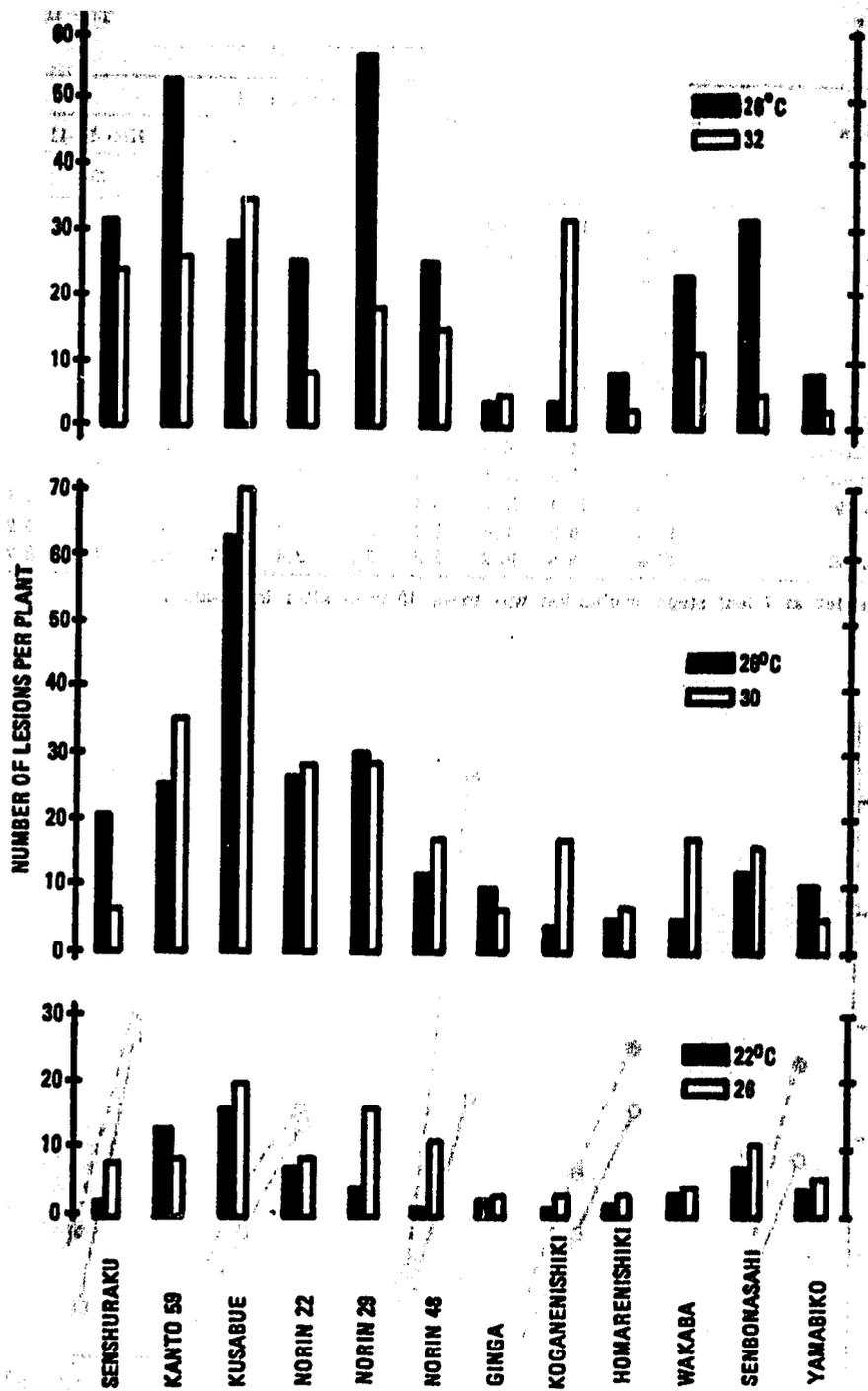


Figure 7. Effects of temperature at time of inoculation on varietal resistance to rice blast disease. Varieties were incubated for 25 hours at selected temperatures. (Central Agr. Exp. Sta. 1967).

Table 5. Effects of air temperature after inoculation on varietal resistance. The figures indicate number of lesions per leaf (Central Agr. Exp. Sta. 1968).

Varieties	Resistant genes	Isolates used								
		Th-63-252			Ken 64-117			Ken 53-11		
		20°	25°	30°C	20°	25°	30°C	20°	25°	30°C
Sanin 68	PI-k	13.8	10.5	8.5	6.6	2.7	2.2	1.6	1.6	0.5
Senahuraku	PI-k	5.1	5.5	3.3	3.6	2.9	1.5	2.0	1.3	0.1
Kanto 59	PI-k	14.7	11.0	11.1	4.0	2.5	3.0	2.3	1.3	0.3
Kasabue	PI-k	10.3	10.5	9.6	7.7	8.1	5.0	3.2	1.4	0.7
Ohu 248	PI-k	5.3	4.4	2.9	1.7	0.9	0.2	0.4	0.1	0.4
Senbonasahi	PI-a	4.9	5.7	1.4	1.8	1.3	2.0	1.2	0.4	0.4
Kinmaze	PI-a	10.1	6.7	2.1	3.1	1.2	0.3	0.7	0.2	0.0
Yamabiko	PI-a	5.6	5.1	2.8	2.1	0.4	1.0	0.6	0.2	0.1
Chiyohikari	+	4.8	4.5	2.6	3.3	2.4	4.5	0.4	0.3	0.0
Kogane-nishiki	PI-a	3.3	2.3	0.9	0.8	2.4	0.1	0.1	0.1	0.0
Norin 29	PI-a	13.4	15.8	8.8	7.6	6.8	2.9	2.0	1.8	0.4
St. 1	PI-a	6.3	2.8	1.0	3.2	1.0	0.1	0.6	0.0	0.2
Tozan 38	PI-a	8.6	10.2	2.3	3.8	2.4	1.7	0.6	1.2	0.2

Inoculated at 7 leaf stage, evaluation was made 10 days after inoculation.

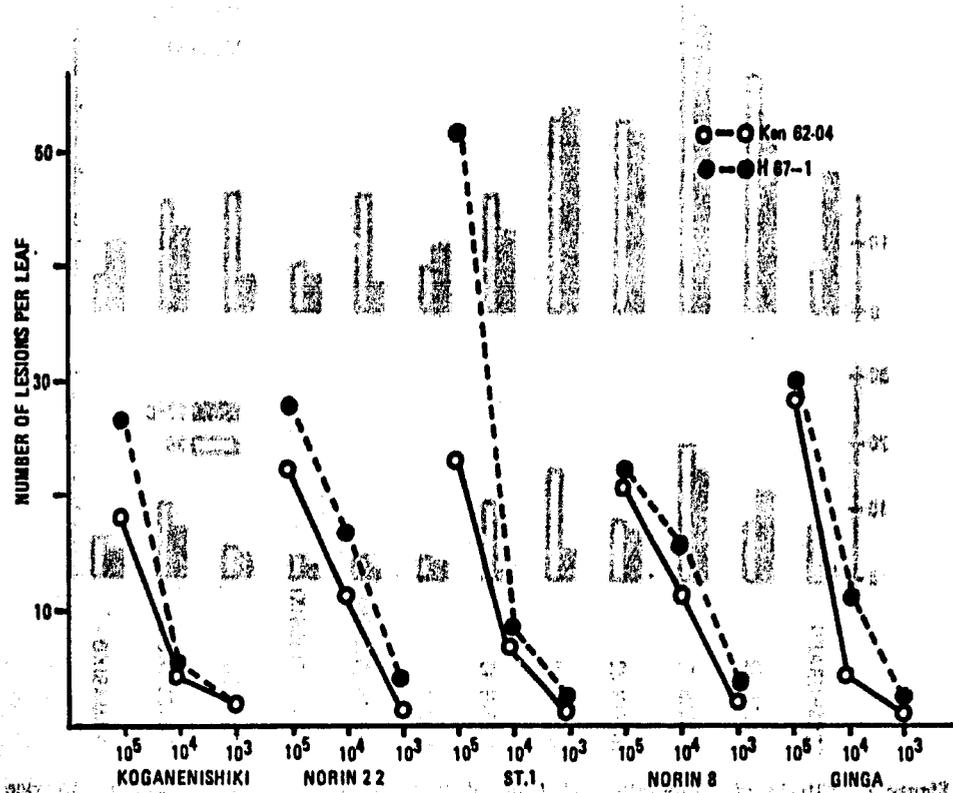


Figure 8. Effects of spore concentrations for inoculation on varietal resistance to rice blast disease. (Central Agr. Exp. Sta. 1967).

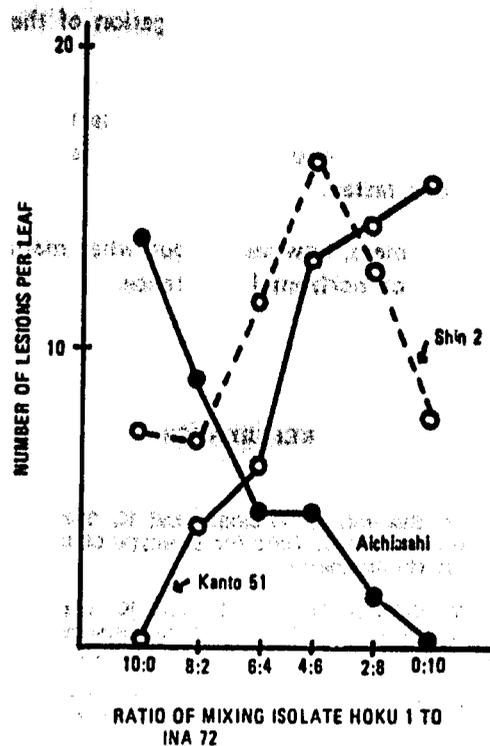


Figure 9. Effect of mixed inoculation with two isolates on varietal resistance. The variety Shin 2 is susceptible to one of two isolates and immune to the other. (Nat. Inst. Agr. Sci. 1968).

#### Inheritance of Horizontal Resistance

A few research varieties, Norin 22, Homarenishiki and Ginga, have been made. These varieties are all very old ones bred in Japan and recognized to be highly horizontally resistant experimentally for a long time. Recent experiments also support this.

Kiyosawa (1970) reported that segregation of the  $F_3$  progenies of the hybrids of Homarenishiki x Aichiasahi and Aichiasahi x Ginga against isolate Ken 54-04 under various environmental conditions could consistently be explained by one major gene and two minor genes. He also reported that Homarenishiki and Ginga have probably at least one common gene, and that a major gene of Homarenishiki and Ginga behaved independently to the race-specific resistance genes  $Pi-a$ ,  $Pi-k$  and  $Pi-l$ , which are carried in Aichiasahi, Kanto 51 and Ishikarishiroke, respectively.

Kiyosawa, Matsumoto and Lee (1967) suggested that the horizontal resistance of Norin 22 against isolate Ken 54-04 was controlled by one major and two

or more minor genes by the analysis of segregation of the  $F_2$  progenies of the hybrids of Norin 22 x Aichiasahi.

These results however, were obtained by a particular isolate Ken 54-04 using an injection method into sheathes. This isolate shows very weak virulence to the varieties tested.

Some questions still remain, however, about what method is most adequate to study the inheritance of horizontal resistance.

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**Factors which may express  
general resistance in rice  
to *Pyricularia oryzae* Cav.**

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Rice blast, caused by *Pyricularia oryzae* Cav., is perhaps the single most destructive disease of rice. Probably half of the world's rice is grown in the tropics (Jackson 1966). In temperate areas where rice is grown such as Japan, chemical control has been emphasized. Although effective chemical control is available for temperate zones, rice blast control in the tropics is limited by social, economic and environmental conditions, and economic chemical control for the tropics is not yet available. Thus, the development of rice varieties resistant to *P. oryzae* is essential.

No single rice variety has shown resistance to all races of the pathogen and in addition, the fungus has been shown to be highly variable, with a single conidium capable of producing many races (Ou et al. 1970). Nevertheless, the major sources of resistance to *P. oryzae* utilized in commercial plantings is simply or qualitatively inherited. Such specific or vertical resistance is rapidly lost with the appearance of new races. General or horizontal resistance has been shown to be stable and is not lost with the appearance of new races. Galvez et al. (1970), Nagai et al. (1970) and more recently Ou et al. (1971), have shown that such resistance is available in rice. If varieties can be bred

with such resistance, the resistance might be maintained indefinitely and not lost with the appearance of new races of the pathogen.

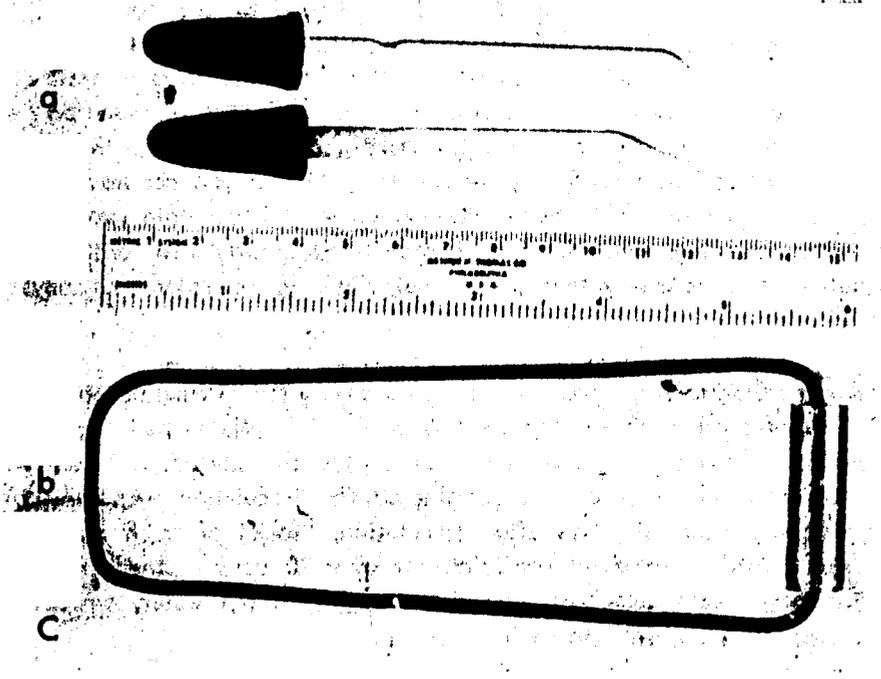
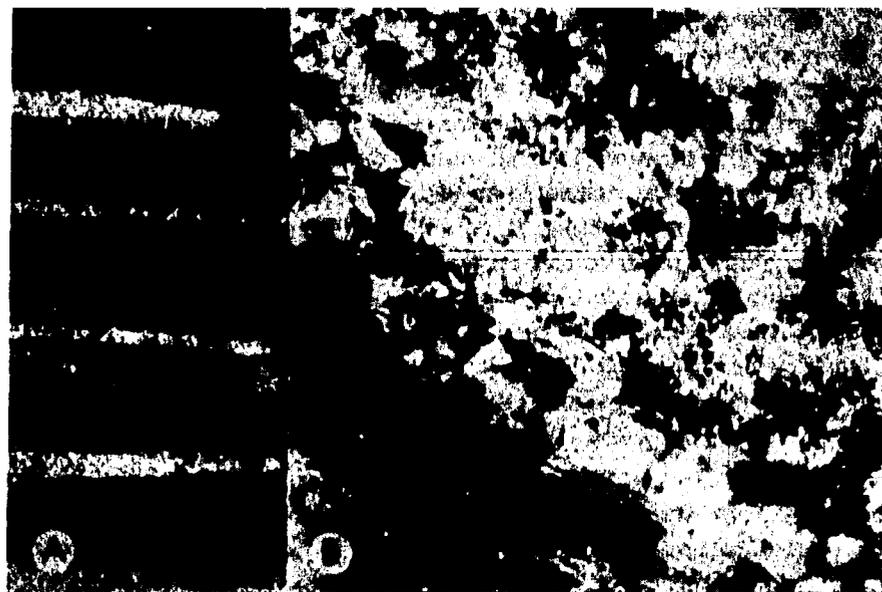
This study was made during 1969-1970 and presented as an M.S. Thesis in January 1971 by the senior author. The object of the study was to determine if consistent differences in characteristics which have been shown to be associated with general resistances in other crops such as potatoes (Thurston 1971), could be found in rice inoculated with *P. oryzae*. These characters might be useful in measuring and identifying relative levels of general resistance in rice. Characters studied were size of lesions, color of lesions, time for sporulation, number of spores produced, and time of ingress.

### MATERIALS AND METHODS

A serious problem in this study was growing healthy rice seedlings in the greenhouses and controlled climate chambers. Iron deficiency was a major problem and varietal susceptibility to the deficiency varied among the varieties used. Another problem, similar to what Latterell et al. (1965) called "winter sickness", was also common.

Numerous soil mixtures and environmental conditions were used in the attempt to grow healthy rice seedlings. The best success was obtained when seedlings were grown in a modification (Yorinori 1971) of the nutrient solutions of Hoagland and Arnon (1950) and Tanaka et al. (1964) for about 10 days and then transplanted to 4-inch plastic pots containing fine white sand. The potted seedlings were watered daily with 60 ml of nutrient solution per pot. Seedlings were grown in a greenhouse with a temperature variation of 26 to 38 C.

The isolates of *P. oryzae* used in the study were received from Dr. J.G. Atkins, U.S.D.A., Beaumont, Texas; Dr. C. R. Adair, U.S.D.A., Beltsville, Maryland, and from Dr. W. N. Harnish, Niagara Chemical Division, Middleport, New York. Cultures were maintained by monthly transfers on 2% rice polish agar slants containing one or two pieces of rice nodes. For initial tests inoculum was prepared using the techniques of Latterell et al. (1965). Of several artificial media tested, rice polish agar (Latterell et al. 1965) gave the best results, but many isolates sporulated poorly and with continued transfers had a marked decrease in sporulation. An observation that profuse sporulation occurred when conidia germinated on an injured part of the rice leaf (Fig. 1) led to the idea that the fungus could be grown and inoculum produced on fresh crushed leaves after surface sterilization. The youngest fully developed leaves of 30 to 40 day-old greenhouse grown plants were cut into lengths 5 to 6 cm long. The sections



**Figure 1.** A) Detached leaves of selection IR8 inoculated with *Pyricularia oryzae* isolate US5; hypersensitive reactions (a) and injured area where high sporulation occurred (b). B) Sporulation on the injured area (a, b). C) Microdrop pipettes used for inoculation of detached leaves (a) and an aluminum roller used for crushing the rice leaves for the culture of the fungus (b).

were surface sterilized by dipping into a 50:50 solution of alcohol and sodium hypochlorite for one minute, and then were washed in running water for 10-15 minutes in a 500 ml beaker covered with cheese cloth. Four or five leaf sections were stretched on a filter paper in a Petri dish moistened with 2 ml of 0.01 M monosodium phosphate and 70 ppm benzimidazole (Jackson 1966). Leaves were crushed by rolling them with an aluminum cylinder (1.3 cm x 3.5 cm long) (Fig. 1). The leaves were inoculated by taking a small piece of the fungus grown on rice polish agar and rubbing it on the leaf after which they were placed in the dark at 24 C. Most isolates produced spores three days after inoculation, and by the fourth day sporulated profusely (Fig. 1). Spore suspensions for inoculation were prepared by cutting the sporulating leaves into sections of about one cm long, placing them in test tubes with 2 to 3 ml sodium oleate-gelatin solution (Anderson and Henry 1946), and dislodging the spores by shaking the test tubes with a Vortex Jr. shaker.

For subsequent transfers pieces of the sporulating leaf were rubbed on newly prepared leaves. Using this procedure, it was possible to grow the fungus continually on fresh rice leaf tissue. Leaves of the varieties Peta, Saturn, Taichung Native 1, Binato and IR5-47-2 were used as the substrate. The varieties Taichung Native 1 and IR5-47-2 were highly resistant (lesion types 1 and 2) (IRRI 1965) to most of the isolates we have used in tests with excised leaves or whole plant inoculation, but abundant sporulation was observed when leaves of these varieties were used for culturing the fungus. The fungus grows better on the upper two thirds of the younger leaves. Older and basal portions of the leaves became brown to yellow within two to three days after crushing, and the fungus sporulated poorly. To obtain good sporulation it is necessary that leaves maintain a green color four days or more after crushing.

When sporulating leaves were dried 5 to 7 days after inoculation and stored at 3°C, spores were viable up to one week. All isolates had maximum sporulation by the fifth to sixth day, but beyond this period, if leaves were kept moist, the spores fell off and germinated. For inoculation purposes spores should be harvested 4-6 days after inoculation. Stocks of fresh rice leaves were maintained in excellent condition for over 20 days in the refrigerator at 3°C. Leaves were detached, washed thoroughly in tap water, wrapped in soaked paper towels, and stored in plastic bags.

The concentration of inocula used in all tests was 180,000 to 250,000 conidia/cc.

### **Inoculation of Detached Leaves**

The detached leaf technique, described by Hsu and Ou (1966), was used with a slight modification in an attempt to find a method of inoculation whereby size, color of lesions, time of sporulation, number of spores produced, and time of ingress could be accurately measured.

In order to test as many isolates and varieties as possible at the same time, the following experimental design was adopted. One isolate was used to inoculate the twelve youngest, fully expanded leaves detached from twelve 25 to 30 day-old plants. From each leaf, a 6 cm long section was cut from the middle, widest area of the leaf and placed in a Petri dish on moist filter paper. Six leaf sections were placed in one Petri dish.

To determine what part of the leaf is most suitable for the detached leaf inoculation method, tests were also made by inoculating the upper (A) and the lower (B) half parts of the same leaf. In this case eight leaf sections from four leaves were placed in each Petri dish, and three dishes were inoculated with one isolate.

Inoculations were made with a special microdrop pipette made by stretching a Pasteur pipette until a needle of 250 to 300 microns in diameter was obtained (Fig. 1). A small eyedropper rubber was fitted on the other end. Inoculation was done by placing three or four droplets (about 1 microliter) on the upper surface of each leaf section. Thus, each variety was inoculated with 36 to 48 microdrops. Due to the high surface tension of the droplets on the waxy surface of the rice leaves the droplets tended to remain attached to the tip of the needle or roll off when too large. This was solved by coating the tip of the needle with a thin layer of Vaseline, which tended to repel the droplets. Placement of droplets was also made easier by preparing the spore suspension with a solution containing Tween 20 (0.20 ml/l), or a gelatin-sodium oelate solution used as wetting agents. The latter was more effective.

To reduce the variation in spore number in each droplet due to settling, the eyedropper rubber was always partially compressed and, after three to four droplets were applied, a small amount of air was allowed to enter the pipette, thus mixing the suspension. One droplet of spore suspension was placed on each leaf section at a time. Inoculated leaves were incubated in the dark at a constant temperature of 21 ( $\pm 0.5$  C) or 24 ( $\pm 1.0$  C). Between 16 to 20 hours after inoculation, the plates were removed to the laboratory at room temperature and the droplets dried for 20 to 30 minutes under a fan after which the plates were again returned to the incubator.

In general the classification of lesion types was made according to the international classification, as proposed by Ou (1965) at the Symposium on

**Table 1. List of colors observed on lesions caused by *P. oryzae* on rice leaves inoculated in Petri dishes and the greenhouse.**

Symbols	Standard 1 color	Key to the Dictionary			Common 2 colors
		a	b	c <sup>3</sup>	
Olg	Olive green	22	B	6	
Opg	Opaline green	17	A	6	
Pg	Pea green	20	G	6	
Sg	Surf green	20	C	7	Green
Fl	Flax	12	B	2	
Ac	Acacia	11	K	1	Grayish-green
Cu	Cub	15	C	1	
Sb	Sandy Beige	14	A	3	Gray
Mg	Mineral gray	20	A	2	
Wj	White jade	10	A	2	
Iv	Ivory	10	B	2	Light pink
W	White				White
Gr	Graphite	48	C	7	Black, purple
Au	Autumn	8	A	12	Dark brown
Bu	Burnt umber	15	A	12	
Ch	Chipmunk	13	L	9	
Cm	Cinnamon	12	B	7	Brown
Sp	Spice	13	A	12	
Tv	Talavera	12	A	12	
Ap	Apricot	10	F	7	Reddish-brown
Gl	Gold leaf	11	K	8	
My	Martius yellow	9	I	1	Yellow
Loy	Light chrome yellow	10	L	4	
Sy	Spruce yellow	12	K	8	

1 According to the Dictionary of Colors (Maerz and Paul 1950).

2 Common names cited in the literature.

3 a- Plate number, b- Column, and c- Line.

Rice Blast Disease. The criteria for rating resistance and susceptibility on detached leaves was slightly changed. The lesion types were classified in 5 scale units and were primarily based on color, relative size of lesions, and on presence or absence of a necrotic center. For standard colors the Dictionary of Colors (Maerz and Paul 1950) was used. A list of colors used is given in Table 1. The necrotic center (area of collapsed cells), as mentioned in Ou's International classification corresponds to the central area of the lesions with a gray (Cu, Sb), opaline green (Opg), olive green (Olg), white (W) to pinkish (Iv, Wj) color which is surrounded by a ring of dark discolored cells. Table 2 gives the five scale units used and the characteristics of each scale.

Lesion color was observed eight days after inoculation and notes were taken under standard conditions from the center of the lesion toward the margin.

**Table 2.** Five scale units of disease reaction on detached rice leaves used in the classification of lesion types caused by *P. oryzae*.

Scale units	Lesion types <sup>1</sup>
1	Lesions limited to the site of inoculation; graphite (Gr) to burnt umber (Bu); no necrotic center; no yellow margin formed.
2	Restricted lesion, about 2-3 mm long; graphite (Gr), autumn (Au) to burnt umber (Bu) center; spice (Sp), gold leaf (Gl) to martius yellow (My) margin; no necrotic center.
3	Lesions up to 6 mm long; small (2-3 mm) opaline green (Opg), pea green (Pg), olive green (Olg), to ivory (Iv) necrotic center; thick graphite (Gr), autumn (Au), burnt umber (Bu) to spice (Sp) zone; and gold leaf (Gl) to martius yellow (My) margin.
4	Lesions up to 10 mm long; frequently with pea green (Pg), olive green (Olg) to mineral gray (Mg) necrotic center; burnt umber (Bu) and occasionally graphite (Gr) zone; gold leaf (Gl), martius yellow (My) to light chrome yellow (Lcy) margin.
5	Lesions more than 10 mm long; pea green (Pg), opaline green (Opg) to olive green (Olg) center; rarely with burnt umber (Bu) zone; spice (Sp), gold leaf (Gl), light chrome yellow (Lcy) to martius yellow (My) margin.

<sup>1</sup> Color of the lesions are from the center toward the margin.

Lesion size was measured only once eight days after inoculation. The criteria adopted for the measurements were as follows: six sections were chosen at random from each variety with twelve leaf sections inoculated; of these, two lesions, the largest and the smallest were measured. The length and width of each lesion was measured and multiplied, and the result given in mm<sup>2</sup>. The results were then presented as the average size (mm<sup>2</sup>) of twelve lesions. Likewise, when the apical (A) and the basal (B) portions of the same leaf were inoculated, twelve leaf sections of each leaf position were inoculated. Recordings of size were made as described above, first, separately for each leaf portion (A and B), and secondly, as the average between both.

Observations on the time of sporulation were made daily from the third day after inoculation up to the tenth day. Notes were taken on the first appearance of conidia using a microscope at 100X. After each observation the plates were put back into the incubator.

The number of spores produced was determined ten days after inoculation. Great variation in lesion types within and between varieties was observed. Some varieties had no sporulating lesions, while others had few to almost all of the lesions producing spores. To measure production, the five best sporulating lesions of each variety were selected using an 80X dissecting microscope. In some cases only two or three lesions had sporulated and spore production could be counted directly under a 100X microscope.

The five lesions were cut from the leaf with a scalpel, washed in a test tube with 1 ml of gelatin-sodium oleate solution, and the spores dislodged by

shaking the test tubes with the Vortex Jr. shaker. The numbers of spores were given as the average of six aliquots taken with a Pasteur pipette and counted with the Spencer "bright line" hemacytometer. Results are given as the average of five lesions.

### **Inoculation of Greenhouse Plants**

Greenhouse-grown plants were also inoculated in order to compare the reactions of inoculations of detached leaves with the reactions on plants in pots. The main objective of this work was to test as many varieties and isolates as possible and then select those varieties showing lesion types 3 or 4 for use in tests with the detached leaf technique. However, due to difficulties in growing the rice plants, both tests had to be made simultaneously whenever healthy leaves were available.

Observations were also made on lesion types, lesion color, time of sporulation, number of spores produced and time of ingress.

For inoculation tests of greenhouse plants, seedlings were grown for 25 to 30 days under the conditions previously described. Six or eight pots, each representing one variety and containing seven seedlings, were sprayed with 20 ml suspension of conidia of one isolate. Inoculum concentration was the same as previously described. When more than one isolate was used simultaneously, the concentrations were adjusted as closely as possible. Inoculations were always made between 8:30 and 9:30 PM, when greenhouse temperatures were lower and the relative humidity higher.

Inoculations were made using a DeVilbiss hand atomizer No. 127, attached to a General Electric 1/6 HP vacuum pump with 10 pounds pressure, and with the nozzle held about 24 to 28 cm from the plants. Before inoculation, the seedlings were sprayed with about 10 ml of distilled water. Since the inoculations were made outside of the humid chamber, this extra spraying was done to prevent drying of the inoculum droplets before the plants were taken to the incubation chamber. Check plants were sprayed only with the gelatin-sodium oleate solution. Following inoculation, the seedlings were maintained in a plastic chamber (1.20 m x 1.82 m x 1.36 m high), which was built by covering a steel frame over a greenhouse bench with plastic. The chamber was divided in three sections using plastic so that three isolates could be tested at one time. The base of the chamber was covered with a plastic sheet to hold a nutrient solution (about 0.5 cm deep), in which the pots were left for 20 to 24 hours after inoculation. After all plants were inoculated, the plastic chamber was sealed and high inside humidity was maintained for 12 to 14 hours by producing

a mist with a DeVilbiss atomizer attached to the vacuum pump and set for 15 pounds pressure. Twelve to fourteen hours after inoculation, before the inside temperature went above 30 C, the chamber was partially opened, so that the cooler air could circulate inside. Temperatures in the moist chamber varied from 21 C at night to 27 C during the day. Although a constant high humidity was maintained throughout the incubation period, the maximum relative humidity recorded was around 96 percent. Following the high humidity period of 12 to 14 hours, the relative humidity in the chamber varied from 54 percent during the day to 86 percent during the night. Two days after inoculation, the plants were watered daily with 60 ml/pot of nutrient solution.

Notes on lesion types were taken eight days after inoculation using the first five scale units of the international classification. Since the readings of greenhouse tests were taken at a shorter time after inoculation, and because greenhouse experiments had a higher inoculum concentration and more favorable conditions for symptom development than tests with detached leaves, the international classification was slightly modified and adapted for the greenhouse tests. In the international classification, the scale units 5 to 7 are based on lesion number and area affected. In these tests, the scale 5 is meant to include all the susceptible reactions beyond scale 4 (Table 3). The color of the lesions given indicate only the most predominant discolorations observed, and are based on the color charts of the Dictionary of Colors. Lesion color was determined eight days after inoculation, and the method was the same as for the tests using detached leaves.

Table 3. Five scale units of disease reaction on rice plants inoculated in the greenhouse, used in the classification of the lesion types caused by *P. oryzae*.

Scale units	Lesion types
1	Only small, brown (autumn to spice) specks, few or many with no necrotic (collapsed cell) spots.
2	Slightly larger (2-3 mm in diam), graphite (Gr), autumn (Au) to spice (Sp) spots, with gold leaf (Gl) margin; no necrotic (collapsed cell) spots.
3	Small, roundish, necrotic, gray (Cu or Sb) to olive green (Olg) center (about 1-2 mm in diam); surrounded by graphite (Gr), autumn (Au) to spice (Sp) and gold leaf (Gl) margin, which is somewhat elliptical.
4	Typical blast lesion, elliptical, somewhat restricted (up to 6 mm long), with large necrotic, gray (Cu or Sb), olive green (Olg) to opaline green (Opg), and sometimes white center; graphite (Gr), autumn (Au), spice (Sp) to gold leaf (Gl) margin.
5	Lesions larger and broader than in scale 4 (more than 6 mm long); large gray (Cu or Sb), olive green (Olg), opaline green (Opg) to white center; graphite (Gr) to autumn (Au) not always present, mostly spice (Sp) to gold leaf (Gl) margin; upper portion of seedling leaves may be killed by coalescence of large lesions.

Adapted from the international classification for field and nursery tests (Ou 1965).

On the same day when notes were taken on lesion type, infected leaves which were fully expanded at the time of inoculation were detached and placed in petri dishes with moist filter paper. The time of sporulation was then checked, considering the time of detaching as the 0 hour, and thereafter, observations were made every hour, up to 10 hours, following at 12, 14, 16, 24, 30 and 48 hours. Notes were taken on time of first emergence of conidiophores and first appearance of conidia. Observations were made at room temperature and between each observation, the plates containing the leaves were kept in the dark at 24° C.

Forty-eight hours after infected leaves were detached, spore counts were made by dissecting out the five best sporulating lesions of each variety, and washing them in 1 ml of gelatin-sodium oleate solution in a test tube. Spores were dislodged by shaking the test tube with a Vortex Jr. shaker. Six aliquots of the spore suspension was taken with a Pasteur pipette and counted with a Spencer "bright-line" hemacytometer.

The number of sporulating lesions varied greatly from one variety to another and the criterion adopted was to take the five best sporulating lesions of each variety, from a population of seven plants (in one 6-inch plastic pot). Each test was made with the same number of plants for all varieties. In some cases, few lesions had developed and only two to three sporulating lesions were available. The results are given based on the average number of spores per five lesions.

## RESULTS

### Lesion Type

On both detached leaves and whole plants inoculated in the greenhouse a considerable variation in lesion types was observed even on the same variety inoculated with a single isolate, as shown in Table 4. The most susceptible lesion type was considered as representative of the actual reaction of the variety.

### Lesion Color

Smaller lesions generally had darker coloration, delayed sporulation, and reduced number of spores. A relationship of lesion color with other characteristics suggested that plants with lesions having green (surf green, olive green, pea green, opaline green or acacia) to gray (cub, sandy beige and mineral gray) centers were more susceptible, had larger lesions, more rapid

**Table 4. Range of lesion types on detached leaves of 13 rice varieties inoculated with five isolates of *P. oryzae*.**

Varieties and selections	Isolate number and lesion types 1				
	US5	27	59L13	68L4	68T1
IR8	1	1	- 2	-	-
Taichung (Native 1)	1	1	-	-	-
IR5-47-2	1 - 2	1	1 - 2	1	-
Fortuna	1 - 2	-	1 - 2	-	-
Padma	-	1 - 3	1 - 2	1 - 2	-
T-141	-	1 - 3	1	1	-
T-Km6	-	1 - 3	1 - 2	1 - 3	-
PI215-936	1 - 3	1	-	-	-
IR154-61-1	1 - 3	-	1 - 3	1 - 3	1 - 4
Bluebella	1 - 3	1	-	-	-
Bluebonnet 50	-	-	1 - 4	1 - 4	1 - 4
Peta	1 - 4	1 - 2	1 - 3	1 - 3	1 - 2
Binato	2 - 5	1 - 3	1 - 4	1 - 4	2 - 5
Saturn	1 - 3	1	1	3 - 5	2 - 5

1 Lesion types: 1 - Highly resistant; 2 - Resistant; 3 - Moderately resistant; 4 - Susceptible; 5 - Highly susceptible.

2 Material not available.

sporulation, greater number of spores formed, and a larger yellow margin. White to pinkish (ivory or white jade) centers occurred in many lesions of types 3, 4 and 5 and on these light-colored areas sporulation was delayed and fewer spores were formed. Lesions with wider black or purple (graphite) to dark brown (autumn and burnt umber) and brown (chipmunk, cinnamon, spice or talavera) areas had lesions with a center with restricted size (1 to 2 mm in diameter) and usually white to pinkish color. The lesion size was restricted by the yellow margin, sporulation was delayed, and there were less spores produced.

Detached leaves generally had more susceptible reactions than plants inoculated in the greenhouse with the same isolates. The lesions were darker in color among the resistant varieties in the detached leaf inoculation as compared to susceptible varieties. The center of lesions on detached leaves was mostly green to gray and generally had less dark areas. Except for lesion type 3, no white or pinkish coloration was present in the center of lesions inoculated by the detached leaf technique while this was common in lesion types 3, 4 and 5 in the greenhouse inoculations.

#### Size of Lesions

Lesion size was only measured on detached leaves. Lesions formed on susceptible varieties and selections had a large yellow margin that often

**Table 5. Size of lesions (mm<sup>2</sup>), measured eight days after inoculation of detached leaves of rice with *P. oryzae*.<sup>1</sup>**

Varieties and selections	Isolate no. and size of lesions (mm <sup>2</sup> ) <sup>2</sup>				
	US5	27	59 L 13	68 L 4	68 T 1
Padma	-	2.87 (3) <sup>3</sup>	1.07 (2)	1.06 (2)	- <sup>4</sup>
T-141	-	3.78 (3)	1.00 (1)	0.77 (1)	-
TKM-6	-	5.11 (3)	1.86 (2)	7.11 (3)	-
IR154-61-1-1	9.37 (3)	-	2.77 (3)	1.45 (3)	4.22 (4)
Bluebonnet 50	-	-	13.42 (4)	14.26 (4)	13.25 (4)
Peta	5.70 (4)	3.09 (2)	11.37 (3)	6.33 (3)	1.14 (2)
Binato	25.74 (5)	6.25 (3)	8.08 (4)	12.70 (4)	16.90 (5)
Saturn	1.53 (3)	1.00 (1)	1.00 (1)	29.99 (5)	25.46 (5)

<sup>1</sup> Varieties, isolates and interaction differed at 1% level.

<sup>2</sup> Average of 12 lesions/isolate.

<sup>3</sup> Number in parentheses indicates the lesion type.

<sup>4</sup> Not tested.

coalesced with the neighboring lesions. In all measurements the entire colored area of the lesions was considered. Results are given in Table 5. Lesion size of varieties and selections showing only hypersensitive reactions did not enlarge beyond the area where the microdrops of inoculum had been placed. The lesions rarely measured more than 1 mm in diameter. Variation was observed within the same lesion type on the same variety when inoculated with different isolates. This may have been due to the varying number of lesions, which were selected as being typical and taken as the basis for classifying the lesion types of each variety and selection. In general the results indicated that the average size of lesions increased with greater susceptibility (Table 6). Analysis of variance on size of lesions made among varieties Binato, Peta and Saturn, and the five isolates shown in Table 5, indicated a highly significant difference among isolates, among varieties and in the interaction variety X isolates. Each variety had 12 lesions measured (mm<sup>2</sup>), representing 12 replicates out of 36 or 48 lesions developed on 12 leaf sections.

Inoculations made on the apical (A) and basal (B) portions of the same leaf of different varieties and isolates to determine whether a difference existed

**Table 6. Range and average size of lesions (mm<sup>2</sup>) of each lesion type on detached leaves.**

Lesion type	Size of lesion (mm <sup>2</sup> ) <sup>1</sup>	
	Range	Average
1	0.77 - 1.00	0.99
2	1.00 - 3.09	1.84
3	1.71 - 11.37	5.07
4	4.22 - 14.26	10.22
5	16.90 - 29.99	24.82

<sup>1</sup> Average of 12 lesions/isolate.

in susceptibility, indicated no difference on the resistant varieties. Differences in lesion size among varieties and selections Padma, T-141, TKM-6, Peta and Saturn using isolates 27, 59L13 and 68 T1 are shown in Table 7. These differences were highly significant for varieties and leaf positions A and B. Size of lesions on position A was significantly smaller than on position B. The average size of lesions on both leaf positions A and B increased with an increase in susceptibility (Table 8). Considerable variation in size of lesion was observed among isolates.

Table 7. Comparison between size of lesions detached leaves, of the apical (A) and basal (B) positions of the same leaf, inoculated with *P. oryzae*.<sup>1</sup>

Varieties and selections	Leaf pos.	Isolate number and size of lesions (mm <sup>2</sup> ) <sup>2</sup>				
		US5	27	59L13	68L4	68T1
Padma	A	- <sup>3</sup>	1.00	1.00	1.00	-
	B		4.66	1.14	1.12	
T-141	A	-	6.14	1.00	0.92	-
	B		1.43	1.21	0.63	
TKM-6	A	-	0.95	1.07	0.64	-
	B		9.28	2.98	14.29	
IR154-61-1-1	A	9.37 <sup>4</sup>	-	1.50	1.45	1.91
	B		-	4.04		6.54
Bluebonnet 50	A	-	-	5.75	7.52	9.66
	B			24.50	21.00	16.85
Peta	A	5.59	1.01	6.75	4.91	1.19
	B	5.82	5.17	16.00	7.75	1.00
Binato	A	25.74	6.25	10.08	8.43	14.66
	B			6.08	16.97	19.25
Saturn	A	1.53	1.00	1.00	21.12	20.22
	B		1.00	1.00	36.87	31.70

1 Varieties, isolates, leaf positions and interactions differed at 1% level.

2 Average of 12 lesions/leaf position/isolate.

3 Not tested.

4 Only one leaf section/leaf was inoculated.

Table 8. Range and average size of lesions (mm<sup>2</sup>) on detached leaves (leaf position A and B), in relation to lesion types.

Lesion type	Leaf pos.	Size of lesion (mm <sup>2</sup> )	
		Range	Average
1	A	0.92 - 1.00	0.98
	B	0.63 - 1.21	0.96
2	A	1.00 - 1.19	1.04
	B	1.00 - 4.66	2.67
3	A	0.64 - 6.75	3.44
	B	1.43 - 16.00	8.79
4	A	1.91 - 10.08	6.99
	B	5.82 - 24.50	13.06
5	A	14.66 - 21.12	18.76
	B	19.25 - 36.87	29.27

### Time of Sporulation

Observations of sporulation on detached leaves were made daily, starting 3 days after inoculation and continuing for 10 days. No spores were formed on lesion type 1, only rarely on type 2, but spores were always observed on lesion types 3, 4, and 5, often as early as 4 days after inoculation on types 4 and 5. Sporulation usually took longer on types 3 and 2.

The results of sporulation on seedlings inoculated in the greenhouse are more useful. A great variation in time of sporulation was observed in the same lesion type and on the same variety. Results on time of conidiophore and conidia formation are presented in Table 9. Conidiophores and conidia appeared as early as 1 hour after infected leaves were detached from the varieties Peta and Saturn inoculated with isolate 68L4. No conidiophores were formed 14 hours after leaves were detached and the longest period to produce conidia was 30 hours. Spores were not formed on lesion types 1 and rarely on type 2.

A comparison of range and average time of conidiophore and conidia formation with lesion types showed that lesion type 5 took the least amount of time to sporulate, and was followed by lesion types 4, 3 and 2 (Table 10). When conidiophores emerged four hours after leaves were placed in the moist chamber, conidia formation generally followed in one to three hours. When conidiophores took more than five hours to emerge, conidia formation was delayed from four to twenty hours (Table 9).

Table 9. Time (hr) of conidiophore and conidia formation by *P. oryzae* on rice leaves detached eight days after inoculation in the greenhouse and observed in moist Petri dishes for 48 hr.

Varieties and selections	Isolate number and time (hr) of conidiophore and conidia formation															
	US5		27		59L13		CSA10		68A12		68A14		68L4		68T1	
	a	b <sup>1</sup>	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Padma	x <sup>2</sup>		x		4	10	4	9	10	14	5	12	x		10	14
IR154-61-1-1	6	14	6	14	- <sup>3</sup>		-		-		-		-		10	14
PI215-936	x		x		x		x		5	14	4	12	x		5	12
T-141	x		10	14	x		8	14	14	24	x		x		4	7
Saturn	6	10	6	24	x		x		x		x		1	1	4	5
Peta	2	6	x		4	8	x		x		x		1	1		x
Binato	10	16	12	16	x		x		x		x		x		4	5
TKM	-		10	14	10	30	5	9	4	7	5	10	10	14	4	7

1 a - Time of conidiophore formation; b - Time of conidia formation.

2 Neither conidiophores nor conidia formed.

3 Not tested.

Table 10. Relationship of lesion types, range and average time (hr) of conidiophore and conidia formation in *F. oryzae*, produced on rice leaves detached eight days after inoculation in the greenhouse and kept in moist Petri dishes for 48 hr.

Lesion type	Conidiophore		Conidia	
	range	average	range	average
	hr	hr	hr	hr
1	x 1	x	x	x
2	8 - 14	11.06	14 - 24	18.00
3	4 - 12	7.16	3 - 30	14.41
4	1 - 6	4.55	1 - 24	11.00
5	1 - 5	3.43	1 - 10	5.85

1 No spores formed.

### Number of Spores Produced

The number of spores produced increased with an increase in susceptibility, both in the greenhouse and detached leaf inoculations. On lesion types 3, 4 and 5, the number of spores varied greatly within the same lesion type. The results obtained with seedlings in the greenhouse are given in Tables 11 and 12.

### Time of Ingress

A study of time of ingress with the detached leaf technique failed to show notable differences among varieties and isolates. Likewise, in a greenhouse test, no relationship was observed between time of ingress and the other characteristics studied in the one test made; therefore, the results of these tests are not given.

Table 11. Number of conidia of eight isolates of *F. oryzae* produced on rice leaves, detached eight days after inoculation in the greenhouse and 48 hr after being placed in moist Petri dishes.

Varieties and selections	Isolate number and number of conidia produced <sup>1</sup>							
	US5 1000X	27 1000X	59L13 1000X	68A10 1000X	68A12 1000X	68A14 1000X	68L4 1000X	68T1 1000X
Padma	0	0	1.66	9.33	1.33	0.66	0	2.50
IR154-61-1-1	1.80	0.55(4) <sup>2</sup>	-	- <sup>3</sup>	-	-	-	-
PI215-936	0	0	0	0	0.33	0.16	0	5.33
T-141	0	0.07(3)	-	0.07(3)	0.10	0	0	18.00
Saturn	6.20	0.26	0	0	0	0	6.14	20.20
Peta	2.00	0	1.75	0	0	0	11.80	0
Binato	0.05	0.14	0	0	0	0	0	18.33
TKM	-	0.03(2)	0.10	9.00	4.50	3.12	0.50	2.70

<sup>1</sup> Results are the average of five best sporulating lesions selected under 80X dissecting microscope.

<sup>2</sup> Numbers in parentheses indicates number of sporulating lesions that were available.

<sup>3</sup> Not tested.

**Table 12.** Relationship of lesion types with range and average number of conidia of eight isolates of *P. oryzae* produced on rice leaves, eight days after inoculation in the greenhouse and 48 hr after being placed in moist Petri dishes.

Lesion type	Range	Number of conidia	Average
1	0		0
2	50 - 100		73
3	30 - 2500		669
4	160 - 9330		4447
5	2000 - 20200		10878

### DISCUSSION

One of the most difficult problems encountered in making this study was the diversity of lesion types observed on the varieties and selections used. This occurred even when a single isolate of *P. oryzae* was used. Such results might be expected when one considers the results of Ou et al. (1970). Kobayashi and Abumiya (1960) found that higher concentrations of inoculum resulted in severe damage to the leaves, and that varieties differed in the number of lesions produced with varying inoculum concentrations. It is possible that the differences in size and inoculum concentrations of the droplets on the leaves may be the explanation for the highly resistant reactions which were observed along with highly susceptible reactions on the same leaf.

Fewer reports on the actual size of lesions and their relationship with color, shape and sporulation were found in the literature. References to size of lesions and their relationship to resistance or susceptibility in the literature were usually made as "small" or "large" lesions.

It was found that the intensity and distribution of lesions colors may be a good indication of the degree of resistance of the rice plant to the blast fungus. Differences in color of lesions observed between detached leaf and greenhouse inoculations should be studied more carefully. Study should also be made on whether or not the color and other characteristics observed on detached leaves actually indicate the reaction patterns that are expressed by plants exposed to greenhouse or field inoculations. As mentioned by Kobayashi and Abumiya (1960) inoculum concentration affects disease severity and may also alter the color intensity of the lesions. In future studies it would be useful to standardize, as well as possible, the concentration of inoculum in the droplets of spore suspension used to inoculate detached leaves and also the inoculum used for field or greenhouse inoculations. The lighter color and higher susceptibility observed in detached leaf inoculations may be related to higher concentration of inoculum in each droplet.

Toyoda and Suzuki (1952) observed that sporulation on greenhouse plants was more rapid and abundant on lesion type 4, less on 5 and 3, and spores were sparsely formed on 2 and were absent on type 1. The present study indicates that the average time (hr) of sporulation on greenhouse inoculated plants was shorter on lesion type 5 with more abundant spores being formed. Also in contrast to Toyoda and Suzuki (1952), the number of spores produced was highest in lesion type 5 when detached leaves were inoculated.

Sporulation was more abundant on detached leaves in Petri plates than on leaves inoculated in the greenhouse and then detached. Detached leaves also gave a more susceptible reaction. This may have been caused by the yellowing which began from the cut ends within four to five days. In many instances the leaves were entirely yellow before any symptoms developed. A more valid reaction would undoubtedly be obtained with leaves from healthy plants.

Differences in susceptibility between the apical and the basal portion of the last fully developed leaves could be related to tissue age. The leaf unfolds or emerges from the tip, thus the basal section has the youngest tissue. This observation agrees with that of Kato et al (1970) that sporulation was greater when leaves were inoculated at the middle stage of expansion. Kahn and Libby (1958) reported that younger upper leaves were most susceptible, with resistance increasing upward. They also found the basal portion of the leaf most susceptible. For future tests it would appear that only the basal half of the youngest, but most fully developed, leaf should be used for the detached leaf inoculation. No relationship was observed between time of ingress and susceptibility, and as experiments made during this study were not replicated they should be repeated with additional varieties before definite conclusions can be drawn.

It should be pointed out that the present studies were made under conditions unfavorable for normal rice plant development. Plant chlorosis and other physiologic disorders, referred to as "winter sickness", were constant problems. Therefore, experiments should be made with healthier plants, before conclusions can be made regarding the relationships among the characteristics observed. Experiments should be repeated with healthy plants, grown together under identical conditions and inoculated with the same spore suspension. Since physiological disorders in rice plants seem to be a common problem in greenhouse culture (Latterell, Marchetti, and Grove 1965), experimental results should be confirmed with healthy plants grown in the field.

No conclusions can be made as to what might represent general resistance in rice to *P. oryzae* from the results of these studies. In addition the results obtained were highly variable. Nevertheless, it has been demonstrated that the characteristics considered can actually be measured, and the study may serve as a useful source of information for further studies.

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**Indications of partial resistance of rice  
to the fungus *Pyricularia oryzae* Cav.**

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The rice (*Oryza sativa* L.) blast disease caused by *Pyricularia oryzae* Cav. is one of the limiting factors in rice production in the tropics. Many efforts have been made to obtain resistant varieties to this pathogen in several countries throughout the world, but without much success.

The use of "major gene resistance" ("vertical resistance" or "specific resistance") has failed in the control of certain diseases due to the great variability of some plant pathogens. Because of this fact, researchers are looking for a different type of resistance usually controlled by minor genes, that hereinafter will be referred to as "partial resistance" ("horizontal resistance", "general resistance", "race non-specific resistance", "stable resistance", etc.). Plants showing partial resistance may develop low disease

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levels, and they should not be affected by specific races (Black 1954, van der Plank 1968).

The fungus *P. oryzae* has a great variability, as shown by the identification of several races from monoconidial subcultures. It suggests frequent genetic changes of the pathogen (Giatgong and Frederiksen 1969). The genetic variability is even more complex when one considers that Ou and Ayad (1968) were able to identify 14 races from 56 monoconidial cultures obtained from just one lesion.

The pathogenic variability is still not understood, as the perfect stage of the fungus has not been found. Nevertheless, Hebert (1971) has obtained artificially the perfect stage of *P. grisea* (Cke.) Sacc., *Ceratospheeria grisea* n. sp., a species very similar to *P. oryzae*.

Type 3 lesions, producing few conidia per day (15-50) in short periods of time, and few numbers of type 4 lesions, have been considered as an indication of partial, stable, field, horizontal, or polygenic resistance in the rice blast disease work (Ou et al. 1971, IRR1 1968, IRR1 1970).

Ou and his co-workers (1971) have suggested that the varieties Carreon and Tetep have horizontal resistance because they showed few lesions of any type per plant. These varieties showed either highly resistant or susceptible type lesions depending on the race. However, type 4 lesion number (susceptible) was always small in these varieties (Ou 1970).

Likewise, in searching for partial resistance in Colombia, it has been observed that certain varieties have shown a high and broad spectrum of resistance including Carreon, Tetep, and Colombia 3 after 20 continuous plantings under highly epiphytotic field conditions in los Llanos Orientales (Galvez, Rodriguez, and Puerta 1970). These varieties and others have also been constantly resistant in Brazil, Panama and Peru.

Size and color of the lesions, amount of sporulation, and speed of penetration of the fungus in the host have been used to evaluate resistance or susceptibility. Suzuki (1965) considers that plants showing pin-point and dark brown lesions are highly resistant, those having lesions of an intermediate size and brown color are moderately resistant, whereas those showing lesions of large size with white, purple, or green-gray color are susceptible.

The amount of sporulation per lesion is an important factor in considering varietal resistance to a plant pathogen. In the case of *P. oryzae* the amount of sporulation has been related to the type of lesion: the highest number of spores has been found in lesions showing a gray central zone and purple to dark brown borders (Kato, Sasaki, and Koshimizu 1970). However, a high

relative humidity, particularly during the night, is necessary for the occurrence of this type of lesion (Barksdale and Asai 1961).

This paper reports the study of some factors that might be involved in the partial resistance of rice to *P. oryzae*.

### MATERIALS AND METHODS

These studies were carried out in the greenhouses and laboratories of the Centro Nacional de Investigaciones Agropecuarias, "Tibaitata", of the Instituto Colombiano Agropecuario (ICA). The varieties used (Table 1) were selected for their high, intermediate and susceptible reaction to the races of *P. oryzae* present in the beds of the C.N.I.A., La Libertad, Villavicencio, in los Llanos Orientales, where the disease is endemic and epiphytotic throughout the year.

Rice seeds were planted in plastic pots 10 cms in diameter and 10 cms high containing loamy-sand soil. The plants were grown in a greenhouse for 20 to 25 days at a temperature of 20-30°C, a photoperiod of 12 hours, and 80 percent relative humidity before they were used.

Table 1. Rice cultivars used in the studies of partial resistance to *P. oryzae*.

Cultivar Name	Origin	Resistance grade (1-7) <sup>a</sup>
Fanny	France	7
Bluebonnet 50	U.S.A.	7
IR 8	Philippines	7
Colombia 3 (T 319 E-2M-2M-1M-5M)	Colombia	3,4
Fa Yui Tsai	China	5
Perola	Brazil	5
Iaca Escuro	Brazil	3
IR 8/2 x Zenith		
IR 1154-106	Philippines	1,2
Nahng Mon S4 x TN1		
IR 160-27-3-1-1-3	Philippines	5
IR 8 x (Dawn x TN1)		
IR 782-24	Philippines	1
Carreon	Philippines	1
Tetep	Japan	Many 1
C4615	Burma	1,3
Dissi Hatif	Senegal	3,4
Mamoriaka	Africa	3,4

<sup>a</sup> 1 = Highly resistant  
7 = Highly susceptible.

IB-1, IC-1, IG-1 and ID-8 of *P. oryzae* from Peru, Colombia (Llanos Orientales and Cauca Valley), and Brazil, respectively, were used. They were grown in rice-polish-agar (RPA) and oats-agar (OA) media at 2 percent of each ingredient. The cultures were kept at 4°C to stop sporulation. The inoculum consisted of an aqueous suspension of conidia (30 to 40 conidia per ml) from a fungus culture grown during 12 days in an incubator at 25°C and a 12 hour daily fluorescent light exposure. Gelatine (0.25 percent) was added to the inoculum suspension as a spreader.

In the laboratory, the Hsu and Ou (1966) technique was used for inoculating four leaf portions 6-7 cm long, previously disinfested by immersing them in a 50:50 solution of alcohol (95 percent) and sodium hypochlorite (5.25 percent) for one minute. Then they were immediately washed in distilled water for 15 minutes.

The leaves were placed in Petri dishes on filter paper moistened in an aqueous solution of monosodic phosphate at 0.01 M. Then the inoculum was sprayed using a manual atomizer De Vilbiss No. 14 at 20 cm. Fanny and IR8 leaves were sprayed only with an aqueous solution of gelatine (0.25 percent) as absolute checks.

Twenty plants/variety in four pots were inoculated in the greenhouse. The inoculum was prepared to contain 30-40 conidia per 100X-microscope field, and sprayed at 10 p.s.i. by a De Vilbiss No. 15 atomizer. Fanny and IR8 were sprayed only with a gelatine (0.25 percent) solution as checks. The plants were kept in the dark for 24 hours in a growth chamber at 20-30°C and 100 percent relative humidity. Then they were supplied with a 12 hour photoperiod for an additional 72 hours under the same conditions.

The evaluation of lesion types (Fig. 1) was made according to the international scale (Ou 1965). The readings were taken eight days after the inoculation.

The lesion in each variety were measured eight days after inoculation. The width and length of eight lesions at random were measured, and the results were averaged in the laboratory tests. The lesions, taken at random from each of the four replications, were measured for each variety in the greenhouse.

The lesion color from the center to the borders was determined under a stereo microscope eight days after inoculation, using Ridgway's scale (1912).

From the third day after the inoculation the sporulation time was daily determined under a light microscope at 100X. Individual lesions of leaves maintained in Petri dishes were observed daily for ten days.

The number of conidia per lesion type was determined from the five best visible lesions in each variety after ten days of the inoculation. The

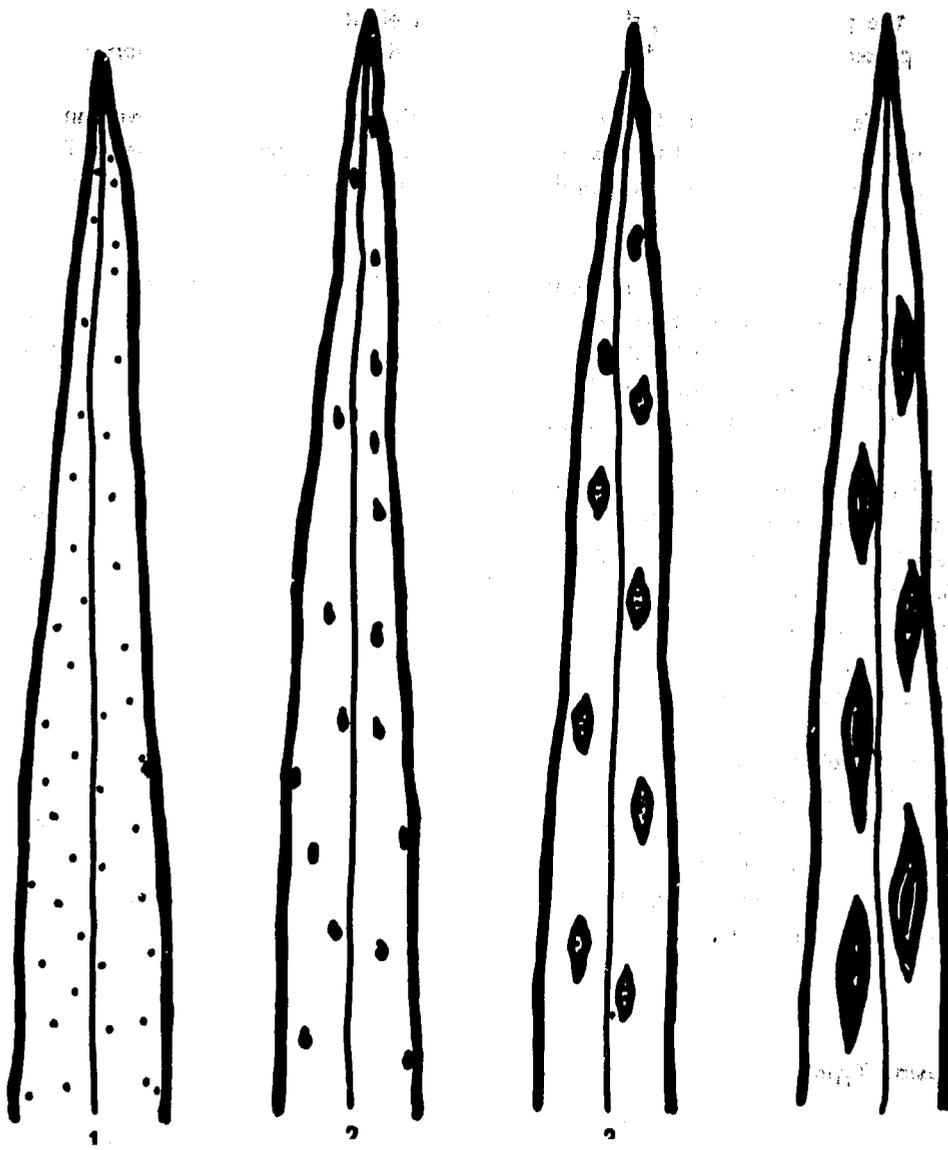


Figure 1. Degrees of reaction to rice blast disease.

lesion was cut and placed in one-ml aqueous gelatine solution (0.25 percent) in a test tube. After one minute of shaking, aliquots were examined in a hemacytometer to determine the number of conidia per ml.

In the greenhouse tests, the leaves were cut eight days after the inoculation and placed on moistened filter paper for two days before examination.

In order to determine the influence of relative humidity in the development of the lesion type, race IB-1 was used. The leaves were placed as usual in Petri dishes and sprayed with a conidia suspension containing 30-40 conidia per 100X microscope field.

The inoculated leaves were kept at 25°C in the dark. IR8 and Fanny were sprayed only with 0.25 percent gelatine. The excess inoculum was dried by hot air after 0, 6, 8, 10, 12 and 14 hours of exposure at 100 percent relative humidity. To avoid moist condensation on the leaves, a dry filter paper was placed at the top of the Petri dish.

The influence of the relative humidity in the development of the lesion type under greenhouse conditions was determined by inoculating 30 plants per variety with a conidial suspension of 30 to 40 spores per 100X microscope field. Groups of 5 plants per variety were exposed to 0, 6, 8, 10, 12 and 14 hours at 100 percent relative humidity and 25-30°C. The excess inoculum was dried as previously described. The lesion type was estimated eight days after the inoculation.

Six varieties showing type 3 and 4 lesions under greenhouse conditions were used to study frequency of sporulation. Leaf samples with type 3 and 4 lesions were placed on the inside top of a Petri dish that contained water agar (WA). The samples were kept in an incubator at 25°C in the dark and at 90 to 100 percent relative humidity. The conidia that fell down on the agar were counted daily under a light microscope (100X) for 18 days.

### **Lesion Type**

The results on lesion type are presented in Tables 2 to 6 for each variety and race. Great fluctuations were observed among varieties when they were inoculated with the different races under greenhouse and laboratory conditions. In general, type 1 lesions prevailed over the other types. Only the variety Fanny showed type 3 and 4 lesions with the race IB-1 in the greenhouse (Table 3).

Table 3. Lesion types on rice cultivars inoculated with 4 races of *P. oryzae* under laboratory conditions.

Cultivar Name	Race Identification and lesion type <sup>a</sup>			
	IB-1	IC-1	ID-3	IG-1
Fanny	2-4	2-4	2-4	2-4
Bluebonnet 50	1-3	1-3	2	1-2
IR 8	1-2	1	1	1
Colombia 3	1-2	1-3	1-2	1
Fa Yiu Tsai	1-2	1-3	1	1
Perola	1-2	1-3	1-2	1-2
Iaca Escuro	1-3	1-3	1-2	1-2
IR 8/2 x Zenith	1	1	1-2	1
Nahng Mon S-4 x TN1	1	1-2	1-2	1
IR 8 x (Dawn x TN1)	1-2	1	1-2	1
Carreon	1	1	1	1
Tetep	1	1	1	1-2
C4615	1-2	1-2	1-2	1-2
Dissi Hatif	1	1-2	1	1
Mamoriaka	1	1	1	1

<sup>a</sup> Lesion type (1-4) according to the International Scale.

Fanny was the most susceptible variety under laboratory conditions to all the races. Bluebonnet 50, Colombia 3, Fa-yiu Tsai, Perola, and Iaca Escuro showed an intermediate reaction (Type 3) only to the races IB-1 and IC-1. The rest of the varieties had a resistant reaction (Table 2).

All the varieties were more vulnerable to the pathogen under greenhouse conditions. With race IB-1, the highest number of type 1 lesions was presented by Carreon, type 2 lesions by Iaca Escuro, and type 4 lesions by Fanny. The rest of the varieties showed a fewer number of lesions (Table 3).

Table 3. Average number of lesions due to race IB-1 of *P. Oryzae* in 15 cultivars of rice under greenhouse conditions.

Cultivar Name	Type and average number of lesions 8 days after inoculation <sup>a</sup>			
	1	2	3	4
Fanny	29.00 <sup>a</sup>	17.25	48.75	57.00
Bluebonnet 50	117.75	34.75	46.50	4.50
IR 8	63.50	2.50	0.00	0.00
Colombia 3	179.50	4.50	1.50	0.00
Fa Yiu Tsai	90.00	13.50	15.50	0.00
Perola	94.25	3.75	2.00	0.00
Iaca Escuro	92.50	52.00	16.75	4.25
IR 8/2 x Zenith	134.25	0.25	0.00	0.00
Nahng Mon S4 x TN1	103.50	0.00	0.00	0.00
IR 8 x (Dawn x TN1)	143.00	0.00	0.00	0.00
Carreon	256.25	14.75	0.00	0.00
Tetep	24.50	0.00	0.00	0.00
C4615	182.50	6.00	0.00	0.00
Dissi Hatif	215.75	1.75	0.00	0.00
Mamoriaka	186.25	0.00	0.00	0.00

<sup>a</sup> Type of lesion (1-4) according to the International Scale.

**Table 4.** Average number of lesions due to race IC-1 of *P. oryzae* in 15 cultivars of rice under greenhouse conditions.

Cultivar Name	Type and average number of lesions 8 days after inoculation <sup>a</sup>			
	1	2	3	4
Fanny	128.00 <sup>a</sup>	79.25	63.50	30.00
Bluebonnet 50	206.00	38.25	49.00	7.25
IR 8	129.75	16.50	0.25	0.00
Colombia 3	296.75	68.50	15.75	0.00
Fa Yiu Tsai	395.00	69.50	33.25	1.25
Perola	378.75	43.75	31.00	2.75
Iaca Escuro	435.75	43.25	24.75	2.00
IR 8/2 x Zenith	119.00	6.00	0.00	0.00
Nahng Mon S-4 x TN1	183.00	10.00	2.00	0.00
IR 8 x (Dawn x TN1)	147.75	3.75	0.00	0.00
Carreon	831.25	9.50	0.00	0.00
Tetep	398.00	6.00	0.00	0.00
C4615	239.00	8.50	2.00	0.00
Dissi Hatif	286.00	7.00	0.00	0.00
Mamoriaka	275.50	0.00	0.00	0.00

<sup>a</sup> Type of lesion (1-4) according to the International Scale.

With race IC-1, the largest number of type 1 and 2 lesions was observed on the varieties Carreon, Iaca Escuro, Colombia 3 and Fanny. The other varieties showed few lesions (Table 4).

With race ID-8, the varieties Colombia 3 and Carreon had the highest number of type 1 lesions whereas Fanny, Bluebonnet 50 and C46-15 had type 2 and type 3 lesions. Fanny also showed type 4 lesions (Table 5).

**Table 5.** Average number of lesions due to race ID-3 of *P. oryzae* in 15 cultivars of rice under greenhouse conditions.

Cultivar Name	Type and average number of lesions 8 days after inoculation <sup>a</sup>			
	1	2	3	4
Fanny	246.25 <sup>a</sup>	58.25	87.00	17.50
Bluebonnet 50	685.50	21.00	4.50	0.00
IR 8	428.00	0.00	1.00 <sup>b</sup>	0.00
Colombia 3	725.25	17.00	0.00	0.00
Fa Yiu Tsai	144.75	6.00	0.25	0.00
Perola	407.25	3.75	0.00	0.00
Iaca Escuro	504.25	13.25	1.75	0.00
IR 8/2 x Zenith	93.50	1.00	0.00	0.00
Nahng Mon S-4 x TN1	220.50	0.00	0.00	0.00
IR 8 x (Dawn x TN1)	39.50	0.00	1.00	0.00
Carreon	720.75	0.00	0.00	0.00
Tetep	82.25	1.25	0.00	0.00
C4615	448.50	13.75	4.50	0.00
Dissi Hatif	383.50	2.50	0.00	0.00
Mamoriaka	533.25	0.00	0.00	0.00

<sup>a</sup> Type of lesion (1-4) according to the International Scale.

<sup>b</sup> Lesions at the tip of the leaves.

With race IG-1, the variety Colombia 3 had the highest number of type 1 lesions whereas Fanny, Bluebonnet 50 and C46-15 had the highest number of type 2 and 3 lesions. Bluebonnet 50, Fanny, and Iaca Escuro also produced type 4 lesions. However, the latter had few type 4 lesions as compared with Fanny and Bluebonnet 50 (Table 6).

Table 6. Average number of lesions due to race IG-1 of *P. oryzae* in 15 cultivars of rice under greenhouse conditions.

Cultivar Name	Type and average number of lesions 8 days after inoculation <sup>a</sup>			
	1	2	3	4
Fanny	94.75 <sup>a</sup>	33.25	41.75	6.76
Bluebonnet 50	237.00	42.75	55.50	10.00
IR 8	83.50	1.50	0.00	0.00
Colombia 3	439.25	0.50	0.00	0.00
Fa Yiu Tsai	75.75	3.50	0.00	0.00
Perola	93.25	5.75	1.50	0.00
Iaca Escuro	240.25	2.75	1.75	0.25
IR 8/2 x Zenith	29.75	1.00	0.00	0.00
Nahng Mon S4 x TN1	113.75	4.00	0.00	0.00
IR 8 x (Dawn x TN1)	8.75	0.00	0.00	0.00
Carreon	273.25	0.25	0.00	0.00
Tetep	20.50	1.50	0.00	0.00
C4615	115.50	12.75	9.75	0.00
Dissi Hatif	254.00	1.75	0.00	0.00
Mamoriaka	246.75	1.25	0.00	0.00

<sup>a</sup> Type of lesion (1-4) according to the International Scale.

Table 7. Lesion size induced by *P. oryzae* 8 days after inoculation under laboratory conditions.

Cultivar Name	Lesion size in mm due to races			
	IB-1	IC-1	ID-3	IG-1
Fanny	7.84 <sup>a</sup>	6.92	6.25	6.35
Bluebonnet 50	3.16	4.03	2.84	1.69
IR 8	0.91	0.97	0.75	-0.50
Colombia 3	0.87	1.85	1.06	-0.50
Fa Yiu Tsai	0.50	2.04	0.69	-0.50
Perola	0.69	3.25	1.47	1.37
Iaca Escuro	1.28	2.16	1.17	1.25
IR 8/2 x Zenith	0.37	0.72	0.62	-0.50
Nahng Mon S4 x TN1	0.50	1.03	0.69	-0.50
IR 8 x (Dawn x TN1)	0.94	0.51	0.91	-0.50
Carreon	-0.50	-0.50	-0.50	-0.50
Tetep	0.37	0.69	-0.50	0.62
C4615	1.69	1.95	0.84	1.56
Dissi Hatif	-0.50	1.16	-0.50	-0.50
Memoriaka	-0.50	-0.50	-0.50	-0.50

<sup>a</sup> Average of the 8 largest lesions.

**Table 8. Lesion size induced by *P. oryzae* 8 days after inoculation under greenhouse conditions.**

Cultivar Name	Lesion size in mm due to races			
	IB-1	IC-1	ID-8	IG-1
Fanny	17.90 <sup>a</sup>	9.88	20.14	13.79
Bluebonnet 50	10.12	8.27	5.81	17.20
IR 8	0.52	1.52	-0.50	1.20
Colombia 3	1.76	3.30	2.35	0.88
Fa Yiu Tsai	3.85	3.80	2.08	1.37
Perola	2.00	4.87	1.16	3.24
Iaca Escuro	4.65	5.32	2.74	3.53
IR 8/2 x Zenith	-0.50	0.99	-0.50	1.07
Nahng Mon S-4 x TN1	-0.50	2.20	-0.50	1.47
IR 8 x (Dawn x TN1)	-0.50	0.92	-0.50	-0.50
Carreon	1.14	1.49	0.41	-0.50
Tetep	-0.50	1.58	0.58	-0.50
C4615	2.31	2.68	3.77	4.83
Dissi Hatif	1.37	1.90	0.77	0.90
Mamoriaka	-0.50	-0.50	-0.50	0.77

<sup>a</sup> Average of the largest 10 lesions/4 replications.

#### Lesion Size

Fanny and Bluebonnet 50 produced the largest lesions under laboratory conditions. The varieties C46-15, Perola, Iaca Escuro and Fa Yiu Tsai showed larger lesions than the other varieties, but never as large as those of the two susceptible ones.

The results were similar under greenhouse conditions. Fanny and Bluebonnet 50 developed the largest lesions. Iaca Escuro and C46-15 showed larger lesions than the other varieties but smaller than the susceptible ones (Table 8).

#### Lesion Color

The results are presented in Tables 9 and 10 for the laboratory and the greenhouse studies. Type 1 lesions showed predominantly light seal-brown and seal-brown colors whereas types 2 and 3 had more variable colors. The susceptible variety, Fanny, always had an oil-green to a cerro-green color.

In the greenhouse, a diversity of colors was observed at the center of the lesion, particularly with types 2, 3, and 4. As will be described later, the color of the lesions was related to the number of conidia produced by the lesions.

#### Sporulation Time

In general the varieties with type 1 lesions seldom sporulated. In this case the conidia production always occurred in lesions by the leaf borders. Type 3

**Table 9. Colors presented by lesions due to different races of *P. oryzae* in rice varieties under laboratory conditions**

Cultivar Name	Color of the lesion by races							
	IB-1		IC-1		ID-8		IG-1	
Fanny	Olg	Lisb <sup>a</sup>	Ceg	Lisb	Ceg	Lisb	Olg	Lisb
Bluebonnet 50	Olg	Seb	Olg	Seb	Olg	Seb	Olg	Seb
IR 8	Seb		Seb		Ma		Seb	
Colombia 3	Seb		Olg	Seb	Seb		Seb	
Fa Yui Tsai	Olg	Seb	Olg	Seb	Seb		Seb	
Perola	Olg	Seb	Olg	Seb	Olg	Seb	Olg	Seb
Iaca Escuro	Olg	Seb	Olg	Seb	Seb		Olg	Seb
IR 8/2 x Zenith	Seb		Seb		Seb		Seb	
Nahng Mon S-4 TN1	Lisb		Olg	Lisb	Lisb		Lisb	
IR 8 x (Dawn x TN1)	Olg	Seb	Seb		Seb		Seb	
Carreon	Lisb		Lisb		Lisb		Lisb	
Tetep	Lisb		Lisb		Seb		Lisb	
C4615	Drg	Seb	Olg	Seb	Olg	Seb	Seb	
Dissi Hatif	Seb		Drg	Seb	Seb		Seb	
Mamoriaka	Seb		Seb		Seb		Lisb	

<sup>a</sup> Olg = Oil green, Lisb = Light seal brown, Olg = Olive gray, Seb = Seal brown, Drg = Drab gray, Ceg = Cerro green, Pa = Marron, following the Ridgway scale (Ridgway 1912).

and 4 lesions, and occasionally type 2 lesions, sporulated earlier, usually 4 to 5 days after inoculation in the laboratory studies (Table 11).

The varieties with intermediate and susceptible types (3 and 4) sporulated 4 to 5 days after inoculation in the greenhouse. C46-15, IR 8/2 x Zenith, Carreon, Dissi Hatif, and Colombia 3 sporulated 5 to 7 days after inoculation,

**Table 10. Colors presented by lesions due to different races of *P. oryzae* in rice varieties under greenhouse conditions.**

Cultivar Name	Color of the lesion by races							
	IB-1		IC-1		ID-8		IG-1	
Fanny	Ceg	Ma <sup>a</sup>	Ceg	Lisb	Ceg	Lisb	Olg	Lisb
Bluebonnet 50	Ceg	Seb	Olg	Seb	Ceg	Seb	Ceg	Seb
IR 8	Seb		Dr	Seb	Seb		Seb	
Colombia 3	Olg	Seb	Olg	Seb	Seb		Olg	Seb
Fa Yui Tsai	Cag	Lisb	Cag	Lisb	Olg	Lisb	Olg	Lisb
Perola	Olg	Lisb	Olg	Lisb	Olg	Lisb	Olg	Lisb
Iaca Escuro	Olg	Seb	Olg	Seb	Olg	Seb	Olg	Seb
IR 8/2 x Zenith	Seb		Dr	Seb	Seb		Seb	
Nahng Mon S-4 x TN1	Lisb		Olg	Lisb	Lisb		Lisb	
IR 8 x (Dawn x TN1)	Seb		Seb		Dr	Seb	Seb	
Carreon	Lisb		Dr	Lisb	Lisb		Lisb	
Tetep	Seb		Dr	Seb	Seb		Seb	
C4615	Dr	Seb	Olg	Seb	Dr	Seb	Dr	Seb
Dissi Hatif	Seb		Dr	Seb	Dr	Seb	Seb	
Mamoriaka	Seb		Seb		Seb		Dr	Seb

<sup>a</sup> Ceg = Cerro green, Ma = Marron, Seb = Seal brown, Olg = Oil green, Cag = Calla green, Lisb = Light seal brown, Olg = Olive gray, Dr = Drab, following the Ridgway scale (Ridgway 1912).

having type 2 lesions which produced fewer conidia. In the other varieties, sporulation occurred 8 to 10 days after the inoculation (Table 12).

### Conidia Number per Lesion Type

The results are shown in Tables 13 and 14 for the laboratory and greenhouse studies, respectively. The highest sporulation occurred in lesions with oil-green,

Table 11. Time of sporulation initiation of *P. oryzae* in rice leaves inoculated with 4 different races under laboratory conditions.

Cultivar Name	Beginning of sporulation after inoculation (days)			
	races			
	IB-1	IC-1	ID-8	IG-1
Fanny	4	4	5	4
Bluebonnet 50	5	4	4	4
IR 8	- <sup>a</sup>	-	7 <sup>b</sup>	-
Columbia 3	6	5	-	-
Fa Yiu Tsai	5	5	8	-
Perola	6	5	8	5
Iaca Escuro	5	6	6	5
IR 8/2 x Zenith	-	-	9	-
Nahng Mon S-4 x TN1	8 <sup>b</sup>	6	6	-
IR 8 x (Dawn x TN1)	-	-	-	-
Carreon	-	-	-	6
Tetep	-	-	-	6
C4615	6	6	5	6
Dissai Hatif	-	6	-	-
Mamoriaka	-	-	-	-

<sup>a</sup> Lesions that did not sporulate.

<sup>b</sup> Sporulation only at the leaf borders.

Table 12. Time of sporulation initiation of *P. oryzae* in rice leaves inoculated with 4 different races under greenhouse conditions.

Cultivar Name	Beginning of sporulation after inoculation (days)			
	races			
	IB-1	IC-1	ID-8	IG-1
Fanny	5	4	6	5
Bluebonnet 50	5	4	5	4
IR 8	7 <sup>a</sup>	-	-	7 <sup>a</sup>
Columbia 3	6	5	6	5
Fa Yiu Tsai	7	5	3	6
Perola	6	5	9	6
Iaca Escuro	6	5	6	5
IR 8/2 x Zenith	- <sup>b</sup>	5	10 <sup>a</sup>	-
Nahng Mon S-4 x TN1	-	5	-	7
IR 8 x (Dawn x TN1)	-	-	8 <sup>a</sup>	-
Carreon	7	5	-	-
Tetep	-	6	-	-
C4615	5	5	6	6
Dissai Hatif	6	5	7	-
Mamoriaka	-	-	-	8

<sup>a</sup> Lesions only at the tip of the leaves.

<sup>b</sup> Lesions that did not sporulate.

olive-gray and cerro-green centers. These lesions have borders light seal-brown to seal-brown. Perola and C46-15 produced few conidia although they had oil-green centers. Lesions with drab-gray, marron and seal-brown colors produced few conidia or none at all.

Under greenhouse conditions the number of spores varied considerably. Lesions with cerro-green, oil-green, olive-green or drab-gray centers produced

Table 13. Number of conidia produced by *P. oryzae* 10 days after inoculation under laboratory conditions.

Cultivar Name	Number of conidia and prevalent type lesion races			
	IB-1	IC-1	ID-8	IG-1
Fanny	7,541 (4) a	16,833 (4)	10,100 (3)	4,833 (3)
Bluebonnet 50	2,125 (3)	9,666 (3)	2,400 (3)	3,400 (2)
IR 8	b	—	250 (2)	—
Colombia 3	733 (2)	1,833 (3)	416 (2)	—
Fa Yiu Tsai	1,231 (2)	2,666 (3)	—	—
Perola	500 (2)	3,000 (3)	333 (2)	2,333 (2)
Iaca Escuro	2,066 (3)	1,500 (3)	250 (2)	2,916 (2)
IR 8/2 x Zenith	—	—	750 (2)	—
Nahng Mon S-4 x TN1	—	500 (2)	1,083 (2)	—
IR 8 x (Dawn x TN1)	—	—	300 (2)	—
Carmon	—	—	—	—
Tetep	—	—	—	—
C4615	1,166 (2)	2,333 (2)	666 (2)	750 (2)
Dissi Hatif	—	666 (2)	—	—
Mamoriaka	—	—	—	—

a The number in the parenthesis is the type of lesion.

b Lesion that did not sporulate.

Table 14. Number of conidia produced by *P. oryzae* 10 days after inoculation under greenhouse conditions.

Cultivar Name	Number of conidia and prevalent type lesion races			
	IB-1	IC-1	ID-8	IG-1
Fanny	9,466 (4) a	20,300 (4)	11,633 (4)	12,700 (4)
Bluebonnet 50	3,433 (4)	12,633 (4)	2,600 (3)	8,800 (4)
IR 8	b	—	—	—
Colombia 3	1,533 (3)	1,500 (3)	966 (2)	1,250 (2)
Fa Yiu Tsai	1,800 (3)	2,700 (3)	66 (2)	—
Perola	1,200 (3)	3,800 (3)	833 (2)	3,666 (3)
Iaca Escuro	3,200 (4)	4,733 (3)	466 (2)	4,800 (3)
IR 8/2 x Zenith	—	100 (2)	166 (2)	—
Nahng Mon S-4 x TN1	—	233 (2)	—	1,700 (2)
IR 8 x (Dawn x TN1)	—	—	444 (3)	—
Carreon	226 (2)	433 (2)	—	—
Tetep	—	133 (2)	—	—
C4615	1,500 (2)	1,366 (2)	1,233 (3)	4,433 (3)
Dissi Hatif	633 (2)	333 (2)	366 (2)	—
Mamoriaka	—	—	—	666 (2)

a The number in the parenthesis is the type of lesion.

b Lesion that did not sporulate.

the highest number of conidia, except for the varieties C46-15 and Colombia 3. The variety Fa Yiu Tsai showed lesions with similar colors to the above, but it did not produce any conidia with race IG-1. In general, lesions showing light seal-brown and seal-brown colors did not produce any conidia.

#### Influence of Relative Humidity in the Development of Lesion Type

The results are presented in Tables 15 and 16. The minimum time necessary for this kind of study and for the development of typical lesions of the fungus

Table 15. Times of relative humidity (100%) necessary for the development of typical lesions due to race IB-1 of *P. oryzae* under laboratory conditions.

Cultivar Name	Exposure time to 100% relative humidity (hours)					
	0	6	8	10	12	14
Fanny	1 <sup>a</sup>	1	3	3	3	3
Bluebonnet 50	1	1	3	3	3	3
IR 8	1	1	1	1	1	1
Colombia 3	1	1	1	3	3	3
Fa Yiu Tsai	1	1	1	1	1	1
Perola	1	1	1	1	2	2
Iaca Escuro	1	1	2	2	2	2
IR 8/2 x Zenith	1	1	1	1	1	1
Nahng Mon S-4 x TN1	1	1	1	1	1	1
IR 8 x (Dawn x TN1)	1	1	1	1	1	1
Carreon	1	1	1	1	1	1
Tetep	1	1	1	1	1	1
C4615	1	1	1	2	2	2
Dissi Hatif	1	1	1	1	1	1
Mamoriaka	1	1	1	1	1	1

<sup>a</sup> Type of lesion (1-4) according to the International Scale.

Table 16. Times of relative humidity (100%) necessary for the development of typical lesions due to race IB-1 of *P. oryzae* under greenhouse conditions.

Cultivar Name	Exposure time to 100% relative humidity (hours)					
	0	6	8	10	12	14
Fanny	1 <sup>a</sup>	1	4	4	4	4
Bluebonnet 50	1	1	1	3	3	3
IR 8	1	1	1	2	2	2
Colombia 3	1	1	1	2	2	2
Fa Yiu Tsai	1	1	1	2	2	2
Perola	1	1	1	1	3	3
Iaca Escuro	1	1	1	1	3	3
IR 8/2 x Zenith	1	1	1	1	1	1
Nahng Mon S-4 x TN1	1	1	1	1	1	1
IR 8 x (Dawn x TN1)	1	1	1	1	1	1
Carreon	1	1	1	1	1	1
Tetep	1	1	1	1	1	1
C4615	1	1	1	2	2	2
Dissi Hatif	1	1	1	1	1	1
Mamoriaka	1	1	1	1	1	1

<sup>a</sup> Type of lesion (1-4) according to the International Scale.

in rice was from 8 to 10 hours, because even susceptible varieties showed only type 1 lesions when they were exposed to 100 percent relative humidity from 0 to 6 hours at 25°C.

### **Sporulation Frequency in Type 3 and 4 Lesions**

The results are shown in Figure 2. The conidia production was abundant after the third day of inoculation, particularly in the susceptible varieties, Fanny and Bluebonnet 50. In these varieties the fungus produced conidia for 10 days, whereas in Colombia 3 with type 3 lesions it sporulated for 11 days. In the varieties Fa Yiu Tsai, Perola and Iaca Escuro, *P. oryzae* produced conidia for 17 to 18 days. However, the number of conidia was lower in comparison with the other varieties.

## **DISCUSSION**

The study of the behavior of rice varieties – susceptible, intermediate, and resistant to blast – under laboratory and greenhouse conditions indicated that the type, size, color of the lesions, and number of conidia per lesion are important factors in determining resistance to *P. oryzae*. Susceptible varieties showed larger size (type 4) and higher number of lesions. Sporulation took place in less time and the number of spores produced was considerably higher. Intermediate varieties in certain cases had type 3 lesions but they produced a higher number of conidia. It implies that lesion size is not a big enough factor in determining resistance. Large size lesions producing few conidia may be less important epiphytologically than smaller size lesions, but may also be active conidia producers. In general, type 3 lesions produced fewer conidia than type 4. Ou et al (1971) found that the varieties Carreon and Tetep produced few lesions to all the races tested, although in some cases type 4 lesions were observed on Tetep.

Sporulation was higher in the greenhouse than in the laboratory. The leaves, because yellow, died in five days and this might be the reason for the smaller number of conidia observed in the laboratory studies. The time from inoculation to sporulation initiation was shorter in susceptible varieties than in intermediate ones. However, the time difference was small. Type 3 lesions produced higher numbers of conidia than type 2 lesions, in general. But, in some cases, a high sporulation occurred in type 2 lesions. These observations differ from Yorinori and Thurston (1971) who found no differences in conidia production between types 2 and 3. These discrepancies might be due to the different varieties as well as the races of the pathogen used in both cases.

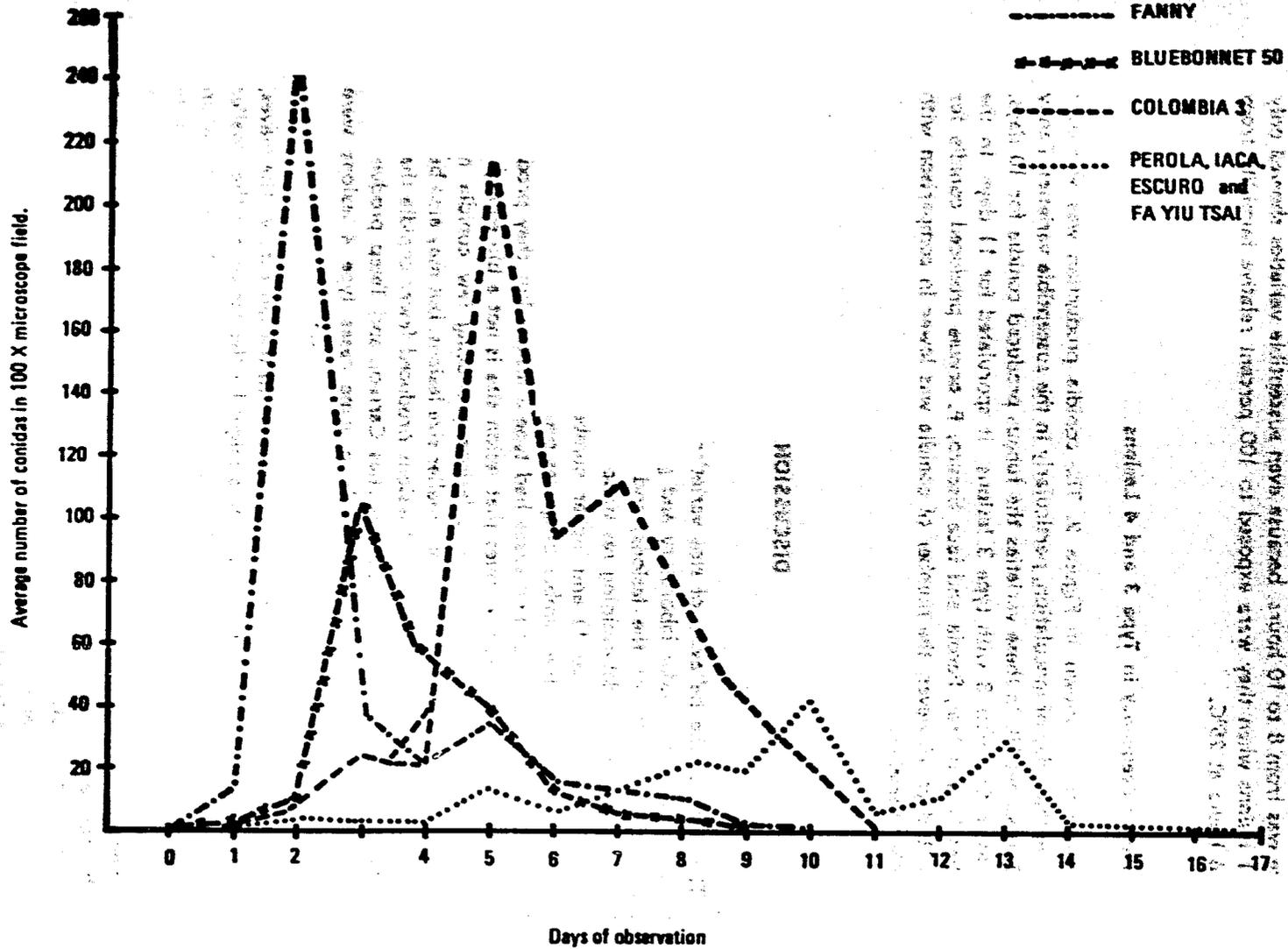


Figure 2. Average number of conidas of *Pyricularia eryzae* (Race 1B-1) produced daily in lesion types 3 and 4 during 18 continuous day on various varieties of rice.

The time required for high humidity in the development of typical lesions of *P. oryzae* was in agreement with Hashioka (1965). The laboratory studies for this factor were not valid because of the condensation present on the surface and borders of the inoculated leaves in the Petri dishes. Furthermore, this factor did not establish any difference among varieties.

Daily discharge of conidia was different for the susceptible and intermediate varieties. High sporulation occurred during the first days for type 4 lesions of Fanny and Bluebonnet 50. However, the type 4 lesions of Iaca Escuro always produced few conidia, an important epiphytological factor in the development of the disease. Colombia 3 showed type 3 lesions but they produced high numbers of conidia after the fifth day. This kind of resistance with a late discharge may be a critical factor in partial resistance of rice to *P. oryzae*.

In the late blight disease of potatoes (*Phytophthora infestans* Mont. de Bary), partial resistance is considered to be present in varieties that show a low number and small size lesions, low and slow sporangia production per leaf area, late penetration in the leaf, and long time for lesion appearance per a given quantity of inoculum (Black 1954, Guzman, Thurston, and Heidrick 1960). Differences in size of the lesion, number of lesions per leaf, number of conidia, and lesion color were observed consistently in these studies for the varieties selected for their reaction under field conditions. Partial resistance, or stable resistance, or general resistance, or horizontal resistance – whatever name is used to express this kind of resistance – is difficult to prove in a short period of time and under limited variability of the fungus. Nevertheless, these studies suggest its probable existence in rice varieties. A worldwide cooperation to establish a uniform partially resistant blast nursery to be tested under different conditions and under standard disease estimation procedures is urgent and necessary. Greenhouse and laboratory studies may be useful in understanding its nature. The finding of a variety with a sufficient number of genes to hold resistance to *P. oryzae*, and not to be broken by new races, will undoubtedly be useful to rice plant breeders.

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**Pathogenic variability and cytology  
of monoconidial subcultures  
of *Pyricularia oryzae* Cav.**

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Many plant pathogenic fungi that lack a known sexual cycle undergo changes in pathogenicity. High rates of variability in *Phytophthora infestans* (Caten and Jinks 1968), *Aphanomyces euteiches* (Beaute and Lockwood 1967), *Ustilago maydis* (Christensen 1963), and *Aspergillus nidulans* (Weisberg and Weijer 1968) are but a few of often cited examples. Many workers have reported high levels of cultural and pathogenic variation, but have not completed the work necessary to define the mechanisms of this variability.

The tremendous economic significance of plant pathogens and the problems that pathogenic variability introduce into disease control programs are closely related. Research in our laboratory demonstrated that the fungus pathogen causing rice blast provides us with a model system for demonstrating pathogenic variability. Several workers have noted an unusually high frequency of pathogenic variability in *Pyricularia oryzae* Cav. (Giatgong 1968; Giatgong and Frederiksen 1967, 1969; Ou and Ayad 1968; Quamawzzaman and Ou 1970). These reports agree as to the rate, type and significance of the pathogenic variability. The variation in pathogenicity is evident on the international set of differential rice varieties (Giatgong and Frederiksen 1969, Quamawzzaman and Ou 1970, Suzuki 1965, United States and Japan Cooperative Study 1967) used to identify physiologic races of the fungus (Galvez and Lozano 1968) as well as on select differentials.

The genetic origin of the variability is not known. Based on Suzuki's (1965) and Chu's (1965) observations, Ou and Ayad (1968) believed that heterokaryosis is responsible, but they recognized that others have found cells of *P. oryzae* to be uninucleate (Yamasaki and Niezeki 1965).

Giatgong (1967, 1969) observed a high frequency of pathogenic variability in single-conidial isolates of *P. oryzae* (Tables 1, 2 and 3). His work indicated that heterokaryosis was probably not responsible since mycelial and conidial cells of *P. oryzae* used in his studies were uninucleate. However, Giatgong was unable, genetically, to determine the mechanism which caused the variability. He also observed that the concentration of inoculum (Table 4) and the temperature (Table 5) during the infection process did not contribute to the pathogenic variability. A 3221:1 ratio of resistant to susceptible lesions was found on one differential host inoculated with one isolate. It is difficult to accept mutation for virulence as the sole explanation of this variability because of the high frequency of variant types within single-conidial populations. This is considerably higher than the mutation rate for most pathogenic fungi.

Table 1. Disease reaction of twenty first-generation monoconidial lines of *Pyricularia oryzae*, U.S. race 1, tested on four differential rice varieties.

Variety or C.I. Number	Reaction Class <sup>a</sup>							
	Parental Isolate	Monoconidial Isolates						
Zenith	S	S	S	S	R <sup>b</sup>			
C.I. 8970-P	R	R	S	R	S			
C.I. 8970-S	S	S	S	R	S			
P.I. 180061	S	S	S	S	R			
	Frequency	13	4	2	1			
	Reaction of Second-Generation							
	Parental Isolate	Monoconidial Isolates						
Zenith	R	R	R	R <sup>b</sup>	S	S	R	S
C.I. 8970-P	S	S	R	S	R	S	R	S
C.I. 8970-S	S	S	S	S	S	S	R	S
P.I. 180061	R	R	R	S	S	S	R	R
	Frequency	7	5	3	2	1	1	1
	Reaction of Third-Generation							
	Parental Isolate	Monoconidial Isolates						
Zenith	R	R	R	R	R			
C.I. 8970-P	S	S	S	R	S			
C.I. 8970-S	S	S	S	R	R			
P.I. 180061	S	S	R	R	R			
	Frequency	15	2	2	1			

<sup>a</sup> R = Resistant, S = Susceptible.

<sup>b</sup> Isolate selected for testing in the next generation.

**Table 2. Disease reaction of twenty first-generation monoconidial lines of *Pyricularia oryzae*, U.S. race 3, tested on four differential rice varieties.**

Variety or C.I. Number	Reaction Class <sup>a</sup>			
	Parental Isolate	Monoconidial Isolates		
Zenith	R	R <sup>b</sup>	R	R
C.I. 8970-P	S	S	R	S
C.I. 8970-S	S	S	S	R
P.I. 180061	R	R	R	R
	Frequency	18	1	1
Reaction of Second-Generation				
	Parental Isolate	Monoconidial Isolates		
Zenith	R	R	R <sup>b</sup>	R
C.I. 8970-P	S	S	S	R
C.I. 8970-S	S	S	R	R
P.I. 180061	R	R	R	R
	Frequency	17	2	1
Reaction of Third-Generation				
	Parental Isolate	Monoconidial Isolates		
Zenith	R	R	R	R
C.I. 8970-P	S	S	S	R
C.I. 8970-S	R	R	S	R
P.I. 180061	R	R	R	R
	Frequency	15	3	2

<sup>a</sup> R = Resistant, S = Susceptible.

<sup>b</sup> Isolate selected for testing in the next generation.

**Table 3. Disease reactions of twenty monoconidial lines that originated from *Pyricularia oryzae*, U.S. race 1, subculture 12 on the international differential varieties.**

Race, Group and Number <sup>a</sup>	Frequency
IB-5	2
IB-21	2 <sup>b</sup>
IB-29	1
IB-33	1
IB-37	2
IB-41	1
IB-45	2
IB-53	3
IB-61	4
IB-62	2
IB-64	1

<sup>a</sup> From Ling and Ou, 1969.

<sup>b</sup> Parental source.

In preliminary work, Glatgong (1968) observed changes in pathogenicity by exposing *P. oryzae* to mutagens (UV and NaF), high temperature, and prolonged periods of culture storage prior to inoculation. However, because of relatively small populations of pathogenic isolates tested, he was unable to conclude what influence these factors had on observed changes in pathogenicity.

Table 4. Effect of conidial concentration on host reaction, race IG-2 of *Pyricularia oryzae*.

Variety and C.I. Number	Concentrations			
	$6.0 \times 10^5$	$2.8 \times 10^5$	$1.6 \times 10^5$	$7.5 \times 10^4$
Zenith	R	R	R	R
C.I. 8970-P	S+++	S++	S++	S+
C.I. 8970-S	S+++	S++	S++	S+
P.I. 180061	R	R	R	R

R = Resistant  
S = Susceptible

Table 5. Reaction of twenty second-generation monoconidial isolates of *Pyricularia oryzae*, U.S. race 1, subculture 3 at three different controlled temperatures.

Variety or C.I. Number	Reaction Class at 20C <sup>a</sup>				
	Parental Isolate	Monoconidial Isolates			
Zenith	S	S	R	S	R
C.I. 8970-P	R	R	R	R	R
C.I. 8970-S	S	S	S	R	R
P.I. 180061	S	S	S	S	S
	Frequency	17	1	1	1
Variety or C.I. Number	Reaction Class at 25C				
	Parental Isolate	Monoconidial Isolates			
Zenith	S	S	R	S	R
C.I. 8970-P	R	R	R	R	R
C.I. 8970-S	R	S	R	R	R
P.I. 180061	S	S	S	S	S
	Frequency	17	1	1	1
Variety or C.I. Number	Reaction Class at 30C				
	Parental Isolate	Monoconidial Isolates			
Zenith	R	R	R	R	R
C.I. 8970-P	R	R	R	S	R
C.I. 8970-S	R	R	S	R	S
P.I. 180061	S	R	R	R	S
	Frequency	10	4	3	3

<sup>a</sup> R = Resistant, S = Susceptible.

It is apparent to us, and I believe others (Ou and Ayad 1968), that any culture of *P. oryzae* may be composed of many pathogenic races that can be recognized only by single-sporing (sampling) from it. In our work, the reaction class of the parental culture occurred most frequently among the monoconidial subcultures. This indicated that the extent of variability within each population was masked, at least under the conditions used in our studies. We believe that the natural selection processes in the field (Quamawzzaman and Ou 1970) would more closely follow the selectivity of single-sporing than the mass inoculation procedures.

We observed cultures of *P. oryzae* with both uninucleate and multinucleate cells. The cultures used in our pathogenicity studies were uninucleate except for an occasional multinucleate cell. Some cultures of *P. oryzae* anastomose with others readily, whereas some do not. We observed both kinds. However, those used in our variability studies essentially failed to anastomose.

Chromosomes, divided in a zipper-like manner, while remaining attached by a thin thread, all within a nuclear envelope. We did not observe or recognize bridging, unequal division or satellite chromosomes. Consequently, we were unable to satisfactorily ascertain the mechanism(s) that might account for the observed pathogenic variability.

Our previous observations (Giatgong 1968, Giatgong and Frederiksen 1967, 1968) do not support heterokaryosis as a basis for variability in *P. oryzae*, and we have no bonified evidence for sexuality or parasexuality. But because there was continual segregation (somatic variation) for pathogenicity in some serial single-conidial subcultures, and because the nuclei in conidia of *P. oryzae* are each derived from a single-nucleate spore mother-cell, it is suggested that there are either unusually high rates of mutation or that virulence is partially controlled by extra-chromosomal or cytoplasmic factors.

The possible role of extra-chromosomal inheritance in *P. oryzae* needs to be clearly differentiated from that of gene mutation and heterokaryosis before the mechanism of pathogenic variability can be studied and understood.

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**Production of the perfect stage  
of *Pyricularia* from rice  
and other hosts**

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The perfect stage of *Pyricularia grisea* (Cke.) Sacc., *Ceratospheeria grisea*, was produced in culture by mating two isolates of the fungus from crabgrass [*Digitaria sanguinalis* (L.) Scop.] from North Carolina (Hebert 1971). An isolate of the fungus from crabgrass from Arkansas also produced a few perithecia when mated with tester lines of fertile ascospore isolates from the original and subsequent crosses. Seven other isolates of *Pyricularia* from crabgrass from North Carolina, one isolate from St. Augustine grass [*Stenotaphrum secundatum* (Walt.) Kuntze] from Peru and thirty-two isolates from rice (*Oryza sativa* L.) from various parts of the world failed to produce the perfect stage in matings with the tester isolates. This paper reports the results of further mating tests with these and with additional isolates of *Pyricularia* from rice and other hosts.

**MATERIALS AND METHODS**

The following *Pyricularia* isolates with origin and race number (in parenthesis) were obtained from Dr. Frances Letterell: 438 rice Taiwan (IE-3), 453 rice

El Salvador (IB-33), 479 rice The Philippines (ID-13), 499 rice India (ID-8), 540 *Oryza rufipogon* Australia (IG-1), 549 rice India (IE-1), 559 rice Malaya (ID-16), 590 rice Japan (IH-1), 603 rice Arkansas (IH-1), 649 rice India (ID-1), 721 rice Japan, 740 rice Sierra Leone (IB-35), 748 rice The Philippines (ID-14), 776 *Digitaria* Florida (II-1), 794 rice Dominican Republic (IG-1A), 825-D-6 rice Costa Rica X-ray (IB-1), 900 rice Louisiana (IE-5), 901 rice Louisiana (IG-1), 904 banana Honduras (II-1), 906 banana Honduras (II-1), 907 platano Honduras (II-1) and 908 *Setaria* Maryland (II-1). Nine cultures of *P. grisea* from pearl millet [*Pennisetum glaucum* (L.) R. Br.] from Georgia were obtained from Dr. Homer Wells. The following additional isolations were made by the author: 19 isolates from crabgrass from North Carolina, 10 isolates from St. Augustine grass from North Carolina and 9 isolates from this host from Peru, 17 isolates from rice from Peru in December 1970 and 74 isolates from rice from Peru in May 1971. Matings were made on Sach's agar as described previously (Hebert 1971).

## RESULTS

Further testing of the isolates listed in the previous report (Hebert 1971) showed that Latterell's isolate 888 from crabgrass from Arkansas, which had produced very few perithecia when mated with certain tester lines, produced an abundance of perithecia when mated with certain other tester lines. Latterell's isolate 883 from rice from Arkansas and isolate 46 from rice from Peru produced perithecia but no ascospores when mated with some tester lines. These three isolates reacted only with lines of the minus-mating type.

The 22 additional isolates obtained from Latterell were mated with each other in all possible combinations. Each isolate was also mated with 10 tester lines of the plus-mating type and with 10 tester lines of the minus-mating type. Isolate No. 776 from *Digitaria* from Florida produced fertile perithecia with 7 of the 10 minus-tester lines. Isolate 904 from banana from Honduras produced two empty perithecia with one of the plus-mating lines. The other matings produced no perithecia. Each of these 22 isolates were then mated with each of the 32 isolates from rice listed in the previous report (Hebert 1971). No perithecia were produced in these 704 matings.

Each of the 17 *Pyricularia* isolates obtained from rice from Peru in December 1970 was mated with 4 plus-tester lines and 4 minus-tester lines from crabgrass and also with 8 other rice isolates from this group. No perithecia were produced. Each of the 74 isolates obtained from rice from Peru in May 1971 was mated with 3 plus and 3 minus-tester lines from

crabgrass and also with 6 other rice isolates from this group. In one of these matings a rice isolate from the north coastal area of Peru produced fertile perithecia with a minus-tester line derived from crabgrass isolates. Single ascospore isolates have been obtained from this cross and tests have been initiated to determine the pathogenicity of these ascospore isolates to rice and to crabgrass.

In other tests, Latterell's isolate 904 from banana produced a few perithecia with ascospores when mated with a plus isolate from crabgrass. Isolate 906, also from banana, produced a few fertile perithecia when mated with a minus isolate derived from crabgrass. A mating of isolate 904 with isolate 906 failed to produce perithecia. In matings with the 9 isolates from pearl millet, 1 isolate produced a few fertile perithecia with few ascospores when mated with a minus isolate from crabgrass. Five of the 19 isolates from crabgrass from North Carolina were fertile; 3 of these were of the plus-mating type and 2 belonged to the minus group. The 10 isolates from St. Augustine grass from North Carolina and the 9 isolates from this host from Peru failed to produce perithecia.

To date, eleven *Pyricularia* isolates have produced fertile perithecia. One of these is from rice, seven from crabgrass, two from banana, and one from pearl millet. The isolates from banana and pearl millet produced perithecia containing only a few ascospores. In addition sterile perithecia (without ascospores) were produced by two other rice isolates.

## DISCUSSION

At the initiation of these studies *Pyricularia oryzae* Cav. was considered to be a specialized pathogenic form of *P. grisea*. It seemed reasonable to suppose that at one time this fungus produced a perfect stage in nature and that with the passage of time the genetic factors responsible for the production of the perfect stage have been disappearing from the fungus population. Since *Pyricularia* isolates from rice appear to be more highly specialized pathogenically than isolates from wild grasses, they are considered to be more highly evolved and therefore should have a lower probability of still retaining genetic factors for producing the perfect stage. Results thus far tend to confirm this idea. Of 140 rice isolates tested, only one was fully fertile, whereas 10 of 62 isolates from other hosts have produced ascospores. Testing of these isolates is continuing and it may be that others will be found to be fertile.

While the emphasis at this symposium is on horizontal resistance to *Pyricularia* in rice, I think that we should not neglect vertical resistance. I believe that our eventual goal should be varieties with combined horizontal and vertical resistance and that we should be working on methods to facilitate the transfer of both types of resistance to rice varieties. The availability of the sexual stage of the causal fungus will enable us to study the genetics of pathogenicity. More information is needed on the genetics of resistance in the host.

It would be highly desirable to have a set of rice lines each with a single major gene for resistance to *Pyricularia* preferably in a common genetic background. A good example to follow is the case of resistance to powdery mildew in wheat (Briggle 1969) where a variety of wheat (Chancellor) susceptible to all known races of the powdery fungus was selected and single major genes for resistance were transferred to this variety in a backcrossing program. Such a set of rice lines with single major genes for resistance would greatly facilitate the monitoring of genes for pathogenicity in *Pyricularia* in the various rice growing areas of the world and would facilitate the identification of races of the fungus.

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## **Rice blast disease in Peru**

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Rice is one of the main basic cereal crops grown in Peru and in the world. About 1.5 percent of the total American area planted with rice is in Peru. This area contributes 3 percent of the total American production. Peru ranks sixth among the highest yield per area countries in the world and second in America after the United States. The national average rice yield is 4,098 kg/ha (Contreras and Giron 1969).

The importance of this crop in Peru is economical and social mainly because of the habitual consumption among the population. Almost 77 percent of the cultivated area and 80 percent of the production is concentrated in the north coast. The jungle region plants 19 percent of the national rice area and supplies 15 percent of national production (Contreras and Giron 1969).

The main characteristics of the rice lands in Peru are (Velasquez, Huerta and Sanchez 1970):

**Coast.** Alluvial soils, plains and variable texture; low organic matter content, nitrogen fertilizer response. Desertic subtropical climate, high solar radiation. The relative humidity average is low although with high dew accumulation on plants during the night. Rice is commonly planted under intermittent flooding conditions.

**High Jungle.** (Zones of Jaen, Bagua and Huallaga Central.) This area has alluvial and vertizolic soils with satisfactory fertility and with good response to nitrogen and phosphorous fertilization. Its climate is classified as dry and tropical high pluvial precipitation, high temperature and good solar radiation. In the Jaen and Bagua zones, rice is sown under lowland conditions while in Huallaga Central zone it is sown under upland conditions.

**Low Jungle.** Its climate is humid tropical forest; studies indicate the predominance of ultisols including those formerly known as red-yellow podzolics and ground water laterites. There is a good possibility of increasing the cultivated areas in this region. Direct sowing method is practiced under upland conditions.

On the coast, blast disease is the most important disease, mainly in the years when there is high dew accumulation on the plant surfaces. Apparently the amount and duration of the dew formed, between 10 p.m. and 9 a.m., is considered the main factor in the spread of blast. Another factor which has increased this disease has been the high nitrogen fertilization used recently. It is common to find blast at seedling stage and then from panicle initiation stage to prematurity. Rice blast disease is not general in the coast; it is only found in certain areas, probably because of the microclimate conditions present in specific areas, type of soils or amount of nitrogen used or plant pathogens distribution -- all apparently governed by an epidemiologic factor still unknown to us.

Stern rot incited by *Leptosphacteria salvinii* and brown spot incited by *Helminthosporium oryzae* are considered as secondary diseases.

As on the coast, blast is the most important disease in the high and low jungle. It is largely helped by rainfall, which is very common. In other geographical areas, brown spot disease is the main trouble. Other diseases found on a minor scale — although certain rice varieties are seriously affected by them — are leaf scald (*Rhynchosporium oryzae*), false smut (*Ustilaginoides virens*), and linear spot (*Cercospora oryzae*).

a. Since its creation in September 1968 the National Rice Program was integrated by researchers from "Pedro Ruiz Gallo University", the Ministry of Agriculture and the North Carolina State University Agricultural Mission to Peru. Among the different work groups, the plant pathology staff is comprised of 17 members and is advised by Dr. T. T. Hebert, Plant Pathology Professor at North Carolina State University.

Rice disease is being studied in the main rice production zones of Peru by plant pathologists of the National Rice Program and great successes have been

obtained in all branches studied. Results have been published in the National Rice Program Technical Reports. As a consequence, our supply now meets the national demand in rice; however, many other factors have also contributed to this self-sufficiency.

The main targets of the plant pathology group can be summarized as follows:

- a) Evaluation of rice varieties and lines resistant to main diseases.
- b) Determination and preponderance of physiological races of *Pyricularia oryzae* in each season and each rice land area.
- c) Study of some epidemiologic aspects from the most important diseases in each rice area.
- d) Control of the main diseases with fungicides.

#### **Studies on Resistance to Blast**

The methods used in these tests were those suggested by Ou (1965) and have been used since January 1969. At first they were started in the jungle (Yurimaguas, Tarapoto and Tingo Maria). At present we have installed other ones in Bagua (jungle) and the coast (Tumbes and Lambayeque). These results will help us to have a wider view and clearer idea of the performance in these areas of the traditional rice varieties as well as the selections made by national rice program breeders.

From the beginning we had rice varieties and lines resistant to blast from national rice selections and from the world collection as well as from the Philippines, Colombia and the United States. We obtained them by interchanging material and information with the International Rice Research Institute (IRRI), the Philippines; from the Centro Internacional de Agricultura Tropical (CIAT), Colombia; and from the United States Department of Agriculture through Dr. J.G. Atkins of Beaumont, Texas.

In Table 1 it is possible to see the number of groups and lines of rice evaluated in each rice zone. The majority of the tests were carried out in the tropical regions where the climatic conditions were the best.

At the beginning we tried to test the higher number of rice varieties and lines in order to know their reaction to rice blast disease in Peru. Our first evaluations began in the 1969-70 growing season, and we introduced collections from a different origin and selection (Table 2). The collections were: World

**Table 1. Number of sets and lines tested in several Peruvian rice zones. 1971.**

Places	No. of sets	No. of lines
Yarimagua	20	4,173
Tingo Maria	15	1,000
Tarapoto	10	2,310
Bagua	7	1,243
Tumbes	1	511
Lambayeque	1	511

**Table 2. Blast reaction types by groups tested in Peru from 1969 to 1970.**

Groups *	No. of lines	Reaction types in %		
		Resistant	Intermediate	Susceptible
CI	250	3	10	87
CEL	508	1	20	79
IBNA	178	14	5	81
IR	292	41	36	23

\* CI = International Collection; CEL = Lambayeque Experimental Station Collection; IBNA = International Blast Nursery for the Americas, U.S.A.; IR = IRRI, the Philippines and CIAT, Colombia collections.

Collection (CI); Agricultural Experimental Station Collection from Lambayeque (CEL), the International Blast Nursery for the Americas (IBNA) and a group from IR lines, brought from the Philippines (IRRI) and Colombia (CIAT). From all these collections we obtained a major number of IR rice types with resistant and intermediate reaction (Table 2).

From the tests made in the 1969-70 growing season, lines with resistance or intermediate reaction were selected; they showed important agronomic characteristics. Those varieties formed the I-PNA group that was evaluated during the 1970-71 season. This group, together with other national and international collections, formed the groups to be evaluated the next season. Origin, number of lines in each group, number of evaluations for each one, the percentage of resistant, intermediate and susceptible varieties are shown in Table 3. PNA IV was the group which showed the highest number of resistant varieties; it mainly had IR lines such as IR 790; IR 1147; IR 1093; IR 1416; IR 667; IR 828; IR 498; IR 1006; IR 1163; IR 825; IR 854; IR 822; IR 879; IR 1157; IR 841; IR 1112; IR 844; IR 835; IR 790; IR 930; IR 1154; IR 1170; etc.

However, in other evaluations many of the resistant varieties became susceptible under our conditions (Huerta 1971). It will be necessary to do other trials to collaborate these results.

Table 3. Blast reaction types by groups tested in Peru from 1970 to 1971.

Group PNA*	No. of lines	No. of sets	Reaction types in %		
			Resistant	Intermediate	Susceptible
I	511	30	—	31	69
II	356	3	31	41	28
III	251	2	20	26	54
IV	923	1	69	27	4
V	150	3	—	68	32
VI	484	1	30	68	2

\* I = Lines IR selected in Peru; II = Rice varieties selected for International Partially Resistant blast nursery, IRRI; III = International Partially Resistant blast nursery, CIAT; IV = International Yield Trials, IRRI; V = Blast Moderately Resistant Varieties, Group III, IRRI; VI = Pedigree II-CRIAN, Lambayeque. Lines F: IR 8 x F, (Fortuna x Minagra).

Table 4. The most resistant varieties selected from the International Blast Nurseries from 1969 to 1971 in more than 30 trials in Peru.

Variety	Variety
IR 4-114-3-2-1	IR 593-1-34-1-3-3
IR 4-114-3-2-2-3	IR 661-17-2-1
IR 5-114-3-1	IR 662-2-7-2-2
IR 480-5-9-2	IR 665-4-4-5
IR 503-1-103-3	IR 667-112-3-3-3
IR 503-1-104	IR 667-113-1-1
IR 532-1-144	IR 682-23-2
Tetep	IR 672-432-2
IR 586-13-2-1	IR 622-432-4
IR 589-66-2-2	IR 622-432-5
IR 589-67-1	IR 648-44-1
IR 589-66-6-1	
IR 589-66-2-1	

I-PNA and V-PNA groups did not show any resistant lines in the evaluations (Table 3). The V-PNA group showed a high number of varieties with intermediate reaction. This was expected because the varieties belonged to blast moderately resistant varieties (III-IRRI group), and were sent by Dr. Ou in the Philippines.

From the I-PNA group, 31 percent of the lines showed intermediate reaction (Table 3) in more than 30 evaluations made in different rice areas since 1969. Table 4 shows a list with selected lines from that group. Selection was made because in most of the evaluations lines had a resistant reaction, and in a few evaluations intermediate reaction was found with type 3 lesions according to the Ou (1965) scale. From those varieties studied, IR 480-5-9-2 has been considered by the National Rice Program as a promising variety for the jungle, while Tetep is considered as potential genetic material.

The last IRRI Annual Report (1970, pp. 73-100) displays a rice varieties list, selected for their permanent resistance to blast on International Nurseries in different countries from 1964 to 1970. The reactions of these varieties in Peru are shown in Table 5. Except CI 7787, which was susceptible, all had resistant reaction or intermediate reaction.

Identification of physiological races of *P. oryzae* was initiated in Peru in 1970. In general, international differential varieties have been used (Atkins et al. 1967). Later Philippine differential varieties were included. From the different identified races, the most common one was IB-1 race. Other identified races were Ia-65 ab; IB-5c; IB-38; and IC-1i. Although the number of trials were few, it is possible to demonstrate that Peruvian races are different from Colombian (Galviz and Lozano 1968) and Filipino races (IRRI 1967, pp. 81-113).

Our present target is the identification of more races of *P. oryzae* from samples brought from different places, varieties and parts of plants.

Another aspect faced was the study of some factors that would be favorable for the sporulation of *P. oryzae* growing on artificial mediums. Diaz (1970), according to those targets, evaluated different mediums, photoperiod cycles, and incubation periods using several monosporic cultures from pathogens of different origin. His conclusions showed that a better sporulation is obtained with potato-dextrose-agar plus coconut water and with B-Takahashi medium made up from rice leaf extract. The best photoperiod was when the plates

Table 5 The most resistant varieties selected from the International Blast Nurseries from 1964 to 1970 around the world\* and their reactions in Peru.

Variety	Reactions to Blast
Telep	Resistant
Nang Chet cuc	Resistant
Takucan	Intermediate
R 87	Intermediate
C 46-15	Resistant
CI 7787	Susceptible
Pah Leud 298-11	Resistant
D 25-4	Resistant
Trang cut I. 11	Resistant
Pah Leud 111	Resistant
Mamoriaka	Resistant
Huan-sen-goo	Resistant
Dissi Hatif (DH 2)	Intermediate
Carreon	Resistant
Ram Tulasi	Resistant
Ram Tulasi Sel	Resistant
Ca 435/B/S/1	Intermediate
DNJ 60	Intermediate

\* IRRI, Annual Report for 1970.

were incubated for the first seven days in darkness and the other seven days under uninterrupted white light. At the same time the skills to produce spores of the *P. oryzae* isolations were different. In a general sense, the photoperiod of incubation did not affect the sporulation as much as the interaction medium x photoperiod did.

### Other Studies on Blast

As Peru does not yet have a rice variety with good agronomic and commercial characteristics and at the same time is resistant to blast disease, we have decided to test many chemicals to control it. Results obtained over the years as well as in the present one have been variable. They have changed with the fungicide and the places where the trials were carried out. The fungicides that seem to give relative control to blast according to Delgado et al. (1970), Jimenez and Mujica (1971), Panizo and Incio (1968) and Panizo and Hebert (1971) were: Hinosan, Duter, Bla-S, Kasumin, Conen, Benlate, Dithane M-45, and Calixin. Other chemicals sprayed on seedling rice that were effective in controlling blast (Huerta 1971) were: Blastin, Kitazin EC, and Antracol (Propineb).

During the last 1970-71 growing season a series of trials were carried out in different parts of the country. One of them was conducted by Eng. Juan Zapata (Ministry of Agriculture, Agrarian Zone II, Bagua) and the fungicides tested were: Kitazin 17 percent granulated, 41 kg/ha; Hinosan 50 percent, 1 liter/ha; Blastin 50 percent, 1 kg/ha; Bla-S 4 percent, 1 kg/ha; Benlate 50 percent, 0.8 kg/ha; Kitazin 48 percent EC 1 liter/ha; Dithane M-45, 80 percent (Mn ethylene bisdithiocarbamate plus ions zinc), 2.5 kg/ha; Manzate D (Maneb 80 percent with a zinc salt added), 2.5 kg/ha; Antracol 70 percent (Propylene bis-dithiocarbamate of zinc), 1 kg/ha; Conen 50 percent (S-benzil-O-butyl S-ethyl thiophosphate), 2.5 liter/ha; P-605 (In code: Farmagro Co.), 1 liter/ha; BAS 3201 F (In code: BASF Co.), 1 kg/ha; Calixin 75 percent (N-tridecyl 2,6-dimethyl-morfolina: Tridemorph), 0.6 liter/ha; Sclex 30 percent (3,5-dichlorophenyl - 5,5-dimethyl oxazolidine-dione- 2,4-Dichlozine), 1 kg/ha; TPTA (Tri-phenyl tin acetate), 1 kg/ha and Triazine 50 percent (2,4-Dichloro-6-0-chloroanilino)-1, 3,5-triazine), 1 kg/ha.

A complete and detailed result of this research work will be reported in the near future by the National Rice Program in a technical bulletin. Partial results are shown in Figure 1. Kitazin 17 percent granulated has displayed the most effectiveness in controlling blast. It attained a significant difference among the chemicals tested and checked.

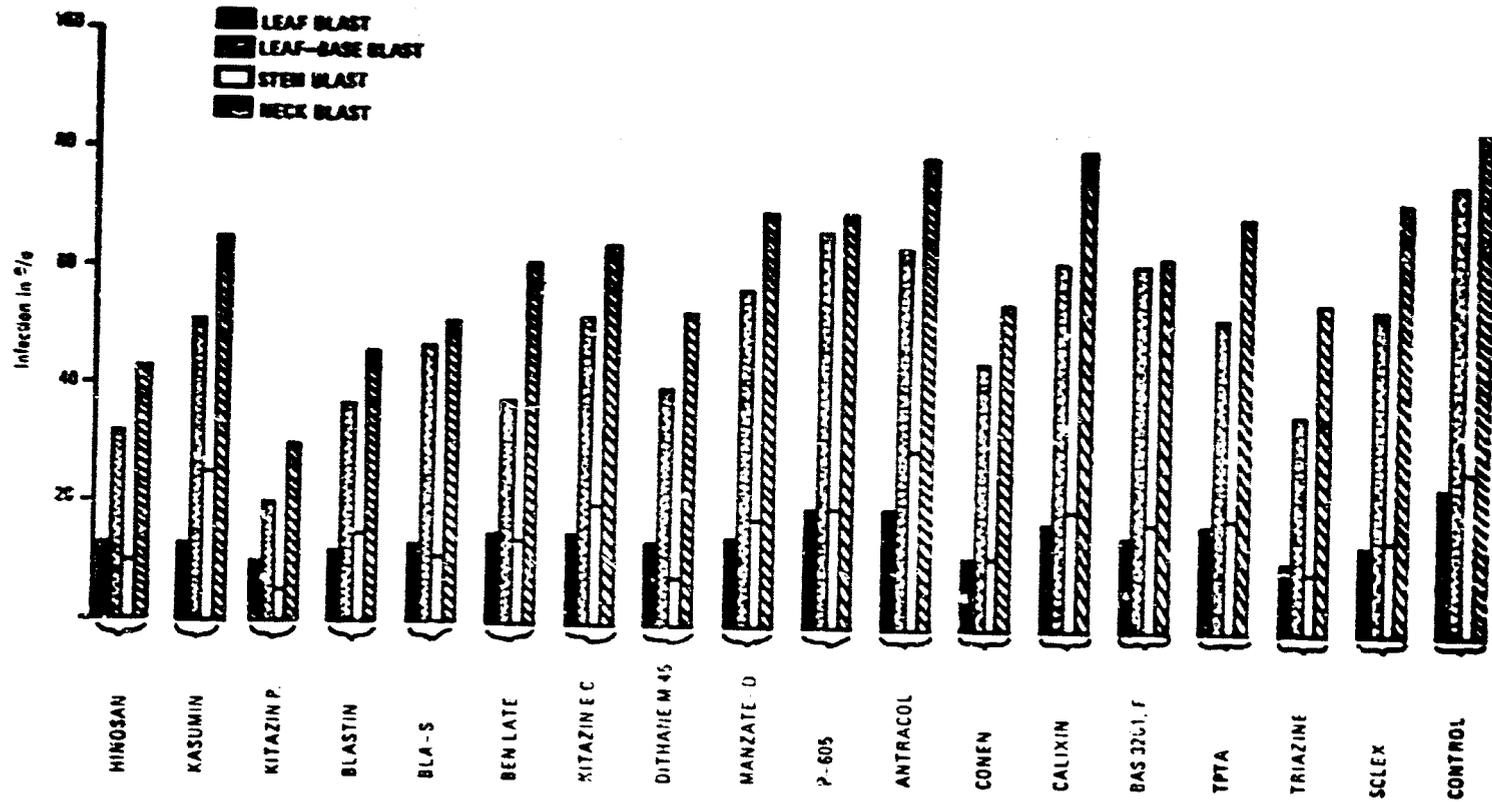


Figure 1. Result of behavior of 17 fungicides on the control of the rice blast disease, evaluated in percent of leaf area, leaf base, stem blast and neck blast affected. (Report by J. Zapata, unpublished data, Bagua 1971).

As a rule, part of the chemicals tested had relatively good control to blast, but their performance changes from place to place or from year to year. At present we have not obtained conclusive results from one chemical. The only one chemical which has a very good performance is Kitazin P 17 percent granulated.

The increase of the blast disease in almost all the rice lands, the use of chemicals in its control and the speculation raised by the salesmen and farmers about the phytotoxicity of certain chemicals, made it necessary to study the influence of them on grain production. During the 1970-71 growing season Eng. Daniel Cumpa from "Pedro Ruiz Gallo" University started a trial on the IR 8 variety. The rates used in our chemical control test and doses of the fungicides used were given by the manufacturer. Each chemical was sprayed on a predetermined rice plant at booting stage, one-third, two-thirds, and three-thirds of the panicle emergence, with two different volumes of water. The water rates were 50 and 300 liters/ha corresponding to airplane and knapsack mist blower sprayers, respectively. The phytotoxicity effects were evaluated by percent of unfilled grains and deformed grains at harvest. Complete results will be reported in the future in a National Rice Program technical bulletin.

Apparently there was no damage from the fungicides when they were sprayed in all of the heading stages (Table 6). However, Bla-S in two water rates has shown the highest (number-percent) of grain damaged. A mercurial

Table 6. Evaluation of damage from 13 fungicides on the formation of rice seeds on IR 8 variety (reported by D. Cumpa, unpublished U.N.P.R.G., Lambayeque, 1971).

Fungicides *	Deformed grains in %		Unfilled grains in %	
	50 L./HA **	300 L./HA	50 L./HA	300 L./HA
Kasumin	3.0(0.2) ***	3.1(0.3)	14.5(15.1)	14.5(11.4)
Blastin	1.6(0.4)	4.8(0.3)	8.9( 6.6)	12.7(13.2)
Bla-S	1.5(5.6)	1.1(1.6)	12.8( 7.1)	12.8( 7.3)
Kitazin EC	4.2(5.9)	2.3(0.8)	11.5(15.6)	13.0( 7.5)
BAS 3201-F	2.3(2.1)	2.0(2.9)	8.2(15.3)	9.9( 6.7)
Benlate	1.2(1.2)	0.6(2.6)	7.4( 6.2)	8.1( 6.2)
Antracol	1.4(1.7)	3.8(0.7)	9.0(12.8)	8.9( 7.4)
Dithane M-45	1.2(1.6)	1.5(2.1)	8.5( 6.1)	13.5(17.7)
Manzate D	1.4(1.6)	1.0(0.0)	8.8( 4.8)	7.9(11.0)
Conen	1.5(0.0)	3.2(2.0)	8.7(19.8)	8.7( 6.6)
P. 608	2.4(1.8)	2.1(2.4)	8.4( 8.3)	9.5(11.5)
Calixin	5.6(4.1)	1.0(2.0)	13.0( 9.4)	8.0(25.4)
Granosan	2.6(2.3)	1.8(4.5)	16.4( 9.5)	7.7( 7.0)

\* Doses used are found in the text.

\*\* The volume of water used per hectare.

\*\*\* The numbers in parenthesis are the percent of damage obtained on the controls.

compound, Grmnosan, used as a check, has also displayed certain damage, but only when it was sprayed at the rate of 50 liters/ha of water. Exact results will be reported after the statistical analysis.

Laberry and Jimenez (1971) have found that *P. oryzae* was capable of infecting *Stenotaphrum secundatum* (American grass) and that *Pyricularia* sp., found normally on *S. secundatum* in Piura (coast), was a rice pathogen too. In our studies, of another 35 weeds inoculated with *P. oryzae*, none displayed reaction. However, *Panicum repens* L., *Sorghum vulgare*, *Cynodon dactylon*, *Paspalum* sp., and other *Echinochloa* species reported as hosts of *P. oryzae* by Asuyama (1965, pp. 9-22), Malagutti et al. (1951), and Revilla (1953) were not infected.

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## Rice blast disease in Brazil

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Brazil occupies the sixth place in the world as a rice producing country, with a cultivated area of approximately 5 million hectares, of which about 35 percent is irrigated and about 65 percent unirrigated, with a total yield of about 7 million tons/year. The main producers are the States of Golas, Rio Grande do Sul, Sao Paulo, Minas Gerais and Maranhao (Fig. 1).

In the State of Sao Paulo 78.8 percent of the cultivated area is on unirrigated soil and only 2.6 percent is on irrigated soil; the remaining 19.1 percent is cultivated on humid soils without irrigation. In the State of Rio Grande do Sul, the situation is the opposite, 98 percent of the cultivated area is irrigated.

The main rice diseases found in the growing areas of Brazil are caused by fungi and their occurrence depends on several factors —mostly the climatic conditions of humidity, temperature and sunlight— which, together with defective agricultural practices, favor the appearance, development and dissemination of the causal agents and increase the susceptibility of the plants.

Rice blast caused by *Pyricularia oryzae*, Cav., is the most common and important rice disease in Brazil. Considerable damage to the crops, limiting national average rice fields, is due to blast.

According to the available literature the first findings were made in 1912 (Averna-Sacca) and 1920 (Hempel) in the State of Sao Paulo, in 1930 (Muller)

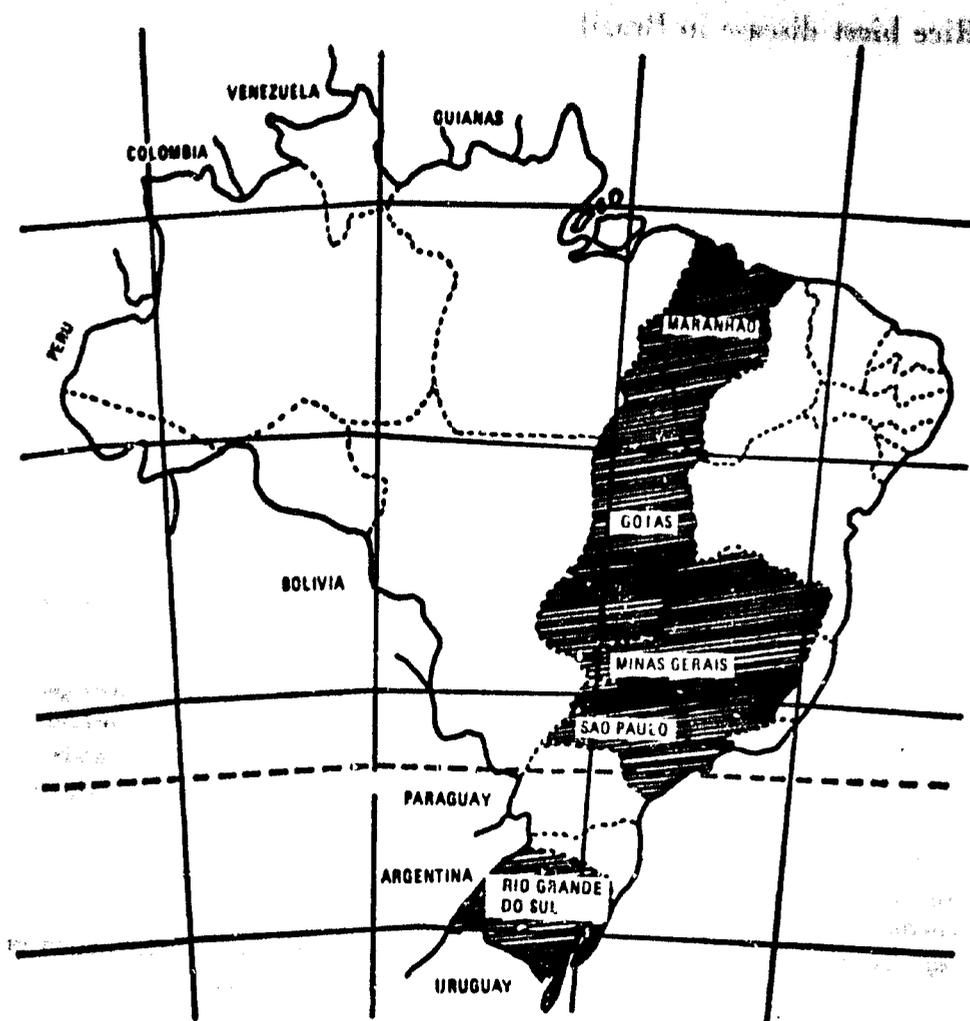


Figure 1. Main rice producing states of Brazil.

**In the State of Minas Gerais, in 1935 (Pimentel) in Rio Grande do Sul, in 1946 (Batista) in Bahia and the northern States, particularly in the São Francisco River Valley.**

The incidence of rice blast has been increasing yearly in the main producing areas. When the environmental conditions are favorable to the development of the disease, it becomes epiphytotic, causing large losses in either irrigated or unirrigated areas (Amaral and Issa 1971, Campacci 1950, IPEAS 1965 and 1967, Machado 1958, Mello 1960 and 1962, Parseval and Costa Neto 1939, Ribeiro 1970 and 1971, Silva 1971, Terra 1957 and 1958, Tocchetto 1947, Viégas 1959).

The symptoms of the disease are usually found on leaves, culms, branches of the panicle and floral structures, but in Rio Grande do Sul one of the most commonly observed symptoms is the "rotten-neck" (Bernardes and Bernardes 1958, Ribeiro 1971).

Research work on rice blast disease has been carried out mainly at the Instituto Biológico, São Paulo, in the State of São Paulo, at the Instituto Riograndense do Arroz, Porto Alegre, and Instituto de Pesquisas Agropecuárias do Sul, Pelotas, both in the State of Rio Grande do Sul, according to the following lines:

- I — Laboratory studies: biology and morphology of the causal fungus *Pyricularia oryzae*.
- II — Identification of the physiologic races of *P. oryzae* in Brazil.
- III — Varietal resistance.
- IV — Control.

#### **Laboratory Studies on the Biology and Morphology of the Fungus *Pyricularia Oryzae***

The results obtained in São Paulo State indicated that in PDA culture medium the optimum growth temperature lies between 27 and 30°C (Rossetti and Mello 1962, p. 47), and that optimum temperature for spore germination is 27°C at pH 5.5 to 6.0 (Amaral and Issa 1971). Isolates can be classified in three morphological groups according to the spore length: 1) 23.2 to 26.2 micra; 2) 17.2 to 20.1 micra; 3) intermediate measures (Rossetti and Mello 1962, p. 47).

### **Identification of the Physiologic Races of *P. Oryzae***

For the identification of the physiologic races, the international methodology proposed by Atkins et al. (1967) has been used.

The race group and classification number was based on the Table proposed by International Rice Research Institute, IRRI (1967, pp. 81-89).

The results obtained showed that in Sao Paulo State physiologic races may occur in large number since a high pathogenic variability has been detected. Four races (ID-13, IA-65, IB-1 and IC-5) were identified in a group of five isolates obtained from upland rice plants collected at Itu, Sao Joao da Boa Vista, Lins, Monte Aprazivel and Ribeirao Preto municipalities. In another group of four isolates from Bebedouro, Santa Barbara do Rio Pardo, Ituverava and Campinas municipalities, three other races will certainly be identified, depending on Raminad Str. 3 reaction, which has not yet been observed (Amaral and Issa 1971).

Four isolates of *P. oryzae* obtained from the basal node of the panicle did not affect leaves of any of the varieties included in the International Differential Group, nor of a set of 42 Brazilian cultivars: Dourado-peludo, Dorado-agulha, Cateto-branco, Jaguarari, Iguape-peludo, 7-V-10, 4 meses, Matao Branco, Espinho, Perola, Cateto-dourado, Iguape-agulha, Bico-branco, Gioano, Pindorama, Guedes, Birigul, Santa America, Agulha ESALQ-12, Ponta-Preta IAMG, Terra Roxa, Paulinia, Dourado-cearense, 3 meses, Dourado-precoce, 7-V-4, 7-V-8, Veranopolis, IAC-8, IAC-9, IAC-4, Come-cru, Chatao, Aviao, Guarapiranga, Guaiba, IAC-120, IAC-435, IAC-1246, IAC-2091, Pratao-precoce and Pratao comercial (Amaral and Issa 1971).

The races IA65 and IB1 were found to be the most pathogenic on 42 Brazilian cultivars, since 35.7 percent and 33.3 percent of these cultivars were respectively infected by the two races. Race IC-5 was less pathogenic on these cultivars since it affected only 11.8 percent of them (Amaral and Issa 1971).

In Rio Grande do Sul State fourteen races (IA<sub>1</sub>, IA<sub>5</sub>, IA<sub>60</sub>, IA<sub>85</sub>, IB<sub>5</sub>, IB<sub>21</sub>, IB<sub>37</sub>, IC<sub>5</sub>, IC<sub>21</sub>, IE<sub>5</sub>, IG<sub>1</sub>, IG<sub>2</sub>, IH<sub>1</sub>, II<sub>1</sub>) were identified in a group of 63 isolates obtained from rice plants cultivated on irrigated soil, located at the Coastal area, SE Slopes, Central Lowland and SE Hills. This result shows that there is a high pathogenic variability in the fungus population in the Rio Grande do Sul State (Ribeiro 1971 a and b).

It has been possible to identify several sub-races by inoculating the above mentioned races onto a group of six local cultivars: Stirpe Sel. Pelotas, EEA 404, EEA 405, EEA 406, Sel. IPEAS 2169 and EEA 201 (Ribeiro 1971a).

Many races, although different, caused similar reactions on these six cultivars. The Stirpe Sel. Pelotas cultivar proved to be the most resistant and Caloro the most susceptible.

In Rio Grande do Sul the predominant races are: IG<sub>1</sub> (19.61 percent of the isolates) less pathogenic to the local cultivars; IA<sub>6</sub> and IB<sub>3</sub> (13.74 and 11.77 percent of the isolates, respectively) which, with the races IA<sub>1</sub> and IB<sub>2</sub>, are highly pathogenic to the cultivars generally grown in the State, i.e., EE-404 and others resulting from interbreeding Zenith and Maravilha (Ribeiro 1971a).

A set of 145 cultivars was inoculated with the prevailing races IA<sub>1</sub> and IG<sub>1</sub>, and with a mixture of both; a decrease of pathogenicity of the race IA-5 was observed when the mixture was inoculated (Ribeiro 1971b).

#### Varietal Resistance

In Sao Paulo State the tests for blast resistance under field conditions on unirrigated soil began in the agricultural year 1964-65 with 519 cultivars (Issa et al. 1967). In the subsequent years another 36 cultivars (hybrids from the Instituto Agronomico of Campinas, and newly introduced ones) were added to these tests.

Two experimental fields were annually established in Campinas, one at Mario D'Apice Experimental Station of the Instituto Biologico, and the other at the Theodureto de Camargo Experimental Station of the Instituto Agronomico.

In the 1964-65 mentioned field, seeds of each cultivar were sown in one 2 meter-long line with a spacing of 0.5 m. In the successive tests two close lines of 2 meters were sown with each cultivar, with two replications. The border lines as well as every tenth line in the field were sown with susceptible cultivars: 7-V-10, IAC-162 and Dourado-precoce. At the tillering stage some of the rice plants from the border lines were inoculated as follows: in the 1964-65 test, inoculum of the fungus was put inside the still-uprolled newest leaf; in the subsequent tests spore suspension of each of the races IC-5, IA-65, IB-1, ID-13 and isolates number G-138 and G-765 were sprayed on all the border lines plants.

The reading of the incidence of blast on leaves and panicles was made according to the type of lesions (R, MR, M, MS, S) and the percentage of the affected area (\*t (trace), 5, 10, 20 ... 100) (Issa et al. 1967).

The same reading method was used for estimating the incidence of other diseases which occurred by natural infection, such as: helminthosporiosis - *Helminthosporium oryzae*; cercosporiosis - *Cercospora oryzae*; "mulata" or bronzing of the stem (unknown cause); "lista parda" or brown stripe - a well marked brown-red longitudinal strip which, beginning at the basis of the sheath, extends to the end of the leaf (unknown cause), although some microorganisms have been consistently isolated from the lesions (a fungus of the genus *Fusarium* and a bacteria of the genus *Xanthomonas* (Amaral and Issa 1971).

After successive screenings, always eliminating the cultivars which showed susceptibility over 10 S ( --- 10 percent of the leaf area affected), only 31 out of the 555 tested cultivars were used in the 1970-71 fields.

As a result 20 of them were found to be resistant to blast. From these only 4 were resistant to all the above mentioned diseases: Binicol, H-4, Down and Hsedni-56 (Table 1).

Six cultivars (64/75 --- (7-V-4 x 59,90), 64/47 --- (IAC-1246 x 1391), IAC-435, IAC-5032, IAC-2091 and Bico-ganga) which showed resistance in the 1970-71 test had been previously found susceptible at the Theodureto de Camargo Experimental Station in 1969-70, where the incidence of blast disease was much more severe than at the Mario D'Apice Experimental Station. Therefore these six cultivars must be included in the next field test in 1971-72 (Amaral and Issa 1971).

The tests at the Theodureto de Camargo Experiment Station in 1968-69 and 1970-71 were lost due to drought.

At the bed-test carried out in 1969-70 with the IAC-435, IAC-1246, IAC-120, Batatais, IAC-106, IAC-146, Pratao-precoce, Dourado-precoce and IR-8 cultivars, which were inoculated with blast infected rice leaves, the cultivars IR-8, IAC-106, and Pratao-precoce were the most resistant. In the 1970-71 bed-test the cultivars IAC-435, IAC-1246, IAC-47, IAC-120, IAC-68, IAC-162 and Batatais were inoculated by spraying a spore suspension of races ID-13, IA-65, IB-33, IC-5 and isolate number G-765, the cultivars IAC-120, IAC-1246 and IAC-47 showed to be more resistant (Amaral and Issa 1971).

Field and greenhouse research work for blast resistance in Rio Grande do Sul State started several years ago (Bernardes 1958, Costa 1961, Irga 1958). The field selection of resistant cultivars was carried out by natural infection under normal conditions of spacing between lines, fertilization and irrigation. Under these conditions, allied to ecological factors, the incidence of the disease was not always favored. In spite of this defective method some cultivars with good blast resistance were selected: Stirpe Sel. Pelotas, EEA 404 and EEA 201 (Ribeiro 1971b).

Table 1. Rice cultivars which showed resistance to blast in the 1969/70, 1970/71 tests and their reaction to other rice diseases.

	Origin	Life period	Cultivation system	Helminthosporiosis	Ceriosporiosis	Mulata	parda
Binicol	Philippines						
H - 4	Ceylon						
Down	USA						
Hsedni-56	?						
IR - 8 - 288 - 3	Philippines			30 MR			
Tremesino	Italy			30 MS		S	
Saturn	USA			70 MR		S	
IAC-1246 = (Pratão x Perola)	Brazil	130	upland		S	S	
IAC-5100 = (Pratão x Perola)	Brazil	130	upland		S	S	S
60/1175 = (Pratão x Perola)	Brazil	130	upland		S	S	S
64/32 = (7-v-4 x 59/442)	Brazil	130	upland		S	S	
64/9 = (6-v-1 x 3 meses)	Brazil	130	upland		S	S	
IAC-5544 = (Pratão x Perola)	Brazil	130	upland		S		
Taiwan no. 1	Formosa				S		S
5047 Days to flower 93	British Guiana					S	S
60/80 = (Palawar x Pratão)	Brazil	140-150	irrigated			S	S
IAC-68 * = (Iguape-agulha x Nira)	Brazil	140-150	irrigated			S	S
IAC-1300 = (Iguape-agulha x IAC-3)	Brazil	140-150	upland				S
Victory **	India					S	
BMT - 22 **	Philippines						

OBSERVATIONS: \* Included in the 1970/71 test.

\*\* MS Reaction to blast on the joint of the leaf sheath and leaf blade in 1970/71 test.

NOTES: Iguape-agulha = Mass selection-Instituto Agronomico of Campinas.  
 Perola = Mass selection-Instituto Agronomico of Campinas.  
 Pratão = Mass selection-Instituto Agronomico of Campinas.  
 3 meses = Mass selection-Instituto Agronomico of Campinas.  
 7-v-4 = (Nira x Dourado-agulha).  
 6-v-1 = (Nira x Dourado-agulha).  
 IAC-3 = (Jaguari x Yola).

In the subsequent tests the reaction to blast incidence on leaves was read using the International scale proposed by the Symposium on the Rice Blast Disease (IRRI 1963). For the evaluation of the panicle basal node infection, the percentage of damaged panicles of each plant was calculated.

During the agricultural year 1968-69, 850 rice cultivars were tested for blast resistance, 90 of which were rated as resistant (Ou, Nuque and Ebron 1970). In the tests carried out in 1969-70 and 1970-71, 49 and 30 cultivars, respectively, were tested and 16 cultivars were found to be resistant: Suwon n° 152, Taichung 65, CI 5309, Down, Tainan 3, Kaoshuing 21, Kaoshuing 24, Chokoto 14, Kanto 106, Kanto 51, Norin 20, Norin 22, Stirpe Sel. Pelotas, IR. 532-1-171, MO-R-500 x Nato and Swon n° 158 (Ribeiro 1971b).

In 1969-70, a set of 256 cultivars from The International Uniform Blast Nurseries was submitted to bed-tests (Ou system). Up to now three of these bed-tests have been completed. As a result several of those cultivars showed horizontal resistance under the prevailing conditions in the State of Rio Grande do Sul although some of them did not flower. Considering the group of the most resistant cultivars in the world, the following ones showed to be susceptible: Pah Leaud 29-9-11, Zenith, Ram Tulasi and Ram Tulasi (sel.); among the resistant cultivars only the following ones flowered: R-67, CI-7787, E-425, Down, KPE 6, Katakara DA 2, Mamoriaka, NP 130, Ca/902/b/2/2, Amritsari HR 22, DT. 10, DNJ-60, 370 Basmati, Pusur, T, Ca/435/6/5/1, 268 b/Pr/8/1/1, E.L. Gopher, Rajbohog N-22 and DV-75 (Ribeiro 1971b).

Local cultivars and some introductions were included in all the tests mentioned above.

The cultivars that presented the greatest number of resistant reactions are mentioned below; the ones which flowered under Rio Grande do Sul conditions are bold-faced. Starred cultivars had more than 50 percent of their panicles infected by "neck-rot".

a) Susceptibility 0/3: Tep-pep, Tadukan, C-46-15, D-25-4, H-4, H-5, H-105, H-501, **Murungakayan**, **Jae Keun (Suwon n° 152)**, Radin Ebo 33, Radin China 4, Lembu Basah, **K.P.F.6**, E.L., B-E-3, FB-86, Milbuer 5, **Chianung-Yu 280**, Taiwan 3, Lewang 28-1-14, Nang Chet Cuc, Trang cut L.11, Doc Phung, O Tre, Nang Quot (floating), Nang Chol, **CP 231 x HO 12 (Dawn)**, Remadja, Sigadis, Ta-poo-cho-z, N-302, **Ram Tulasi (sel)\***, Ram Tulasi \*, Unblatuzi Val. Sugar Co., **Mamoriaka**, Sorna Vari, Ramgarh, T<sub>3</sub>, **NP-97**, Milketan - 20, J-519, Laka, Huan-sen-goo, **Taichung line 47720**, Badshabag, Jhug Paddy n°7, Acheh Puteh, Leter 08, **Katakara DA 2**, **Rajbhog N. 22**, Thava lakkannan Ptb 9, T9, T1, T23, **Basmati T3**, **PI 4**, **CP 231 x HO 12 (215)**, **CP231 x HO 12 (216)**, C-46-15, **S20J.K.W.**, **S.39.J.K.W.**, **268b/Pr/8/1/1**, Pusur, **DF-1**, **DN-J-60**, **DNJ-52**, **DZ-78 \***, **D-74**, **DL-5**,

**DL-8 \***, **DL-9 \***, **DD-63**, **DD-80**, **DD-89**, **DD-99**, **DD-113**, **Ctg 250**, **Ctg 480**, **Ctg 1316**, **UCP 6**, **UCP 27**, **DM-32**, **DV-2**, **DV-12**, **DV-68**, **DV-73**, **DV-107**, **DV-109**, **DV-112**, **Samba**, **Murungakayan**, **Basmati (C 5836)**.

b) Susceptibility 0/2 : 1 intermediate reaction (3.0 — 4.0): **CI 5309**, **PI 231128**, **Norin 22**, **Taichung 65**, **Pal-kan-tee**, **C-33-18**, **M-302**, **Co25**, **N<sup>o</sup> K-60**, **Norin 22**, **Kongo (Br. n<sup>o</sup> 1)**, **Acheh**, **Hagi Haroun**, **Eng Katek**, **Padang Trengganu 22**, **R 67**, **Taichung 181**, **Kaoshiung 24**, **Kaoshiung 21**, **Kaoshiung-Ta-Lichin-Yu**, **Tam Vout**, **Samo Trang**, **Doc Phung Lun A**, **Nang Dum to (floating)**, **Pah Leuad 111**, **Pah Leuad 29-8-11**, **Kanto 53**, **= 79**, **T1**, **CI 6037-4**, **Basmati 370**, **N 12**, **N 32**, **CI 7338-5**, **CI 6914**, **Bm: 53 R 3540**, **Rad Shabbag (scented)**, **Ahmed Puteh**, **W.R.C. n<sup>o</sup>4**, **818-3 BR 9**, **Carreon**, **Madao 30My 137**, **Chou Kayama Ptb 26**, **Chuvanna Modan Ptb 30**, **Ca 902/b/3/3.**, **Ca 902/b/2/1**, **Tun Start**, **Surjamukhi DA 4**, **Amritsari HR 22**, **370**, **Basmati**, **46 Palman**, **Karla**, **Saraya**, **PI 3**, **S 18 J.K.W.**, **PI 184575-4**, **PI 184675-2**, **Donduni Kunluz**, **268b/Pb/22/1 1**, **268/Pb/22/2/3**, **Td 68**, **DNJ-128**, **DNJ-101**, **DJ-74 \***, **DJ-41**, **DZ-192**, **DK-11**, **DL-10**, **DD-6**, **DD-54**, **DD-100**, **Ctg 1206**, **DV-52**, **DV-83**, **DV-101**, **Basmati (C5875)**.

c) Susceptibility 0/1 + 2 intermediate reactions (3.0 — 4.0): **Ram Tulasi (sel)**, **Hashikalmi**, **Kataktara**, **Tan Chet Cut**, **Zilanica \***, **Pendok**, **Charnock (Aust)**, **NP-130**, **T9**, **J-109**, **Ca 435/6/5/1**, **Ca 902/b/2/2**, **406**, **41 Mushkan**, **3 month variety**, **E.L. Gophar**, **Nang Sawm**, **268 b/Pb/22/1/2**, **268 b Pr/22/3/2**, **268 b/Pr/50/2/2**, **Dissi Hatif**, **DZ-105 \***, **DK-13**, **DL-2**, **DL-11 \***, **DD-24 \***, **DD-120**, **DM-30**, **DM-59**, **DM-68**, **DV-114**, **DV-150**, **T412 (W349)**, **JC-170**, **St = 1**, **Columbia 1**, **MO-R-500 x Nato**.

d) Moderate susceptibility (3 intermediate reactions — 3.0 — 4.0): **E-425**, **TPxB 3812A** and **DNJ 171**.

Several new screening tests are planned to be established in the next agricultural year in the States of Sao Paulo, Rio Grande do Sul, Parana and Para. Local cultivars will be tested in each of the mentioned States along with introduced ones.

It seems very important that standard uniform reading methods for evaluating rice blast infection be established, so as to permit the comparison of results obtained in tests carried out at different parts of the country.

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## **Rice blast disease in Africa**

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At the beginning of the 1960's, annual rice consumption in Africa amounted to about 2.7 million metric tons. In the FAO Indicative World Plan the quoted estimate for annual rice consumption in Africa by 1985 is in excess of 5 million metric tons. Rice production in most tropical African countries is inadequate to meet the present consumption, and imports of rice add a heavy burden to foreign exchange balances. If the demand for rice is to be met in the coming years, there must be either an excessive increase in rice importation or a massive increase in home rice production. The importance of rice in the kitchens and economies of the various countries of tropical Africa is shown in Table 1. With the exception of Madagascar the East African countries are in general not so rice-oriented as are many of the Central and West African countries.

Present unit yields of rice in Africa are estimated to be very low, with an average of 1 metric ton per hectare. There is obviously a great deal of improvement to be made in the technology of production on the present area cropped with rice. Also there are huge areas which have the potential for rice production which have not yet been developed. Certain West African countries (e.g., Ivory Coast, Ghana, Nigeria) have initiated programs to increase rice production through both the expansion of the area cropped and promotion of improved technology (provision of new varieties, provision of fertilizer subsidies, etc.). However, considerable efforts will have to be made on many aspects (production, marketing, prices, supply of seed to growers, extension services, etc.) for rice production to meet present and projected demands.

Table 1. Rice production, trade and consumption data for tropical African countries \*

Country	Population <sup>1</sup>	% in Agric.	Rice Prod. <sup>2</sup>	Area <sup>3</sup>	Imports <sup>4</sup>	Exports <sup>4</sup>	Annual per capita consumption (kg)
Sierra Leone	2514	80	400	300	300	-	115.35
Liberia	1159	80	152	19 <sup>n</sup>	344	-	114.75
Gambia	384	88	20	18	86	-	60.44
Senegal	3916	75	126	89	1534	2	60.01
Guinea	3988	85	330	250	187	-	58.43
Ivory Coast	4553	81	336	300	241	-	52.71
Mali	5058	90	140	162	-	-	17.99
Ghana	8839	60	43	36	401	-	7.69
Upper Volta	5438	86	42	50	38	-	5.70
Niger	3851	91	33	12	14	-	5.97
Nigeria	63578	79	391	240	15	-	4.03
Togo	1863	79	32	34	27	-	1.29
Congo (K)	16353	70	120	115	450	-	7.52
Chad	3410	95	33	25	-	-	6.16
Gabon	473	84	1	1	16	-	4.24
Angola	5239	83	33	22	20	30	3.82
Cent. Afr. Rep.	1459	85	7	7	1	-	3.43
Cameroun	5470	84	13	13	69	1	3.11
Congo (B)	860	65	3	3	14	-	2.33
Zambia	3968	81	-	-	35	-	1.01
Madagascar	7224	83	1700	820	-	400	147.43
Tanzania	12173	95	115	110	24	4	6.33
Mozambique	7124	69	74	51	-	53	6.04
Uganda	7934	91	8	3	82	1	1.64
Kenya	9928	84	16	3	-	1	1.01
Burundi	3490	95	4	3	-	-	0.88
Sudan	14355	77	2	1	69	-	0.56
Malawi	4130	80	4	5	14	-	0.48

1. 1,000 people
2. 1,000 metric tons
3. 1,000 hectares
4. 1,000 metric tons

\* Table prepared by Dr. D.D. Hedley, IITA Economist.

The various types of rice culture in tropical Africa are differentiated in terms of the way water is made available to the crops. They include:

- (1) Upland rice, which is entirely dependent upon precipitation for its water supply;
- (2) Swamp rice, which is grown in flood plains or poorly-drained low lying areas and stands in surface water for much of its development, the level of which is not controllable;
- (3) Rice grown in irrigated paddies where the water level is closely controllable by the farmer;
- (4) Floating rice, which is grown in areas where the water level rises greatly throughout the season, and varieties are needed which are able to elongate rapidly to keep 3-5 leaves above the water level.

Upland rice accounts for over 60 percent of rice production in West Africa (Cooper 1970) and swamp rice makes up most of the remainder.

Research into rice production has been carried out at a number of centers for many years in West and Central Africa. At the end of the second World War regional rice research bodies were set up by the various colonial powers, for example, the Centre Des Recherches Rizicoles at Koba, Guinea, whose activities covered all countries of the former French West Africa; the Rice Research stations at Rokupr (Sierra Leone) and Badeggi (Nigeria) for the Commonwealth countries; and the Yangambi Research Station of the then Belgian Congo. When the countries gained independence, the research stations met with various fates. For the Francophone countries the Institut de Recherches Agronomiques Tropicales et des Cultures Vivrieres (I.R.A.T.) was created in 1960 and conducts research into rice in Madagascar, the Ivory Coast, Mali, Senegal, Niger, Cameroon and the Central African Republic. In the Anglophone countries rice research is undertaken at the Rokupr Station, Sierra Leone (which has undergone several changes of administration and is now part of Njala University of Sierra Leone); Kumasi and Kpong Stations in Ghana; Badeggi Federal Rice Research Station, Nigeria; and very recently the International Institute of Tropical Agriculture (IITA) at Ibadan, Nigeria. Within the last two years an attempt has been made to establish a West African Rice Development Association (WARDA) but at the present time WARDA is still very much in the planning stage.

### **Blast Disease in Africa**

The first report of blast disease in Africa was made by Small (1922 a and b) who stated that the blast of rice consistently reduced yields in Uganda and was the only major disease of rice in that country. Since 1922, blast has been reported wherever rice has been grown in Africa (CMI. 1968) and many workers cite blast as the most severe rice disease problem encountered (Bunting 1924 pp. 19-23 and 1928; Deighton, 1936, pp. 22-26; Hansford 1943; Inst. Nat. Etu. de Agron. 1951; Lawrence 1951; FAO 1960; Abo-El-Dahab and Michails 1966; Awoderu 1970).

In Africa, as in Asia, blast can occur in the rice crop at all stages of growth. As much of the rice crop in Africa is dependent upon direct precipitation for its water supply, and as rainfall is unreliable, especially at the beginning of the wet season (Cocheme 1971), rice can be expected to experience periods of drought stress, which according to the evidence of Suzuki (1934 and 1935) will increase its susceptibility to blast. Lamey (1971) noted relatively little blast in an irrigated nursery at IITA, whereas in an upland nursery nearby, several

varieties (which were also present in the irrigated nursery) developed severe leaf blast. The effect of drought stress on varietal susceptibility will have to be carefully considered when techniques for the assessment of varietal reactions to blast are being developed. Periods of drought alternate with periods of heavy rains with overcast skies for many days, and during these periods the rice plants continuously have free water on their leaves and culms. Rice which is heading in these wet, overcast conditions is particularly vulnerable to the rotten-neck phase of blast. In a summary of rice research by IRAT in West Africa (1970) it is stated that when rice is heading during heavy rains without sun, neck-blast is so severe that chemical treatment is the only way to save the crop. At IITA, varieties have been observed with very little leaf blast and extremely severe neck-blast (e.g., IR22, M7-28). Clearly, both the leaf and panicle phases of the disease need be examined in evaluation of varietal resistance to blast.

The variability within the population of *Pyricularia oryzae* in Africa has not been studied in any great detail. The International Blast Nursery has been tested in Sierra Leone and Nigeria and the results indicate a wide spectrum of pathogenicity within *P. oryzae* in these countries. The results of IBN tests in

Table 2. The blast reactions at IITA of those varieties found most resistant in the International Blast Nurseries.

No.	Group I Vars.	Test		
		1	2	3
11	Tetep	2(4)	3	1
88	R67	2(3)	1	2
41	C48-15	1	4	1(2)1
105	Nang Chet Cuc	2	1+	1
12	Tadukan	2	2(4)	1
1	C17787	3	2	3
98	Pah Leuad 29-8-11	2	1+	1+
42	D25-4	2	1+	1+
108	Trang Cut L 11	2	1	1
122	Pah Leuad 111	2	1+	1+
44	M-302	2	2	1
76	Pandang Trengganu 22	2	1+	1(2)
91	E-425	5	4	2(4)
50	Ram Tulasi (SEL)	1	1	1(2)
119	Ramadja	2	1+	1
46	H-5	2	1+	1
117	Dawn	-	-	-
63	No. K-60	4	3	1(3)
123	MO-R-500 x Nato	-	-	-
79	K.P.F. 6	3(4)	3	3(4)
78	Kataktara DA-2	-	-	-
45	H4	1	2(3)	1(2)
101	Zenith	2	4	2

A minus sign following lesion type indicates very few lesions.

A plus sign following lesion type indicates very many lesions.

A figure in parenthesis following another figure, e.g., 3(4) indicates that although there were a few of the more severe reactions the predominant reaction was that given by the first figure.

Table 3. The blast reaction at IITA of those varieties found most resistant in the International Blast Nurseries.

No.	Group II Vars.	Test		
		1	2	3
137	Mamoriaka	2	1	2
244	Dissi Hatif (DH-3)	2(3)	1	2(3)
126	Fah Leuad 29-8-11	2	1+	1(2)
128	Ram Tulasi	2	1	1(2)
149	NP 130	2(3)	1+	1(2)
164	Huan-Sen-Goo	1	1-	1
189	Thavalakkannan Ptb 9	2	1	1
201	Ca 902/b/2/2	2	2	1(2)
203	Amritsari HR22	2	3(5)	1
127	Ram Tulasi (sel)	1	1	1(2)
291	DL-10	3(4)	5	3
217	C46-15	2	3(4)	1(2)
257	DNJ-60	2(3)	3(5)	1(2)
136	Unblatuzi Valay	2(3)	2	1(2)
147	T3	1(2)	2	1(2)
255	DB-3	4	2(3)	3
205	370 Basmati	2(3)	2	1
254	Pusur	3(4)	5	1
135	T1 (3392)	2	1	1+
265	DNJ-146	2(3)	2	3-
180	Carreon	2	1	1+
191	TI (6294)	2	1	1(3)
196	Ca 435/b/5/1	2	1(4)	2
233	2686/Pr/8/1/1	2	1+(4)	1+
207	T23	2(3)	1(2)	1(3)
224	E.L. Gophar	2	1	2
188	Rajbohog N22	3	4(5)	3
328	DV-73	3(4)	5	3-

A minus sign following lesion type indicates very few lesions.

A plus sign following lesion type indicates very many lesions.

A figure in parenthesis following another figure, e.g., 3(4) indicates that although there were a few of the more severe reactions the predominant reaction was that given by the first figure.

Nigeria (at the Badeggi Federal Rice Research Station and at IITA, Ibadan) reveal the presence of virulence within the local population of *P. oryzae* to several varieties (E425, Amritsari HR22, DL10, NDJ-60, Rajbohog, DV-73, CI 7787, CI 27-3, Tua Sart, 3 month variety (Entry 211), C5561 and TD 70) that have been consistently resistant in most other parts of the world (Ou et al. 1970, Tables 2 and 3). Awoderu (1970) described eleven physiologic races of *P. oryzae* in Nigeria, but from the evidence of Ou and Ayad (1968) on the extreme variability within monoconidial cultures of *P. oryzae* it is probably more valid to describe a spectrum of virulence within a local population of *P. oryzae* than to attempt to define distinct physiologic races. The IBN tests should be established in many more locations throughout the rice-growing areas of tropical Africa to provide comprehensive information on the spectrum of virulence genotypes of *P. oryzae* in the continent.

Resistance to blast has been a selection criterion in several rice-breeding programs in West Africa for many years, and varieties have been developed with

quite a high degree of what appears to be horizontal resistance (e.g., the OS6 and OS4 varieties developed at the Yangambi Research Station in the Congo). However, these varieties have a relatively low yield ceiling, with poor plant type and poor quality grain, and improved varieties are being introduced from Asia and elsewhere. Several of the IRRI varieties (IR5, IR8, IR22) have been found particularly susceptible to blast in Africa, and resistance to blast continues to be one of the major selection criteria in most of the rice-breeding programs in Africa.

### **Blast Disease at IITA**

The rice program is one of the major crop improvement programs already underway at IITA. The objectives of the rice pathology team are stated as:

- (1) Identify and develop broad spectrum (horizontal) resistance to the major African diseases of rice;
- (2) Explore methods of chemical control of rice diseases;
- (3) Study pathogen variability, ecology and biology, and in collaboration with other international bodies organize and stimulate interest in Pan-African blast tests (both the International Blast Nursery and horizontal resistance nurseries).

During 1970, 874 entries were planted in both upland and irrigated nurseries for comparative evaluation and seed increase. Frequent disease observations were made in both nurseries and diseases encountered included blast, leaf scald (*Rhynchosporium oryzae* Hashioka and Yagoki), brown leaf spot (*Cochliobolus miyabeanus* Ito and Kuribayashi), narrow brown leaf spot (*Cercospora oryzae* Miyake) and false smut (*Ustilagoide virens* (Ck.) Tak.). The only serious disease problem was blast. Of 539 entries assessed for leaf blast reaction in the upland nursery in 1970, 216 were highly resistant (reaction types 1 or 2), 78 were moderately resistant (reaction type 3 or very few type 4 lesions), 189 were moderately susceptible (reaction type 4) and 58 were highly susceptible (reaction type 5 and above). During 1971, 387 entries were grown in upland nurseries and again a wide spectrum of varietal reactions was observed. The varieties that consistently develop reaction type 3, or very few type 4 lesions, will be the candidate varieties for further testing for horizontal resistance.

As stated above, the International Blast Nursery (IBN) was tested at IITA on three occasions, and varieties were observed to be susceptible at IITA that

are resistant in most other locations where the IBN has been tested. Several varieties were highly resistant in one planting, and highly susceptible in another, indicating a shifting spectrum of virulence in the local population of *P. oryzae*. In the future the IBN tests will be planted more frequently at IITA, and the IITA rice team will promote the establishment of many testing locations throughout tropical Africa for both the IBN and horizontal (or partial) resistance nurseries.

#### **Studies of Horizontal Resistance to Blast at IITA**

The program to develop varieties of upland rice with a high degree of horizontal resistance to blast is just getting underway at IITA. Ideas on methods for the selection of the resistance are being developed and tested. One idea is to obtain a precise measurement of those host controlled factors that affect the rate of development of an epidemic (e.g., incubation period, sporulation capacity related to lesion size, number of lesions per unit leaf area, efficiency of spore production per unit lesion area) and compare varieties for these parameters. This would be done with controlled inoculation with a standard concentration of conidia, a standard drop size, and a controlled environment for maintenance of seedlings – the use of detached leaf segments offers the greatest precision in such an operation. However, degree of varietal susceptibility in the field will depend upon the interaction of many factors including:

- (i) the complement of major resistance genes the variety possesses;
- (ii) the complement of polygenes for resistance in the variety;
- (iii) the complement of virulence genes in the inoculum;
- (iv) the aggressiveness of the races in the inoculum;
- (v) environmental conditions including soil-water relations, soil nutrient status, temperature, humidity, etc.

The questions that arise when considering precise measurement of host-controlled epidemic factors include:

- (i) what nutrient levels are to be used in raising the plants;
- (ii) should the plants be subjected to water stress, and if so, how much;
- (iii) do seedling leaf reactions tally with the reactions of adult plant leaves;
- (iv) what is the spectrum of virulence in the inoculum;

(v) even if leaf blast resistance is good, is the variety resistant to neck rot phase of the disease.

Precise measurement of the host-controlled epidemic factors mentioned above will be conducted in conjunction with field testing at many locations, with several dates of planting at each location. In this way an evaluation will be made of the usefulness of the controlled precise measurement method for identification of horizontal resistance.

### **Chemical Control of Blast in Africa**

Many fungicides are known that can control the blast disease organism, but their usefulness depends upon the economics of their application. In Africa, yield ceilings for rice are very low compared with those in many parts of Asia; there is no chemical industry, and pesticides are very expensive; there is a low availability of hardware for the application of pesticides and the general level of technical skill at the farm level is low. At the present time, therefore, there is little practical use of chemical control of blast in Africa. Tests are being made on the effectiveness of various chemicals for blast control at various research stations in Africa (Dept. Agr. Res., Nigeria 1968b, IRAT 1969, Dept. Agr. Nigeria 1971) and at IITA in 1971 over threefold increases in yield were obtained with two applications (at 50 percent flowering and again 12 days later) of Benlate (600 g in 1000 l per ha) with the rice variety IR22. At the present time the information gained on chemical control can be usefully applied to control blast in experimental crops of rice, but it is the efforts of the geneticists and plant breeders that can be expected to have the major technological impact in the control of rice blast disease in Africa in the next decade.

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**Phenotypic stability of pathogenic races  
of *Pyricularia oryzae*,  
and its implications for breeding  
of blast resistant rice varieties**

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Our studies on the rice blast disease have been concerned primarily with the extent of pathogenic variation that is inherent in the causal fungus, *Pyricularia oryzae* Cav. Following recognition of pathogenic specialization in this organism in 1952 (Latterell et al. 1954), we undertook to develop a series of differential rice varieties as an essential tool in exploring this variation. In the course of testing varieties as candidates for differentials and as sources of resistance to the races characterized, we became familiar with the range of rice leaf reaction types that were characteristic of infection of different varieties by different races. As we tried to select varieties of rice that would show consistent and definitive reactions to given isolates of the pathogen, we observed the range of variation in lesion type that a variety may show to a single race under different nutritional and environmental conditions. Some varieties showed less variation with environment than did others respecting blast reaction, and such varieties, if they showed differentiating qualities, were selected as candidates for a differential series.

As we began to evaluate leaf reactions in terms of panicle reactions to determine the significance of the former in terms of crop damage, we saw the need to standardize growing conditions for plants, and inocula. The former was approached through experimentation with test plant culture and fertilization practices, and through the development at our laboratories of special facilities

for controlled conditions of plant growth, inoculation, and incubation (Kingsolver and Mitchell 1954, Lange 1954, Mitchell and Cherry 1954). As means for quantitative inoculation were developed (Lange 1954, Tervet et al. 1951), the advantages of dry spore inocula were recognized in evaluating rice varieties for resistance and for studying the relation of various environments and levels of nutrition to infection and disease development. Dry spores of the fungus, which can be stored in relatively stable form for a number of years, also offer the advantage of continuity and reproducibility of experiments, even though years may elapse between tests of different varieties to a given race, or of comparisons among races to given varieties. Today we are able to obtain varietal reactions identical with those of leaf specimens collected from greenhouse inoculations 12 years ago, inoculated with spores produced at that time.

Our search for differential varieties was facilitated by the use of dry spore products in that any number of candidate varieties could be tested repeatedly with identical inocula. The goal was to select a minimum number of varieties that would differentiate a maximum number of races of the pathogen. Ten varieties were selected as a result of this effort (Latterell, Tullis and Collier 1960), and six of these were chosen by the United States—Japan Study Group along with two of the Japanese differentials (Goto and Yamanaka 1961) as the International Differentials (Atkins et al. 1967, Goto and Atkins et al. 1967). Although these varieties have been useful in identifying races, they do not entirely fulfill our hopes for unequivocal differentiation. The present controversy as to the rate of pathogenic mutability in *P. oryzae* may be at least in part related to the difference in interpretation of borderline varietal reactions that are significantly affected by environment and nutrition. The goal of obtaining a series of differential varieties that would yield unambiguous reactions under all conditions is probably unattainable. As suggested by Hebert (1971b), the development of lines of rice known to be isogenic for resistance to individual races is a worthwhile goal. Considerable pathogenic variability has already been demonstrated in *P. oryzae*. Knowledge of the extent of this variability can be approached by identification of all the genes for resistance potentially available in the rice plant.

Apparent mutations from one race to another were observed early in our work, and have occurred occasionally throughout our studies, but the more substantial evidence indicates that phenotypic stability of pathogenicity is more common than variability. The question of racial integrity has important implications in consideration of the type or types of breeding programs that should be emphasized in developing resistance to the blast disease. Some of the techniques that have been developed in our laboratories and their application to a search for sources of "horizontal resistance" to blast will be described.

### **Range of Variation: Cultural and Pathogenic**

Since the demonstration of pathogenic specialization in this fungus in 1952, recognizing specific differential reactions of selected varieties to different races of the pathogen (Latterell et al. 1954), we have cultured and tested several thousand isolates of the fungus from specimens representing most of the rice-growing regions of the world. Early in our work we observed the variety of colony types characteristic of this fungus. We recognized the importance of sector selection as a means of improving sporulating characteristics, thus facilitating preparation of inoculum.

Summarizing the types of growth habit with which most workers are familiar, we encountered on 2 percent rice polish agar every gradation of shades of gray between white and charcoal-black, with occasional buff-colored mutants. Textures ranged from cottony (non-sporulating) to smooth-velvety (highly sporulating) with intermediates of mosaic-patterned cushions (moderately sporulating) and flat "melted-out" colonies lacking aerial mycelium and forming few or no spores. Other colony types included normally smooth colonies that develop deep furrows, white mycelial colonies with black extracellular pigment in the agar (poorly sporulating), and "perspiring" cultures - gray colonies covered with liquid globules. Our experience has indicated no correlation between pathogenicity and colony type except for the following weakly- or non-pathogenic types: (1) buff mutants (non-pathogenic), (2) white cottony colonies that produce no spores and therefore cannot be readily tested, (3) "melted-out" cultures that may be virus-infected and produce no spores, and (4) deteriorated cultures that produce few and abnormal spores, most of which produce appressoria in agar culture (an abnormal behavior). These findings are in contrast to those of Ling and Chinte-Sanchez, who reported a high degree of correlation between colony type and pathogenicity (1968). Some of our broadest-range races include both (1) dark-colony, highly sporulating, and (2) light-gray, poorly sporulating types, plus even the mixed types with cottony-white mycelium, black agar pigment, and sporadically sporulating gray surface growth. Some of our narrowest-range isolates are among the strongest sporulators.

Most of the races (pathotypes) we have characterized and of which we have cultures and stored inoculum in stable form are shown in Table 1. The type cultures of fifteen of these races have retained their original cultural and pathogenic characteristics for more than 10 years, four of them for nearly 20 years in continuous culture. The races are not arranged as under the international system (Ling and Ou 1969), but according to their pathogenicity toward two key varieties, Caloro and Sha-tiao-tsao (S). Japanese and Chinese workers have also grouped races according to their pathogenicity to types of varieties from the same gene pool (Goto and Yamanaka 1961; Chien, Lin and

Table 1. Races of *P. oryzae* of which dry spore inocula have been produced.

ORIGIN OF U.S. CULTURE	RACE 1 GROUP										RACE 2 GROUP										RACE 3 GROUP					RACE 7 GROUP																									
	U.S. TYPE CULTURE NO.	873	429	802	499	748	559	468	712	476	740	600	602	551	367	439	733	520	438	661	603	590	923	760	479	640	196	455	825	710	899	463	793	908	695	580	504	699	696	649	737	566	549	900	820						
INT'L RACE NO.	IA	IB	ID	ID	ID	ID	IG	II	IA	IB	IB	IB	IC	IC	IC	ID	ID	IE	IE	IH	IH	IA	IB	ID	IG	IG	IA	IB	IB	IB	IB	IC	ID	ID	IE	IE	IF														
U.S. RACE NO.	110	54	6	8	14	16	2	1	111	35	47	63	19	19	23	7	15	3	3	1	1	30	45	13	1	1	65	1	5	17	33	45	1	9	17	17	25	25	1	1	5	9	1	5	1						
VARIETY	50	1	12	14	5	10	32	39	30	48	34	37	24	20	22	41	26	23	44	2	4	11	36	6	3	18	25	13	40	49	7	35	9	42	16	29	45	43	8	47	38	28	51	46							
RAMINAD STR. 3	+								+														+				+																								
ZENITH		+								+	+	+												+					+	+	+	+	+																		
NP 125														+	+	+												+	+	+	+	+	+	+	+	+	+	+	+	+											
USEN	+		+	+	+	+			+	+	+					+	+						+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
DULAR		+	+	+						+			+	+	+	+		+	+									+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
KANTO 51										+			+	+				+	+									+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
SHA TIAO TSAO (S)	+	+	+		+	+																	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
CALORO									+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
AICHI ASAHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = susceptible

blank = resistant

Jong 1963) rather than to single varieties listed in order of their assumed decreasing number of genes for resistance, as in the international system (Ling and Ou 1969). The Japanese classify their races into "T" races for those that attack such highly resistant Indica varieties as Tetep and Tadukan, "C" for those attacking such Chinese types as Usen and Ishikari Shiroke, and "N" for those races essentially limited in their pathogenicity to the Japanese (Norin type) Japonica varieties (Goto and Yamanaka 1961; Kiyosawa 1971; Ezuka et al. 1969; Yamada, Matsumoto and Kozaka 1969). The races identified in this laboratory appear to segregate naturally into 4 groups: Race 1 group—races that are non-pathogenic to Caloro; Race 2 group—races that are strongly pathogenic to Caloro but which induce only a "brown-speck" resistant reaction in Sha-tiao-tsao (S); and Race 3 and 7 groups—races that are pathogenic to both Caloro and Sha-tiao-tsao (S), the majority of which (Race 7 group) are generally associated with a broad host range. When tests were extended to include additional varieties of different geographical origins, these patterns recurred consistently, suggesting that the grouping of races as shown in Table 1 does reflect basic similarities in their genotypic potential for pathogenicity (Latterell and Marchetti 1968).

Our experience has been that vertical pathogenicity shows a strong negative correlation with varietal host range. Most of the races comprising groups 1 and 2 plus certain races from group 3 are characterized by narrow host ranges and definitive host-pathogen interactions. Conversely, the majority of races in group 7 have relatively broad host ranges and often produce less definitive responses in host varieties. The clear-cut effects of the narrow-range races suggest few genes for virulence individually having large effects. Alternatively, less definitive pathogenicity among the broad-range races indicates polygenic (horizontal) components.

The goal of recent investigations has been to (1) gain insight into the probable significance of "intermediate" type leaf lesions in terms of panicle reactions and ultimate crop damage, and (2) examine the pathogenic stability of the various races. This information is critical to meaningful evaluation of the responses of rice varieties of agronomic interest to defined races of the blast pathogen and to determination of the relative merits of future emphasis on horizontal versus vertical resistance in breeding programs.

During the course of our efforts to test varieties from many sources of germ plasm for their race-differentiating ability, we excluded varieties that consistently showed intermediate reactions to any race. Depending on the "state of vigor" of test plants and greenhouse temperatures, most varieties varied by 1 or 2 lesion rating points in reaction to a given race at one time or another. Those that showed predominantly intermediate reactions were assumed to represent more complicated factors for resistance; i.e., they probably differed from either highly susceptible or highly resistant varieties

by at least several genes for specific races, and so were not suitable as differential varieties. We assumed that they would be in the "tolerant" category (with polygenic resistance) if exposed to those races in the field. This assumption was supported by field tests with Nato and Calrose exposed to race IG-1, and Rexoro to IB-54. Although these varieties could yield susceptible ratings when tested in the greenhouse under ideal conditions for plant growth at optimal age for infection, their reactions to the same races under less favorable conditions were altered to a resistant category by adverse field conditions.

This same phenomenon was even more striking when such broadly resistant varieties as Tetep, Tp Saigon 229, Tam Den, Chiem Chanh 198, Rikuto kogane hata mochi, and CICA-4 were tested. Plants of these varieties in the 3- to 5-leaf stage could show susceptible reactions to their respective pathogenic races during spring testing at moderate temperatures, yet resistant reactions at the same growth stage tested one week later at higher greenhouse temperatures. Plants of the same varieties and same growth stage tested to the same races in mid-winter were highly resistant. In this case, the resistant reaction was the result of poor host condition (unfavorable conditions for pathogen invasion). Lush growth of the host favors infection by *Pyricularia*, and plants growing under less than optimal environment are, ironically, more resistant to blast. Relative to this, there is a saying in Surinam that blast is the "rich man's disease," but *Helminthosporium* brown spot, which flourishes in plants under poor nutritional conditions, is called "the poor man's disease." Our results reflect the observations of Hashioka (1965) in defining the parameters for blast severity in different locations and seasons. His basic premise, with which we agree, is essentially that what is good for the rice plant is good for *Pyricularia*.

It is difficult to grow healthy rice in the greenhouse during the winter months at our latitude, due to what we interpret as a light-water-nutrition imbalance (Latterell, Marchetti and Grove 1965). This is the reason that a variety such as Tetep may show a susceptible reaction to race IB-33 in young plants under optimal conditions for infection and lesion development, yet will be quite resistant to that race a short time later under conditions that favor a shift in the growth-differentiation balance of the host. It is for this reason that races IG-1 and ID-13 may not be readily differentiated during our winter season: Usen, the differentiating variety for these races, is so sensitive to the nutritional-environmental balance that it may show resistance to ID-13 to which it is normally susceptible under good rice-growing conditions. Races such as ID-15 and ID-7, which depend upon Dular for differentiation, are sometimes difficult to separate because of a similar characteristic of this variety. We do not wish to malign these or any of the differentials unduly, but only to stress the importance of providing optimal growing conditions for test plants. It is appropriate to refer again to the work of Hashioka

(1950), in which he showed from field observations over a number of years in different locations on Taiwan that blast is not severe when either of two situations prevails: (1) temperatures are too high (or too low) for the lush vegetative growth of the host that *P. oryzae* requires or (2) nitrogen level is too low. His conclusion was, "When nitrogen is given excessively, its effect may override that of temperature, but if it is given not to exceed that which allows normal growth, temperature is more influential in susceptibility than is nitrogen." That the nitrogen-temperature-susceptibility relationship is extremely complicated is evident in the scholarly analysis of the interrelated chemical, anatomical, and environmental factors reported by Sadasivan et al. (1965). The basic studies of Otani (1959) on the relationships between nutrition and susceptibility include detailed and definitive work on the positive correlation between blast severity and nitrogen in the nutrition of the rice plant.

Hashioka (1963), in the final report on his work for FAO in Thailand, noted that whereas most of the resistant varieties tested in different locations remained resistant, and all of the susceptible varieties were susceptible in all regions, more variations were observed in the intermediate group. He speaks of a concept to explain wide variations in blast resistance due to local environments in terms of an equilibrium between qualitative (genetic) resistance, broken down only by new races of the pathogen, and quantitative resistance that is affected easily by environmental conditions. The literature includes a number of reports such as those of Ou and Nuque (1963) and Hsieh and Chien (1968) indicating a high positive correlation between **leaf resistance** to blast at an early stage and eventual **panicle resistance**, and **leaf susceptibility** with **panicle susceptibility**. Reports to the contrary are mostly based on field data obtained before the existence of pathogenic races was recognized. That is, a difference in reaction at the two stages can now usually be explained by changes in race population as the season progresses. As far as we have been able to determine however, little or no information is available on the precise correlation between **intermediate** type **leaf** reactions and **panicle-stage** reactions to the same race; that is, how severe is panicle blast in variety-race combinations that yielded 2+ or 3- to 3+ leaf reactions in the seedling stage? Our inclination has been to consider these intermediate leaf reactions as indicative of resistance that could be overcome only by exceedingly favorable conditions for growth and reproduction of the pathogen: high level of ammoniacal nitrogen, high relative humidity with frequent precipitation, and moderate temperatures, especially with low night temperatures (Sadasivan, Suryanarayanan and Ramakrishnan 1965; Ramakrishnan (1967). Our recent studies comparing reactions of varieties selected for either high or intermediate resistance to a broad spectrum of races in the leaf stage with their reactions to these races in the panicle stage have led us to somewhat different conclusions. Efforts to determine whether these discrepancies are real or attributable to some deficiency in inoculation and/or plant culture techniques are in progress. The methods used in various

phases of these studies (strain selection, inoculum production, harvest, and storage, plant inoculation, and disease evaluation) will be described followed by some of the kinds of information we have acquired through application of these techniques.

## METHODOLOGY

This section is entitled "Methodology" to indicate that we are not only presenting "methods," per se, but a study of methods, along with results necessary to evaluate the various techniques.

It became evident during our first year of work on the blast disease that *P. oryzae* exhibits a broad range of cultural growth types. Unlike some species of *Helminthosporium*, *Pyricularia* mycelium (without spores), either freshly macerated or dried, was unsatisfactory as inoculum. The most important cultural characteristic with respect to evaluation of pathogenicity, therefore, was sporulating capacity. A great number of natural and synthetic agars and substrates were tested for efficacy in supporting sporulation. Among some 100 media tested, several substrates were selected, either as superior for certain strains, or for general utility in providing good spore inoculum. Along with the testing of substrates, environmental conditions — including light, temperature, and relative humidity — were evaluated.

A need existed for spores in two forms: (1) spores freshly harvested from recently isolated cultures for the purpose of pathogenic race identification, i.e., for qualitative determination of pathogenicity; and (2) dry spores that could be dispensed quantitatively onto plants for greenhouse or field studies of spore-to-lesion ratios among different strains and effects of various environmental factors on infection. Dry spore products are of paramount importance here in providing continuity among experiments and reproducibility of results even though years may elapse between tests involving specific isolates. This capability is especially significant in light of recent reports that this pathogen is so mutable (or variable) as to make testing of rice varieties for resistance to specific races meaningless (Bandong and Ou 1966, Giatgong and Fredriksen 1967 and 1969, Ou and Ayad 1968, Quamaruzzaman and Ou 1970). Before discussing the implications of these reports and our findings to the contrary, we will describe the approaches, processes, and techniques developed to standardize inoculum production, testing, and evaluation methods.

The first isolate of *P. oryzae* tested (1952) was one collected in Arkansas in 1947. Following these first tests, in the same season, an epiphytotic of blast occurred in Florida on varieties of rice that had been resistant in

Arkansas (Zenith, Bluebonnet, Century Patna 231). Isolates from these varieties proved to be opposite in pathogenic pattern to the isolate already on hand from Arkansas. They were pathogenic to Zenith and Rexoro but non-pathogenic to Lacrosse and Caloro, whereas the Arkansas isolate showed the reverse pattern (Latterell et al. 1954). This first indication that *P. oryzae* comprised more than one pathogenic race led to an intensive study of the pathogenic potential of this fungus.

As isolates were acquired from worldwide sources, we began testing a wide range of natural materials as substrates for growth and sporulation of *P. oryzae*. The first of the two processes developed for producing dry spores is a biphasic process in which, during "phase 1," corn grain is steeped, sterilized, seeded with fungus, and incubated in aerated Fernbach flasks under aseptic conditions. For the "phase 2" incubation, the fungus-covered grain is shaken from the flasks onto wire mesh trays and incubated under clean but only semi-aseptic conditions in a cabinet with 97-98% RH at 26 C, with continuous light. Following several days of incubation, the sporulated grain is dried in a cabinet with circulating air at 40 C for 24 hours. After the corn is thoroughly dry (ca. 7% H<sub>2</sub>O), it is agitated with an organic solvent, the resulting suspension of spores is filtered, the spore "cake" is dried at 40 C, crumbled and put through a sieve, and the spores are placed in appropriate containers for storage.

An alternate "mycelial mat" process, developed more recently, is less wasteful of substrate and more efficient, but it has the drawback of not yet being amenable for use with all strains of the fungus. It is especially good for highly sporulating strains. The procedure involves the following steps: (1) mycelium is grown in liquid shake culture for several days; (2) the growth is filtered off (shortly after pigmentation starts) onto filter paper in a Büchner funnel, maintaining suction only until the liquid has disappeared; (3) the mycelial mat is separated from the paper; (4) the mat is incubated on a wire screen in a cabinet with 97-98% RH at 26 C with continuous light for 2 or 3 days; (5) it is then dried, broken into a Waring blender with an organic solvent, blended, filtered through a fine sieve to remove mycelial clumps, and filtered a second time through a Büchner funnel, leaving a cake of spores that is then dried; and (6) the dry cake is pressed with a spatula through a coarse screen to convert it to a powder. The product is packaged and stored as described subsequently.

Although the steps of these processes appear to be relatively simple, a surprising degree of refinement in technique is necessary to insure that optimal conditions and treatment are carried out at every stage of the operation for both processes. Corn, sorghum, and rice grains all were good substrates for supporting sporulation, but sorghum, and rice tended to be too sticky, so that clumping of grain during the steeping and sterilizing resulted in wasted surface

area for fungus growth. The corn grain should have a minimum of cracked or broken grains, because exposed endosperm causes stickiness and promotes mycelial growth rather sporulation. Cooking time for corn in relation to volume is critical: it must be steeped long enough for moisture to penetrate into the center of the grains, yet not so long that when it is pressure-cooked for sterilizing it will be too soft with excess bursting of grains, exposing too much starchy endosperm. Shake cultures of mycelium are used as starting inoculum. The rate of air flow (through sterile air samplers used as filters) should be nearly minimal to prevent rapid drying-out of the steeped corn. The aeration increases pigmentation and sporulation of the fungus in "phase 1" growth and, although these spores germinate following the daily agitations or after transfer of the grain to wire mesh trays for "phase 2" incubation and are thus not part of the final spore product, growth that results from pigmented sporulating mycelium is more likely to yield spores than is that from white vegetative mycelium such as that grown in non-aerated flasks. The optimal growth period for "phase 2" incubation varies with the strain: highly sporulating strains can be incubated for as long as 4 or 5 days with increasing numbers of spores throughout the period; poorly sporulating strains are likely to be overtaken by mycelial growth, or spores that are formed tend to germinate in situ, so such strains are usually best dried after about 48 hours. It is very important that the corn be thoroughly dry (ca. 7% moisture); otherwise, moisture reacts with the solvent used in harvesting spores to give the spore cake a rubbery consistency that does not crumble uniformly into powder. The solvents of choice are vythane (1, 1, 1-trichloroethane) and Genetron 113® (Freon 113®). Spore harvest in the corn process is performed by shaking the corn and solvent in a stoppered flask either manually or on a mechanical shaker for 10 minutes. Harvest of spores from dried mats should be performed under a hood to avoid excess breathing of fumes. The solvent can be redistilled following each spore harvest and used repeatedly. We have prepared a detailed write-up of both protocols that we will gladly furnish upon request. The main steps in each procedure are illustrated in Figs. 1 through 29.

By both processes we have attained a level of spore concentration exceeding 2 billion spores per gram, with viability greater than 90 percent. A photomicrograph of a suspension of spores from a superior product is shown in Fig. 29. Our calculations indicate that a pure spore product would exceed 3 billion sp/g. Impurities resulting from the "steeped corn" process consist of minute particles of corn and mycelial fragments, whereas those of the "mycelial mat" process consist only of the latter. We have spore products of a high degree of purity that were produced as long as 16 years ago and are still more than 60 percent viable. Among some 500 spore samples produced over a 16-year period, not a single instance of qualitative change in pathogenicity from that of the seeding inoculum has been observed.

The products have been stored variously in sealed ampoules and bottles under partial vacuum with nitrogen, and in screw-cap jars at atmospheric pressure. Long-time storage temperatures have ranged from room temperature down to that above liquid nitrogen, ca.  $-170^{\circ}\text{C}$ . Samples stored in sealed containers retained viability better than did those stored in screw-cap jars that were opened frequently for testing. All the colder temperatures were better than room temperature for preserving viability. Tests of liquid nitrogen storage over a 6-year period have shown no significant loss in viability. The tolerance of these spores to extreme cold, — in contrast to data reported by Abe (1955) in which spores died within a day if frozen at  $-10^{\circ}\text{C}$  — is assumed to be attributable to their dry state (ca. 7%  $\text{H}_2\text{O}$ ), whereas Abe probably was testing partially hydrated spores.

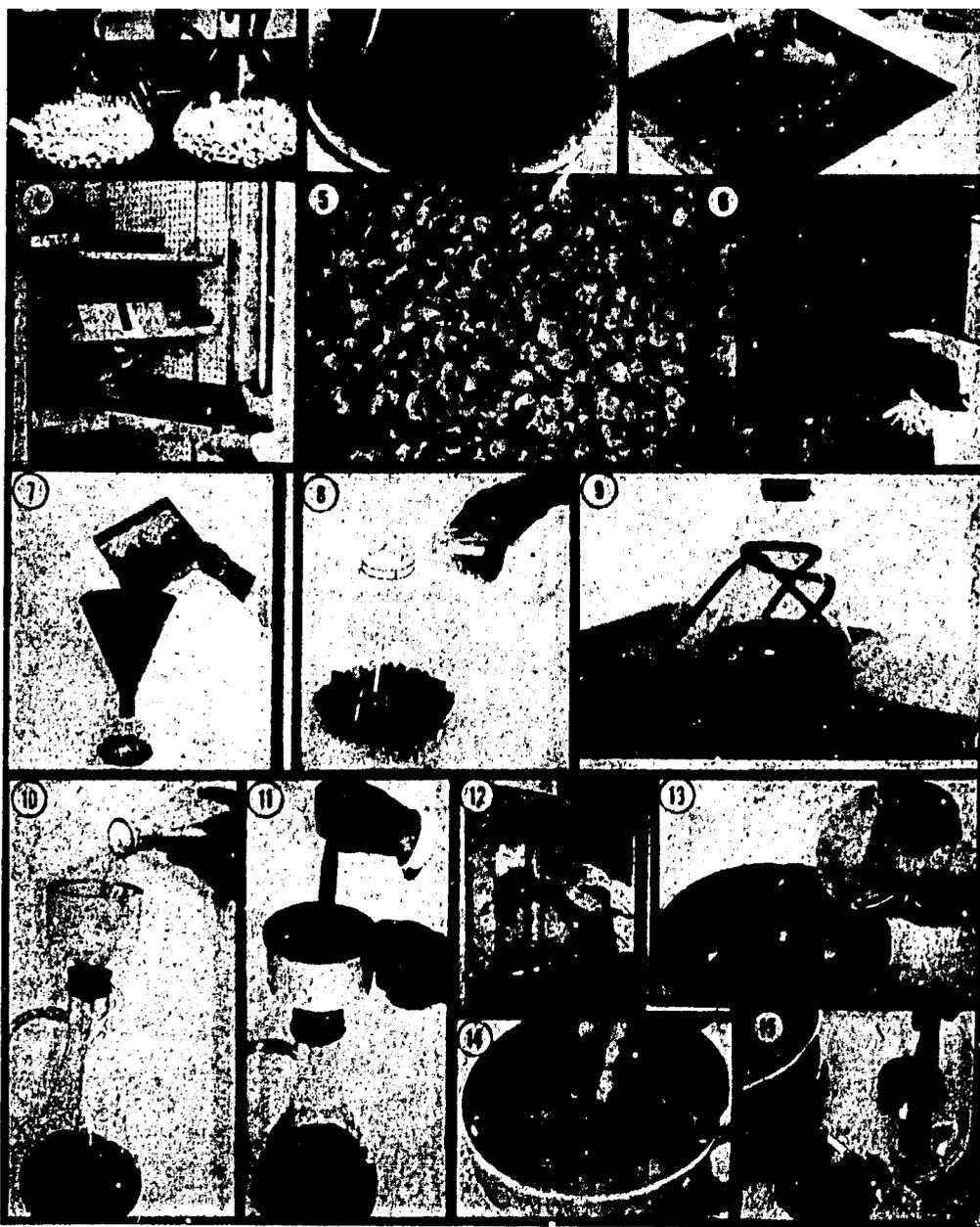
The best and most convenient procedure for preserving inoculum of a given race and strain is to divide the spore product into small lots for sealing in ampoules under partial vacuum with nitrogen and store them at  $4^{\circ}\text{C}$ . Then spores can be removed from small samples as they are needed for greenhouse or field inocula without exposing the whole product to frequent changes in temperature by removal from the refrigerator for sampling. This is not to say that a sealing apparatus is essential. Even if spores are divided into small quantities and stored in screw-cap vials at atmospheric pressure (at  $4^{\circ}\text{C}$ ), the viability of a good product may be retained at a high level for years.

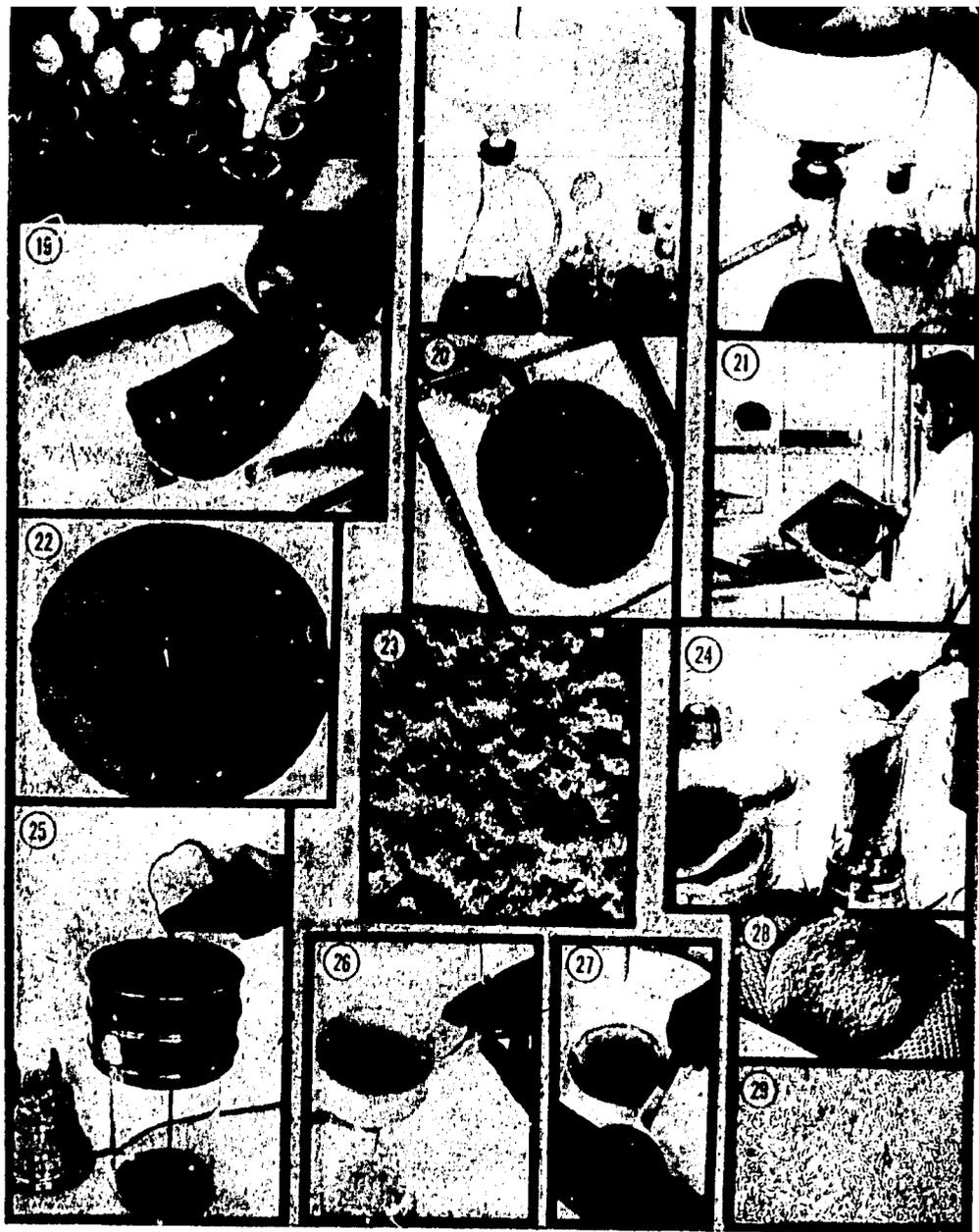
Another prerequisite for producing a superior spore product is a highly sporulating strain as seeding inoculum. We mentioned earlier that we had found no correlation between cultural growth habit and pathogenicity, except for non-pathogenic buff mutants (Neufeld et al 1958). We have been successful in selecting highly sporulating strains of many races representing a broad range of pathogenic patterns. Occasionally a culture sporulates densely upon first isolation and retains this characteristic indefinitely. More often, however, our experience has been that "wild-type" cultures are only moderate sporulators when first isolated onto agar. A few strains, however, have sporulated poorly from their first isolation onward and have retained this habit for years. Because of our interest in dry spore inoculum, we have attempted to increase the sporulating capacity of all cultures by continuously selecting from better sporulating sectors whenever they occur. Densely sporulating cultures yield the best quality spore products. When such sectors are selected, they are tested to compare pathogenicity with that of the parent culture. Among several hundred cultures thus selected and tested, less than a dozen instances of pathogenic change have accompanied the changes in growth habit.

We have also employed X-radiation of both germinating and dry spores in an effort to improve sporulating capacity and to determine the amount of pathogenic variability resulting. Dosages used for germinating spores ranged

**Figures 1 to 15:** Fig. 1. Fernbach flasks fitted with aeration tube, vent, and seeding orifice through which steeped corn grain has just been seeded with mycelial inoculum of *P. oryzae* grown in shake culture. Fig. 2. Fernbach flask containing fungus-covered grain 3 days after seeding; flask has been shaken once daily to redistribute growth. Fig. 3. Fungus-covered grain is transferred to wire mesh stainless steel tray that has been autoclaved to provide semi-aseptic conditions for 2nd phase incubation. Fig. 4. Fungus-covered grain in tray is placed in high-humidity (ca. 97% RH) cabinet with light at 26 C for static incubation. Fig. 5. Corn grains bearing dense sporulation after 3 days in the high-humidity incubator. Fig. 6. Sporulated grain is placed in a drying oven with circulating air at 40 C for 24 hours. Fig. 7. Dry sporulated grain (ca. 7% H<sub>2</sub>O) is dumped into Fernbach flask for spore harvest. Fig. 8. Vythane solvent is added to flask. Fig. 9. Flask containing grain and solvent is shaken 10 min. on reciprocating shaker, after which time solvent suspension of spores is poured into another flask. Fig. 10. Spores in solvent suspension are poured into Büchner funnel fitted with filter paper. Fig. 11. After filtration, spore cake is removed from funnel. Fig. 12. Spore cake on filter paper is placed in 40 C oven for drying overnight. Fig. 13. Dried spore cake is scraped from filter paper into sieve (40-mesh). Fig. 14. Cake is pressed through sieve to break up spore clumps. Fig. 15. Powdered spore product containing  $2 \times 10^9$  spores/gm is transferred to storage container.

**Figures 16 to 29:** Fig. 16. Shake cultures of *P. oryzae* in 1-liter Erlenmeyer flasks containing 400 ml yeast extract-cereulose medium; cultures are grown for about 72 hours, a few hours past the beginning of pigmentation. Fig. 17. Pigmented mycelial suspension is filtered onto filter paper in Büchner funnel. Suction is stopped the instant that "wetness" disappears. Fig. 18. Mycelial mat is removed from funnel. Fig. 19. Mat is removed from filter paper and placed on sterilized stainless steel mesh tray. Fig. 20. Mat on tray ready for incubation. Fig. 21. Mat is placed in high-humidity (97% RH) cabinet with light at 26 C for sporulation. Fig. 22. Mycelial mat after 3 days' incubation, showing "velvety" surface bearing dense sporulation. Fig. 23. Close-up view of sporulating mat. Fig. 24. After drying at 40 C overnight and storage if necessary at room temperature in a desiccator, mat is broken into pieces and blended with Freon-113® until a slurry is formed (ca. 1 min.). Fig. 25. Suspension of spores and macerated mycelium is poured through 2 sieves, first through 40-mesh (0.42 mm) and then through 80-mesh (0.177 mm). Fig. 26. Resulting suspension containing almost pure spores is filtered onto filter paper in Büchner funnel. Fig. 27. Spore mat is removed from filter paper and placed on stainless steel screen to dry. Fig. 28. Spore mat after drying. Fig. 29. Photomicrograph of concentrated suspension of spores from dry mat after having been pressed through sieve as in Fig. 14. Note purity of spore product.





from 800 to 10,000 r, and those for dry spores from 10,000 to  $2 \times 10^6$  r. Among 10 experiments for each range and spore condition, several cultural changes were observed, but only one change in pathogenic pattern. This involved two irradiations of germinating spores from a poorly sporulating culture of race IB-1; first at 800 r, then a moderately sporulating single-spore isolate from this treatment was irradiated at 10,000 r. Two densely sporulating mutants appeared among the 60 single-spore isolates obtained from the second treatment. One of these was of the same race as the parent culture, IB-1, and the other represented ID-8, a race apparently having a much narrower host range. These cultures have maintained their growth habit and race type since they were selected from the X-radiated cultures in 1959 as has also the poorly sporulating parent isolated in 1954 from Costa Rica. The parent (825) and densely sporulating X-ray mutant of the same race (825-D6) are shown in Fig. 35.

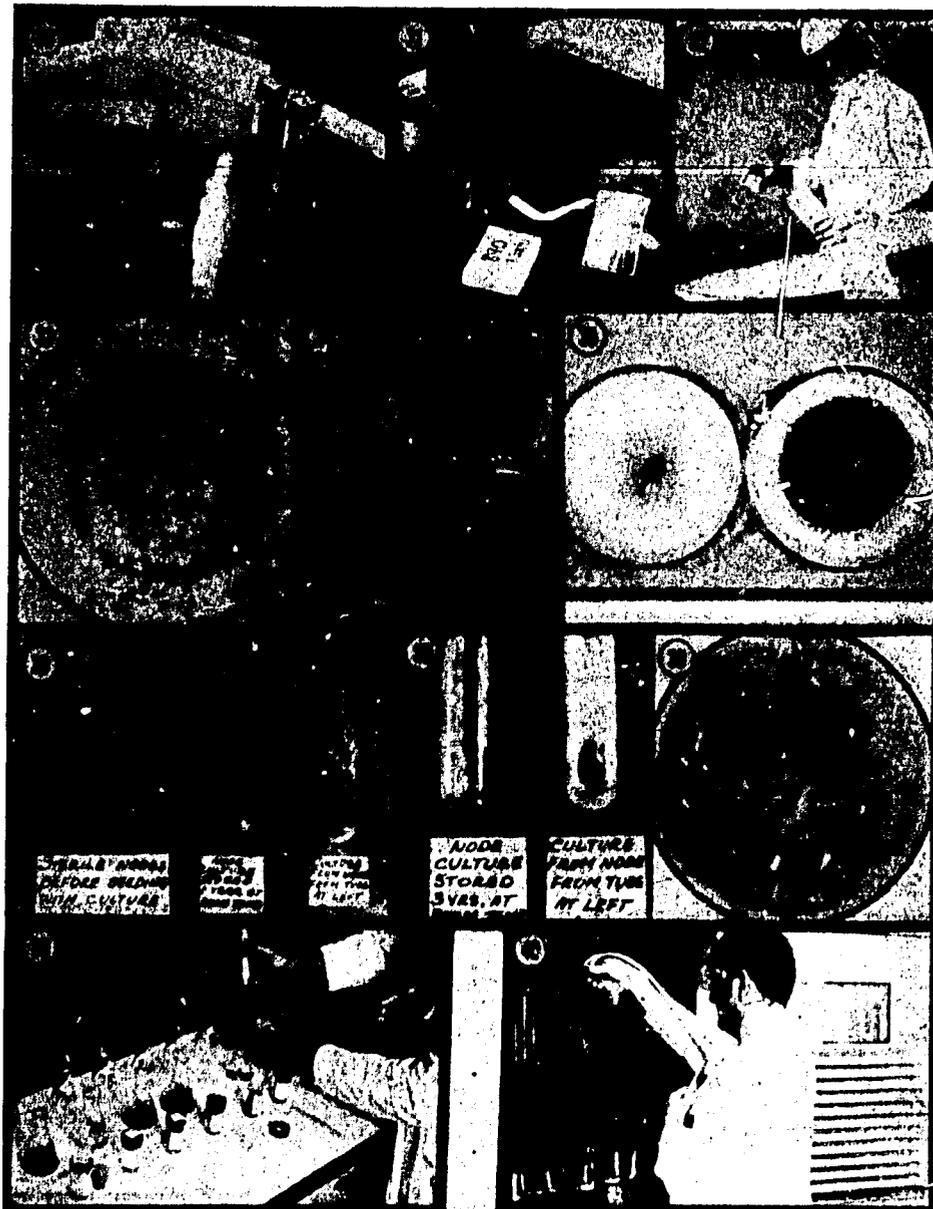
Because some of our cultural techniques were described in a previous paper (Latterell, Marchetti and Grove 1965), we will mention only briefly certain variations of those techniques. We have continued to use rice polish agar (2%) as a basic medium but have found that the range in quality of rice polish is considerable and may account for the poor results reported by other workers with this medium. Excessive amounts of bran, bran oil, and rancidity are some of the undesirable qualities. We have followed the recommendation of Dr. J. G. Atkins in obtaining rice polish that is especially prepared for use in cereal for infants (source upon request). Certain strains sporulate better on yeast-extract dextrose agar (0.5% dextrose, 0.25% yeast extract).

We have found no better medium than sterilized rice nodes for maintenance of stock cultures at -18 C, as previously described (Latterell, Marchetti and Grove 1965). Because of our large number of cultures, however, we have changed from 50-ml Erlenmeyer flasks to small culture tubes (13 x 100 mm). Rice nodes are cut from field- or greenhouse-grown rice culms, ca. 2 cm long, placed in tubes (2 nodes each), soaked in water overnight, then nearly drained and sterilized either with cotton or stainless steel (Bellco®) closures. The nodes are seeded with small plugs from young cultures and incubated at 26 C until nodes are moderately well covered with growth. The tubes are then allowed to dry for two weeks in a low-humidity room (<15% RH) or low-temperature drying oven (35-40 C). They are then stored in the freezer.

When fresh cultures are required from a stored node culture, one node is removed by specially modified forceps and transferred to agar (Fig. 36-37). As soon as a culture is isolated and identified either as a new race or as a vigorous strain of a race already characterized, we transfer it to node culture for long-term maintenance. The use of agar as a medium for plug-seeded cultures continues to be necessary for the purpose of recognizing cultural

Figures 30 to 40. Fig. 30. Dry spores of *P. oryzae* are dispensed into 1-gm cryles for storage. Fig. 31. After having been sealed by melting orifice with a torch, vials are packed in storage boxes. Fig. 32. Spores are being placed in liquid nitrogen storage tank at ca. -170 C. Fig. 33. Agar plate culture of a non-sporulating strain of *P. oryzae* in which a highly sporulating sector has developed spontaneously. Fig. 34. The three highly sporulating cultures shown with the sectoring culture are strains that arose 20 years ago almost simultaneously from such a culture and have retained their characteristic growth habit until the present. Upper right culture is a buff-colored non-pathogenic strain; lower left is a nearly black strain of race ID-14, and the lower right is a dark gray strain of ID-13. Fig. 35. Culture on the left is a poorly sporulating strain of IB-1 that has not changed in appearance or pathogenicity since its isolation in 1954. Culture on the right is a densely sporulating X-ray mutant of culture on the left, selected in 1959, and has remained constant in growth habit and pathogenicity since that time. Fig. 36. Left to right: sterile moist rice culm nodes before seeding with culture; dry culture after maintenance on nodes for 1 year at room temperature; agar culture growing from node taken from middle tube. Fig. 37. Dry culture maintained on rice nodes for 3 years at room temperature, and agar culture growing from node taken from that tube. Fig. 38. Agar plate showing 11 young colonies growing from single spores isolated from one lesion. Black pigmented lines are "incompatibility barriers" developed between some single-spore isolates but not between others. Regardless of their biochemical "incompatibility," all 11 cultures were of the same pathogenic race. Light spots are holes remaining where plugs have been taken for transfer of cultures. Fig. 39. Preparation of spore suspensions for greenhouse inocula from dried spore products. Fig. 40. Spraying spore suspensions onto test plants inside "dew chamber." Walls and door of chamber are lined with cooling coils in which refrigerated water circulates, and plants in pots are placed on a stainless steel mesh circular turntable (that can be rotated during inoculation) over a reservoir of warm water (heated by an immersion heater). Dew is formed on the leaves through the interaction of warm saturated air from below and cool air from above.

Figures 41 to 51: Fig. 41. View of inoculated plants in greenhouse. Note severity of reaction of plants in left foreground in response to highly toxic strain of IG-1. Fig. 42. Close-up view of severe reaction of plants to toxic strain of IG-1; note the few resistant varieties. Fig. 43. "Susceptible" type "4" lesions. Fig. 44. "Intermediate" type "3" lesions. Fig. 45. Two pots of variety "Frances:" plants on left inoculated with race IA-111 show susceptible type 4 lesions, but no toxic effect; plants on right show severe effect of toxin from strain of IG-1. Fig. 46. Technique for preserving leaf specimens for record. Lucite mounting frame is attached to back board with plastic tape hinges so it can be flipped over, first for placing strips of transparent tape (3M Magic), then flipped back to lay leaf specimens on sticky side on tape. Fig. 47. Mounting frame has been flipped over again for removal of tape strips bearing leaf pieces for mounting on standard specimen cards. Fig. 48. Specimens mounted on card ready for storage in file cabinet. Specimens retain nearly normal color for years if kept in dark. Fig. 49. Withdrawing spore suspension from serum bottle by hypodermic syringe. Fig. 50. Injecting several drops of suspension into space between emerging panicle and flag leaf sheath without puncturing tissue. Fig. 51. Panicles in 6 pots of Stirpa 136.7 that were injected with races that had been only moderately pathogenic to that variety in the seedling stage (3- to 3+). All panicles were severely blasted.





changes and selection of sectors, but a simpler substrate has been utilized for culture of fresh spore inoculum for greenhouse testing. We use young corn leaves from 2- to 3-week-old plants (that are routinely available in the greenhouse), cut them in 2-inch pieces, and arrange them flat on the surface of wet filter paper that has been placed in the bottom of 300-ml Erlenmeyer flasks. The flasks are plugged with cotton or plastic foam, autoclaved, and seeded either by "plug" or "flood" culture. Spores are removed from the corn leaf pieces by abrading with water and glass beads.

Plant inoculation techniques remain essentially the same as previously described (Latterell, Marchetti and Grove 1965) except for the containers. We now use 3-inch clay pots rather than plastic food containers for young plants, because better aeration is provided for the roots and healthier plants result. Twelve varieties (nine differentials + three additional varieties of interest) are planted in 12 3-inch pots per galvanized tray, which is fitted with a wire mesh false bottom to minimize overwatering by plants standing in water. These trays are placed in "dew chambers" (Mitchell and Cherry 1954) for inoculation by spore suspension as shown in Fig. 40. For quantitative inoculations plants are placed randomly at stations in a settling tower with a rotating turntable and inoculated with dry spores discharged from a CO<sub>2</sub> pistol (Lange 1954). Inoculum quantities range from 1 to 5 mg per shot, depending upon the quality of inoculum. This technique is especially valuable for studies comparing changes in susceptibility with age or with different nitrogen levels or in comparing different varieties as to numbers of lesions resulting from a single dosage rate.

For growing plants to panicle stage, we transplant from the clay pots either to ¼-gallon glazed pots or to the 4-inch food containers described previously (Latterell, Marchetti and Grove 1965). We have utilized several techniques for panicle inoculation, the most nearly natural of which we believe to be the best. This was conducted in a large greenhouse in which diurnal temperature variations and nightly artificial "dew" could be provided and controlled so that large plantings of many varieties could be inoculated at seedling stage and inoculum could build up naturally, becoming available for infecting panicles as they emerged over a period of time among the different varieties (Kingsolver and Mitchell 1954). We also inoculate panicles either by spraying them in various stages of emergence with spore suspensions or by injecting them in a manner somewhat similar to that described by Ou and Nuque (1963), followed by a 16-hour incubation in a "dew chamber" (Fig. 40).

Panicles inoculated by the spray technique were the most difficult to evaluate. Often there would be blast of some spikelets, pedicels, glumes or other panicle tissue, but no lesions of the main rachis severe enough to cause "rotten neck," and some grains would fill. On the other hand, all the panicle tissues might show blast of unnatural severity. When some spikelets were blasted and some not, did this mean only that no spores alighted on the

healthy spikelets? And did complete kill of all tissues mean that the variety was extremely susceptible? Evaluation of such tests in terms of true susceptibility or partial resistance of a variety to a specific race was often difficult or impossible.

Because such comprehensive and definitive studies as those of Ono and Suzuki (1960) and Hirano and Goto (1963) have been conducted on the significance of blast of the various panicle tissues and influence of environment, we will not attempt to add to this volume of work. We are especially concerned with consideration of the validity of results obtained by the injection technique as we have practiced it. We fill a hypodermic syringe with a spore suspension of known concentration, place the needle gently between the emerging panicle and flag leaf sheath pulling slightly on the blade to make space for the needle without puncturing any tissue, and release 2-3 drops of spore suspension (Fig. 50). By this technique a clear-cut resistant or susceptible reaction is usually obtained, but we are concerned whether this technique is too drastic and overcomes levels of "tolerance" in certain variety-race combinations that would prevail in nature.

For disease evaluation we have continued to use the lesion-type scale and techniques described in a previous paper (Latterell, Marchetti and Grove 1965). A specimen-mounting board facilitates the preservation of leaf pieces bearing lesions under transparent tape (3M Magic Transparent Tape®) (Fig. 46), so that the actual reaction of a certain variety to a specific race at a known age and time of year can be permanently recorded. The technique can also be used for mounting panicles or parts of panicles. Sample specimens from all significant plant inoculations are collected in coin envelopes, dried, and frozen. Among such "significant" inoculations are those of some 500 single-spore isolates from which dried-leaf specimens (and/or nodes) have been preserved as part of studies on racial stability.

The various techniques we have described and illustrated for culturing and harvesting spores for dry products and for preservation and inoculation of cultures in various forms have made possible the study of the pathogenic range of specific isolates over a period of years. Some of the strains that we have studied most intensively arose as highly sporulating cultural variants or as X-ray mutants and were selected for further study because they were thus amenable to spore production by one or both of the techniques described. Some strains have been used in field studies year after year and have shown their same characteristic growth habits and pathogenic patterns upon reisolation at the end of each season. Our techniques have facilitated the study of a number of cultures that have retained complete constancy of both growth habit and pathogenicity for many years.

### Application of Techniques to Problems of Current Interest

There are at the present time two questions of primary concern to rice breeders and pathologists with respect to the blast disease. Those are (1) to determine the nature of the pathogen regarding the integrity of its pathogenic races—or the degree of racial stability and (2) to determine thereby what kind of breeding program will be likely to yield the most lasting and therefore the most valuable types of blast resistance.

There is obviously a dichotomy of opinion regarding the answer to the first of these questions. The results of Giatgong and Ou and their respective co-workers (Bandong and Ou 1966, Giatgong and Frederiksen 1967 and 1969, Ou and Ayad 1968, Quamaruzzaman and Ou 1970) have indicated that the organism is so variable that a number of pathogenic patterns can be expected to arise among as few as 20 single-spore isolates from a parental culture. Ou and Ayad (1968) have suggested that each spore may indeed represent a different pathogenic race.

In contrast, we have maintained a number of isolates for more than 20 years without observable changes either in cultural habit or pathogenicity. (The instances in which we did observe such changes already have been discussed.) Moreover, in an effort to test the hypothesis of these workers regarding pathogenic variability, we have specifically tested some 500 single-spore isolates from both lesions and cultures and found no changes in pathogenic specialization from that of parent cultures.

That *P. oryzae* exhibits extensive variability in patterns of pathogenicity is not in question. Our own work has demonstrated some 50 pathogenic races of this fungus. Undoubtedly the inclusion of additional varieties along with the standard differentials would enable an even finer resolution of pathogenic diversity. However, phenotypic instability is not an inevitable consequence of the genotypic variability that exists within populations of the pathogen. Proper perspective requires consideration of the adaptive significance of pathogenic races as products of the evolutionary history of both pathogen and host. The present diversity of races could have been produced by "average" or even "low" rates of gene mutation and/or recombination, providing that host populations were sufficiently diverse in susceptibility that many of the races had a unique selective advantage. An inevitable corollary to the hypothesis of regular and recurrent segregation of a spectrum of pathogenic races of *P. oryzae* from single isolates of the fungus is that the genetic determinants of pathogenic potentialities are correspondingly labile. For the genetic basis of pathogenicity to be so unstable is largely unprecedented.

Considering that all workers involved in this controversy are experienced with both the host and pathogen, the answer to this anomaly must lie either

in technique or interpretation or both. We believe that an effort to resolve these philosophical discrepancies is imperative if the second question cited in the first paragraph is to be answered. Already the general acceptance of the "constant variability" theory has influenced the opinion of a number of pathologists and breeders who have not worked intensively with the pathogen. There is a growing assumption that, if the fungus is so unstable in its pathogenic specialization, it is hopeless to attempt to breed or select varieties for resistance to specific races. An alternative would be to reduce a breeding program for blast resistance to selection of varieties that "tolerate" blast in epidemic areas. It was stated in the preface to the CIAT seminar program (October 1971), that the reported extreme variability of the fungus (e.g., >25 races from a single lesion) would make vertical resistance quite unstable, and that the goal of workers searching for horizontal resistance would be to detect varieties "which must be resistant to present and future races everywhere." This is indeed a worthwhile and ambitious goal, but our data contradict the premise on which it is based and cast doubt on the probability of its fulfillment.

Through the use of techniques developed in these laboratories, we are inclined to continue to approach a determination of the limits of pathogenic variation in this organism. Our data indicate that the races may exhibit a high degree of phenotypic stability—culturally, biochemically, and pathogenically. An outstanding example of such stability is a moderately sporulating strain of race IG-1 that we isolated from Nicaraguan specimens in 1953. This isolate, although representing a race having a generally narrow host range, has a toxin-producing capability such that the varieties it does attack are usually killed without exposure to dew periods beyond the night of initial infection (Figs. 42 and 45). This isolate has retained its racial identity, toxic action, and growth habit without apparent change for nearly 20 years in frequently transferred cultures.

During recent years we have not actively pursued the collection of isolates from foreign sources; hence, we have not added many races since 1967. Our characterization of 50 races (6 of which have been lost) from more than 2000 isolates represents roughly an average rate of 1 new race in 40 isolates. We estimate that each isolation from a lesion involves ca. 50 to 100 spores, because we make our isolations for race identification by touching a sliver of agar (adhering to the tip of a handmade "microknife") to a densely sporulating lesion (following incubation under light in a moist chamber). Thus 2000 to 4000 spores may be represented among the 40 cultures that yielded on an average 1 new race; or, among our 2000 isolates from which we defined 50 races, between 100,000 and 200,000 spores were sampled. We know of only one isolation among these that has yielded a mixed race culture in 20 years' use of this technique. Following the initial testing, a number of single-spore isolates are made as a means of selecting a maximally sporulating strain and reducing

potential genetic variability before dry-spore inoculum is produced. The point of this apparent digression is that we have isolated only 50 races from 100,000 to 200,000 spores, and this involved the efforts of very few persons. We believe that an active cooperative sampling and testing program among worldwide laboratories would go a long way toward determining the limits of variation in this organism, and could result in a breeding program that would utilize the best of vertical and horizontal resistance. With present methods dry spore inoculum of any culture supplied could be produced so that workers in all areas of the world could test their varieties with identical inoculum. As expertise is developed at other laboratories, "spore banks" could be produced. Combined inocula of all races known to be present in a certain area could be used in blast nurseries to supplement natural inoculum and thereby hasten the process of eliminating varieties that do not qualify as sources of horizontal resistance. The same inocula could also be tested as individual races on varieties in the greenhouse for determination of vertical or specific resistance.

The increasing interest in finding sources of horizontal resistance to blast need not preclude the continued search for vertical resistance in greenhouse or nursery testing with specific races. In such tests, as already discussed, moderate resistance or intermediate-type reactions are readily discernible, as well as the extreme reactions, and can be indicative of horizontal or polygenic resistance. Padmanabhan (1964) described the successful testing program at the Central Rice Research Institute in which varieties in pots are artificially inoculated and screened for both moderate and high resistance to specific isolates. Rigorous tests in the field under conditions favoring blast development follow the screening tests, and are supplemented by observations of reaction of the varieties to natural infection at seedling, post-transplanting, and flowering stages. Reporting on a conference of Indian rice workers in 1962, he noted the emphasis on gaining knowledge of the number and distribution of pathogenic races, with recommendations that breeding programs for resistance be oriented towards building up moderate resistance to a group of known pathogens screened through "disease gardens." The conference further concluded that to all major diseases, a high degree of "tolerance" is to be desired in evolving varieties for release. Robinson (1971) has formulated a number of rules regarding the value of vertical resistance based on concepts set forth by van der Plank (1968), among which is the principle that vertical and horizontal resistance are best used in combination— "that one helps the other." The difficulty in field testing of varieties for both kinds of resistance simultaneously is, according to van der Plank (1968), that field plantings of varieties with high vertical resistance reduce the selection pressure for the buildup of many races and thus interfere with the recognition of horizontal resistance when it occurs.

Because of the antiquity of the rice crop in the Asiatic rice growing areas, a wider variety of race genotypes is likely to be indigenous there than in the Western Hemisphere. This does not give assurance, however, that an Oriental variety selected for broad resistance in Oriental blast nurseries will be resistant elsewhere. Two examples in point are Tetep and Tep Saigon 229. Two races in our collection from Sierra Leone and El Salvador were decidedly more pathogenic to these varieties than were any of our broad-range Oriental races. This is the basis for our proposal that dry spore inocula of all available races be produced. We are suggesting that the order of testing in a breeding program should be to (1) select those varieties that appear to have horizontal resistance and (2) expose them to known inocula (from the "spore bank") of all available races. This "vertical challenge" of varieties showing apparent horizontal resistance should provide the ideal combination of both types of resistance.

In preparation for the CIAT seminar, we undertook a testing program to discover sources of horizontal resistance among 126 varieties selected as being of potential interest in the Americas. Utilizing the techniques described in the previous section, the 126 varieties were inoculated in the seedling stage with 40 races of *P. oryzae*, and varieties that reached panicle stage during the five months of testing were re-inoculated at that time to compare seedling- with panicle-stage reactions. In the beginning of this testing we were concerned primarily with selecting varieties that showed predominantly intermediate reactions, which we believed would indicate polygenic sources of resistance. When we failed to find more than two or three of the 126 varieties that fell into this category, we returned to the broadly resistant varieties that seem properly to fall in the category of having many genes for vertical resistance. Some of these such as Tetep, Tep Saigon 229, and CICA-4 may have a broad basis of horizontal resistance that is usually masked by their epistatic vertical resistance to many races, but which "shows through" as intermediate reactions to a few races, strongly influenced in severity by host nutrition, age, and environment. According to van der Plank (1968), horizontal resistance must be evaluated in the field, so our standards may not precisely coincide with his. We have had the opportunity through a number of seasons, however, to observe the reactions of varieties to specific races in the field in comparison with the same host-race combinations in the greenhouse. These comparisons have provided a basis for evaluating the greenhouse reactions of many varieties in terms of their field performance.

Vertical or specific resistance is defined as that resistance which is not influenced by environment. That is, a variety that has a specific resistance to a race will not become susceptible, no matter how favorable the environment for pathogen development. Horizontal resistance, on the other hand, like any polygenically determined trait, can be strongly influenced by environment; it can be reduced by providing a favorable environment for the pathogen. This

is the phenomenon that we described earlier for Tetep and Tep Saigon 229 in their seedling responses to races IB-33 and ID-5. We have seen it in other race-variety combinations, but have not had the opportunity to carry these observations through to the panicle stage. Are panicle tissues as sensitive to environment and nutrition as are seedling-to-early-tillering-stage tissues? If so, horizontal resistance in the panicle stage may be less valuable and dependable than vertical resistance. Padmanabhan (1964) cites evidence to indicate that resistance to leaf infection and panicle (neck) infection are independently inherited.

As mentioned earlier, we have been especially concerned with the ultimate significance of the intermediate seedling stage reaction. That is, with what type of panicle reaction, and thus yield-reducing potential, is the intermediate seedling reaction correlated? On our scale of leaf lesion types ranging from 0 (immunity) to 5 (killing), reaction 1 to 2+ have been considered resistant, 4- to 5, susceptible. Intermediate reactions, 3- to 3+, have been the imponderables, very dependent in their severity or potential damage upon nutrition, temperature, moisture (humidity, rain, mist, or length of dew period), and host-plant growth stage. The work of Wills et al. (1968) was the first indication at our laboratories that seedling reactions of 2 or 2+, such as that of Zenith to race IA-109, could be directly correlated to susceptible reactions in the panicle stage. Recent field studies of Marchetti (1971) have supported these findings by the reverse technique. Isolates of IG-1 and IG-2 from severely blasted panicles of Belle Patna, Bluebelle, and Nato caused only low-intermediate reactions when inoculated onto those varieties in the seedling stage. Our results from panicle-stage inoculations of the 50-plus varieties that have matured during the test period for the present study indicate that the rapidly developing tissues of the immature panicle are more susceptible than the youngest leaves of 3- to 5-week-old seedlings. Race-variety combinations that yielded seedling reactions as low as 2+ often became susceptible in the panicle stage. The mitigating effect that we know this degree of resistance can confer can probably be attributed to the early onset of leaf resistance characteristic of varieties showing the intermediate seedling reaction. These varieties are typically strongly sensitive to nitrogen nutrition, temperature, and effects of aging (primarily silicification) (Yoshii 1936; Kahn and Libby 1958; Volk, Kahn and Weintraub 1958). The main result of the presence of these factors is the failure of lesions to enlarge and support sporulation for a significant period. Hence, although the tissues of emerging panicles of these varieties may be highly susceptible, their predecessors, the leaves, may have provided poor substrate for sporulation of the pathogen, and a severe epidemic may thus have been averted due solely to the lack of buildup and/or persistence of inoculum.

Our results in comparing panicle- with seedling-stage reactions corroborate those reported by Kozaka (1971): "Generally, panicles, especially spikelets,

glumes, and panicle branches are found to be more susceptible than leaf." Further, he finds that "...isolates which are nonpathogenic to leaf are able to cause considerable infection on panicles including neck nodes."

We conclude, therefore, that varieties showing leaf reactions as low as 2+, and certainly 3-, 3 or 3+, must be considered potentially susceptible in the panicle stage even though their intermediate seedling reactions are indicative of a level of horizontal resistance. We have considered the possibility that our technique of panicle inoculation is too drastic; yet instances of high vertical resistance were not overcome by supplying spores to the "moist chamber" of the emerging panicle: Tetep, Tep Saigon 229, IR-8, CICA-4, and other broadly resistant varieties were still highly resistant in the panicle stage to the races to which they were highly resistant in the seedling stage.

The 126 varieties were "screened" by the initial testing to 40 races. From the results thus obtained, one-fourth of the varieties were eliminated from further consideration because of their high susceptibility to many races. The remaining varieties, listed in Table 2, were planted again and subjected to a second testing with 40 races. Following these tests, all but 29 varieties were eliminated, and these were planted for testing to 28 of the 40 races (selected for both pathogenicity and relevance). A summary of results is shown in Table 3. Twelve varieties among these that appeared to have the broadest bases for resistance are shown in Table 4 as they reacted to their respective pathogenic races.

Most of the varieties in Table 4, especially Tetep, Tep Saigon 229, Corerepe, and Rikuto kogane hata mochi, showed high vertical resistance to most of the 40 races. The races to which they were susceptible could attack them only during a brief period of the seedling stage under ideal conditions for growth and also in the panicle stage. This degree of "tolerance" would probably be even stronger in the field where "hardening" of plants would occur sooner, and sporulation on the leaves would be restricted. If no highly susceptible varieties were in the vicinity, there would be minimal buildup of inoculum for attacking the "tolerant" varieties in the panicle stage, even though they would be susceptible. This "brief susceptibility" then is effectively the same as horizontal resistance. The threshold of susceptibility in these varieties is so narrow that the variety escapes except under artificial conditions when inoculum is supplied in abundance at precisely the vulnerable stage and state of vigor.

Table 2. Varieties \* tested for seedling reaction to 40 races of *P. oryzae* b

Variety	Country of Origin
No. 18. K8C/140/16	Surinam
No. 27. K12C/48	Surinam
B. G. 60/47	Surinam
E.E.A 404 x Zenith x Maravilha I	Brazil
E.E.A 406 x Zenith x Maravilha I	Brazil
IR-8	Philippines
IR-20	Philippines
IR-22	Philippines
Corenape A67	Mexico
Apura	Surinam
Magall	Surinam
Washabo	Surinam
Stirpa 136 x Chiapelli	Italy-Sicily
Stirpa 136	Portugal
Stirpa 136 7	France
Bbt 50/2 x Jojutla	Mexico
Piedras negras A67	Mexico
Perlita jalapa	Guatemala
Agulja branco TM 821	El Salvador
Agulja	Bolivia
Mojito	Bolivia
Mojito colorado	Bolivia
Noventa dias blanco	Bolivia
Palo morado	Bolivia
ICA 1 T 11 2E 6T 4P 2T 1P	Colombia
ICA 2 T 145 C 1P 4T 1P 1P	Colombia
ICA 7 T 18 1E 6P 1T 2P	Colombia
ICA 5 T 11 2E 6T 1P 2T 1P	Colombia
ICA 10 T 11 2D 7P 5T 1P 2T	Colombia
Honduras	Peru
Eas 3	Peru
140/4/1/2/5	Surinam
140/4/1/2/10	Surinam
131/6/1/1	Surinam
VD 5096/73/6	Surinam
VD 5096/73/36	Surinam
KX/5	Surinam
K8C/263/12	Surinam
K8C/634/10	Surinam
K10 B/28/1	Surinam
K8C/140/16	Surinam
Short straw Starbonnet	U.S. (Arkansas)
Nato x Nova	U.S. (Arkansas)
Vegold-C.I. 9556 x Dawn Stg 67 6291	U.S. (Arkansas)
Vista	U.S.
Nova 66	U.S.
Nato	U.S.
Saturn	U.S.
Della	U.S.
Dawn	U.S.
Bluebonnet 50	U.S.
Starbonnet	U.S.
Belle Patna	U.S.
Bluebelle	U.S.
CI 9453.Bbt 50 x CI 9187	U.S.
Belle Patna x Dawn B6311A	U.S. (Texas)

Table 2 - Continued

Variety	Country of Origin
Calrose	U.S.
Tono Brea 208	Haiti
Tono Brea 439	Haiti - Dominican Republic
Tep Saigon 229	Indochina
Zenith	U.S.
NP 125	India
Usen	China-Japan
Dular	India
Kanto 51	China-Japan
Bau 157	Indochina
Tetap	Indochina
Rikuto kogane hata mochi 27	Japan
15 0121	No. China
Chu-To	So. China
Ko-To	No. China
Basmati 370	India
Enshiko-Kyuko 1 Ku-san Mochi	So. China
Waka-Ba	Japan
Chusel-Shin-Sembon	Japan
15-0101	No. China
15-0173	No. China
Fon Yaku Man Goku	Japan
Napal	Colombia
Bbt 50 x Gulf 8P-5P B579 A2-13-1	Colombia
Tep Trang 176-T1	Indochina
292230 Fukel - 53	Japan
291667 Susono Mochi	Japan
SML 467	Surinam
Nig 727 Chin-Tsao	So. China
15 0044 Kannon-Sen	No. China
Dalshukoku 15-0006 (Mochi)	No. China
SML 242	Surinam
SML 352	Surinam
SML 359/4	Surinam
Tari 9638 RTS 24	Indochina
CICA-4	Colombia

a In selecting these varieties for testing, as being of potential interest in the Americas, consideration was given only to their being either of climatically analogous origins or of probable interest as breeding material. That is, agronomic or other characteristics were not considered.

b Results of tests for specific varieties and races are available upon request.

Table 3. Varieties selected for moderate to high resistance after two screenings to 40 races; distribution in reaction categories to 28 of the most broadly pathogenic races.

Variety	Origin	Number of races to which		
		Susceptible	Intermediate	Mixed (Impure var.)
No. 18 K8C/140/16	Surinam	4	5	1
No. 27 K12C/48	Surinam	6	1	3
B. G. 60/47	Surinam	5	7	0
E.E.A. 404 Z x Mar. I	Brazil	7	0	0
IR-8	Philippines	3	1	0
IR-20	Philippines	1	5	0
IR-22	Philippines	6	1	1
Corerepe	Mexico	1	7	0
Apura	Surinam	1	1	0
Magall	Surinam	2	6	0
Washabo	Surinam	1	0	0
Stirpa 136 x Chiapell	Italy-Sicily	2	8	2
Stirpa 136	Portugal	7	8	3
Bbt 50/2 x Jojutla	Mexico	3	1	0
Agulja branco TM 821	El Salvador	1	0	0
Agulja	Bolivia	1	0	0
Noventa Dias Blanco	Bolivia	2	4	1
Palo Morado	Bolivia	2	8	12
ICA 1 T11 2E 6T 4P 2T 1P	Colombia	1	4	0
ICA 5 T11 2E 6T 1P 2T 1P	Colombia	1	4	0
Honduras	Peru	5	0	0
140/4/1/2/10	Surinam	2	3	1
131/6/1/1	Surinam	2	3	0
Saturn	U.S.	8	6	0
Tono Brea 208	Haiti	3	2	0
Tep Saigon 229	Indochina	2	3	0
Tetep	Indochina	0 <sup>a</sup>	4	0
Rikuto Kogane	Japan	1	0	0
Hata Mochi				
CICA-4	Colombia	2	0 <sup>b</sup>	0

a Tetep is susceptible to races IB-33 and ID-5 in the panicle stage, and is moderately susceptible to these races in the seedling stage under ideal conditons for host growth.

b Tests are incomplete for this variety.

Table 4. Reactions of varieties showing maximal resistance (among 126) to 20 broadly pathogenic races.

VARIETY	ORIGIN	RACE																				
		INTL US	IG 2	IA III	IB 47	IC 19	IA 109	ID 13	IG 1 g	IA 65	IB 1	IB 5	IB 33	IB 49	IC 1	IC 17	IC 17 b	IC 25	ID 1	ID 5	ID 9	IE 1
IR-20	PHILIPPINES		±					■	±	±		±	±									
COREREPE	MEXICO	±						±	±		±	±	±					±				■
APURA	SURINAM												+									
WASHABO	SURINAM																					
AGULJA BRANCO TM 821	EL SALVADOR																					
AGULJA	BOLIVIA																					
6T 4P 2T 1P ICA 1 TII 2E	COLOMBIA							±							±				±		±	
ICA 5 TII 2E 6T 1P 2T 1P	COLOMBIA						±			±									±	±		
TEP SAIGON 229	INDOCHINA																					
TE TEP	INDOCHINA																					
RIKUTO HATA- KOGANE MOCHI	JAPAN		±	±	±	±	±	±		±												
CICA-4	COLOMBIA																					

□ = RESISTANT (0 to 2+)

± = INTERMEDIATE (3- to 3+)

■ = SUSCEPTIBLE (4- to 5)

Table 4. Footnotes

- a US 3A differs from US 3 in being pathogenic to IR-8, IR-20, IR-32, and other varieties but both must be classified as IC-1 by the international system.
- b US 16 differs from US 29 in being pathogenic to Alchi umhi, but both fit IC-17 in the international system.
- c Tetepe is susceptible to IB-33 and ID-5 in the panicle stage.

## CONCLUSIONS

We have attempted to elucidate some of the major questions and problems confronting rice pathologists and breeders concerned with developing varieties that are resistant to the blast disease. Two questions that should be resolved, and over which there is considerable controversy, are (1) what is the true nature of the pathogen with respect to integrity of its pathogenic races, or the degree of racial stability; and (2) what kind of breeding program will be likely to yield the most valuable types of blast resistance? In efforts to contribute to the answering of these questions, we have discussed the effects of environment and nutrition on host reaction to blast and the discrepancies in evaluation of reaction that may result thereby. Our experience has indicated that the current controversy as to stability of races may be traced at least in part to (1) variation in reactions of differential varieties under different conditions and (2) personal differences in evaluation standards. The answer to this philosophical dichotomy must lie in technique or interpretation or both.

We have presented the developments in our research that support our conclusions regarding degree of racial stability and a description of techniques that have evolved during our 20 years' work on the pathogen. As we acquired isolates from worldwide sources we needed to find ways to maintain the many cultures in a state such that they would retain their original spectrum of pathogenicity and degree of virulence in a form that would be amenable to testing at any time. We have developed techniques for producing dry spore inocula that, properly stored, remain pathogenically stable over a number of years. We have described these techniques and recommended the use of dry spore inoculum for quantitative dispersal (e.g., in a settling tower) for comparing the effects of different environmental conditions and nutrition on host susceptibility as well as for qualitative determinations of varietal reactions by spraying the spores in water suspensions. Reproducibility of results and continuity among experiments is thus greatly enhanced.

That there is great pathogenic diversity within the species *P. oryzae* is not in question. In our own work we have characterized 50 pathogenic races of the pathogen. However, phenotypic instability is not an inevitable consequence of the capacity for genotypic variability that may exist within populations of this pathogen. We have maintained cultures for as long as 20 years in periodically transferred culture with no apparent changes in pathogenic specialization. On the other hand, we have encountered occasional instances of striking pathogenic change during growth in culture. Our thesis is that, although mutation certainly occurs, as well as asexual recombination, and perhaps even sexual recombination in light of Hebert's recent work (1971a), the rate of pathogenic change has been exaggerated in some reports. We believe that the widespread

acceptance of the concept of "constant variability" is misleading with respect to the value of breeding for specific resistance. We do recognize a valuable type of resistance that differs from specific resistance in not being clear-cut, probably because it is polygenically controlled. This type, which may be "horizontal" resistance, is probably involved in many of the host-race combinations in which maximal susceptibility is characterized by the so-called "intermediate" reaction; that is, leaf lesion types of 3-, 3, and 3+. These reactions are strongly influenced by environment and nutrition of the host, as is the amount of sporulation that occurs and contributes to disease development and spread. The significance of the "intermediate"-type leaf lesion in terms of subsequent panicle reaction and ultimate crop loss is, we believe, an important part of the picture in evaluation of types of resistance. One of the goals of our studies has been to determine this significance over a broad range of varietal types. Our efforts along this line included a testing program in which 126 varieties of potential interest in the Americas were tested for reaction to dry spore inoculum of 40 races of *P. oryzae*. Although we found a number of varieties that must have a broad spectrum of vertical resistance genes and some that appear to have residual horizontal resistance in those instances where their vertical resistance breaks down, we found no varieties that were resistant to all races in the panicle stage. This bimodal susceptibility complicates the analysis of resistance to blast.

We conclude from these studies that all promising sources of resistance should be considered in tests to select potential breeding material. We agree with Chien (1967) in his conclusion regarding future research plans, that "...It is important to select field resistance together with true resistance," and that "...a breeding program should be conducted under both controlled and natural conditions." There appears to be no obvious reason why the use of sources of horizontal resistance should necessarily preclude the use of valuable sources of specific resistance to virulent broad-range races when such are found. The two forms of resistance, for example, can be combined by using a horizontally resistant line as the recurrent parent in a program of backcrossing. We have proposed that, through utilization of our spore production techniques, an international cooperative program could be set up such that varieties selected for "field," "general," "horizontal," or "partial" resistance in any country of the world could be further screened by testing to dry inocula of specific races from all areas. These could be provided by any of several laboratories set up to receive blast specimens, isolate cultures, and produce dry inoculum of the different races. We can foresee, through the implementation of such a program, greatly increased communication and knowledge regarding sources of resistance and their incorporation as breeding material.

A review that would do justice to the enormous volume of critical investigations contributed by Asian workers, especially the Japanese, on

relationships of environmental factors, nutrition, and stage of host development to prevalence and severity of blast would require a longer and more comprehensive work than has yet been offered and would be a Herculean task. We believe, however, that these relationships are deserving of such critical study and are intricately woven in the fabric of blast resistance, be it vertical or horizontal.

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**Geographical distribution  
and predominant races  
of *Pyricularia oryzae* Cav.**

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Several countries other than Japan, the United States and Taiwan have carried out studies on the pathogenic races of the rice blast fungus since the symposium on the rice blast disease held at the International Rice Research Institute (IRRI) in 1963.

It will be useful to the study of horizontal resistance to the disease of rice to take a view of geographical distribution of pathogenic and predominant races in certain countries from the data which have been reported.

**Geographical Distribution of the Pathogenic Races**

According to S. Matsumoto et al. (1969) nearly two hundred specimens of diseased plants in thirteen countries were tested on their pathogenic races at the National Institute of Agricultural Sciences of Japan from 1962 to 1966. At the same time workers at the Japan-United States Cooperative Blast Project conducted inoculation tests of the isolates which had been exchanged between both countries (Figure 1 and Table 1).

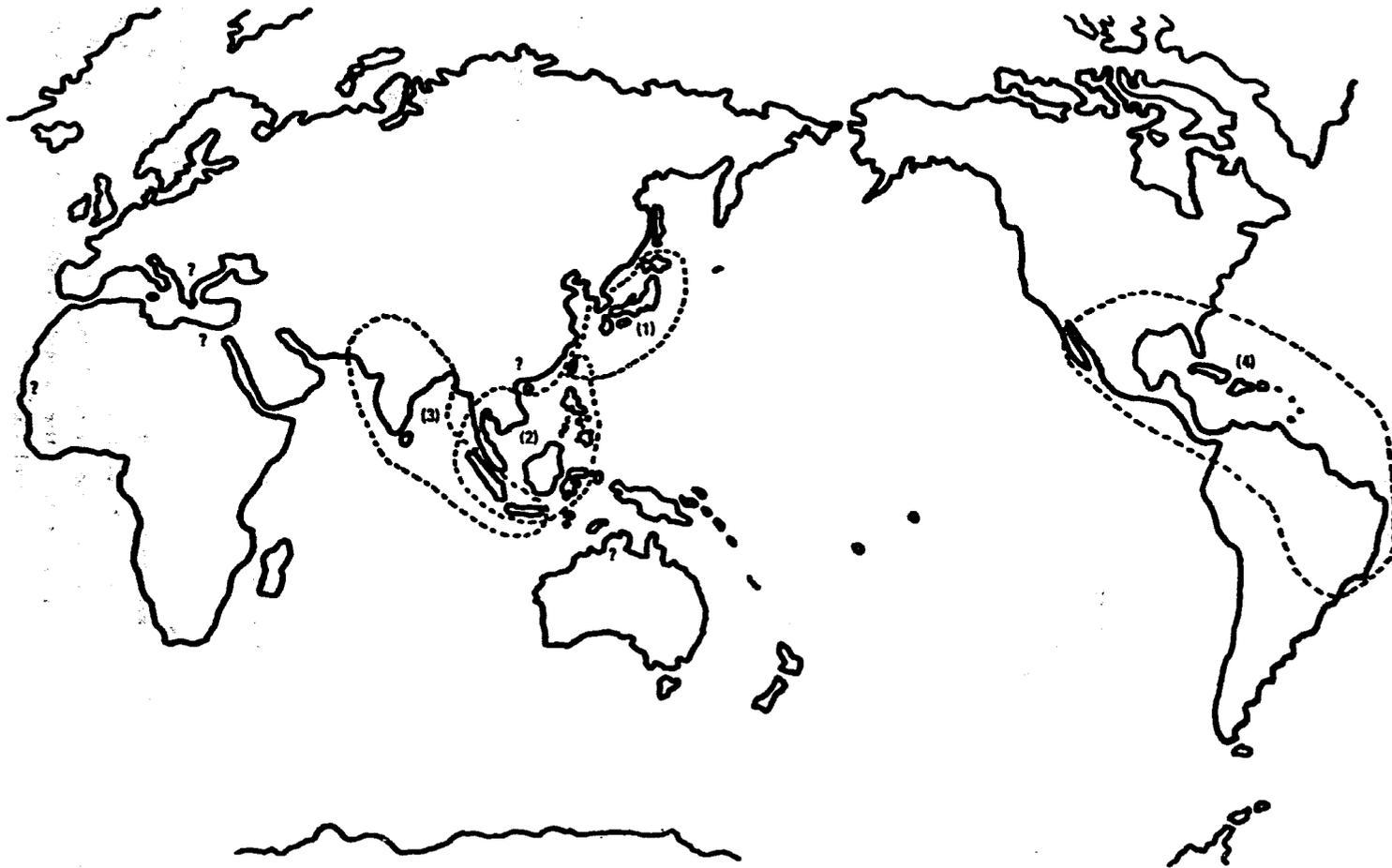


Figure 1. Geographical grouping of pathogenic races of *Pyricularia oryzae*.

Table 1. Geographic distribution of the representative races of rice blast fungus

	Reaction of races																			
	a <sup>1</sup>	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t
Zenith	+					+	+	+					+	+	+	+	+	+	+	+
Rexoro	+	+				+	+	+	+	+	+		+	+	+	+	+	+	+	+
Lacrosse	+	+	+			+	+	+	+	+	+		+	+	+	+	+	+	+	+
Caloro	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
Sha-tiao-tiao P	+	+				+	+	+	+	+	+		+	+	+	+	+	+	+	+
Sha-tiao-tiao S	+	+				+	+	+	+	+	+		+	+	+	+	+	+	+	+
C.I. 5309	+																			
Dular	+																			
NP-125																				
Raminad Str. 3																				
Wag-wag																				
Taichung 65	+		+	+	+	+	+								+	+	+	+	+	+
Tetep																				
Tadukan																				
Usen																				
Chokoto	+																			
Yakoko	+																			
Kanto 51	+																			
Ishikari-shiroke	+	+		+		+	+	+												
Homare-nishiki	+	+	+																	
Ginga	+	+	+	+	+															
Norin 22	+	+	+	+	+															
Aichi-sahi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Norin 20	+	+	+	+	+															
Japan	X <sup>1</sup>	X	X	X	X															
Korea		X	X	X	X															
Taiwan		X		X	X															
Philippines						X	X						X							
Vietnam						X	X	X	X	X	X									
Thailand						X	X	X	X	X	X									
Cambodia						X	X	X	X	X	X									
Indonesia						X		X				X	X	X		X	X	X	X	X
India													X	X	X	X	X	X	X	X
West Pakistan								X												
Ceylon															X					
U.S.A.																			X	X
Brazil																				X

1) existence of race

2)	Reaction	Name of races	Reaction	Name of races	Reaction	Name of races
	a	Japan C-1	h	ID-13 (JU-2)	o	IE- 1 (JU-2)
	b	Japan N-1	i	ID- 13 (JU-3)	p	IC- 17 (JU-2)
	c	Japan N-2	j	1A-109 (JU-2)	q	IC- 17 (JU-1)
	d	IG- 1 (JU-3)	k	ID- 15 (JU-1)	r	IB- 54
	e	IH- 1 (JU-1)	l	ID- 14 (JU-1)	s	IB- 33
	f	ID-13 (JU-1)	m	ID- 1 (JU-1)	t	IB- ?
	g	1A-109 (JU-1)	n	ID- 1 (JU-3)		

International race numbers are based on the proposed number by IRRI.

From the results of these tests, four geographical areas are distinguishable on the basis of race distribution: 1) Japanese, 2) Philippine, 3) Indian and 4) American. The races found in each of these areas have some pathogenic characteristics while showing differences from each other. The common characteristics in the pathogenicity of the races in each area are summarized below:

Of those races found in the Japanese area, the major races encountered in Korea and Taiwan belong to those characterized in Japan, namely, Japanese race N-1, N-2, N-3, N-4 and N-5. They are pathogenic to all varieties of the Japonica type, that is, Homarenishiki, Ginga, Norin 20, Norin 22 and Caloro, but are nonpathogenic to the majority of the Indica type varieties, that is, Tetep, Tadukan, Usen, NP-125, Raminad Str. 3 and Wag wag. Many varieties of Japonica type are cultivated widely in this area.

Most major races found in the Philippines, Vietnam, Cambodia and Thailand and to some extent in Taiwan and Indonesia are identical to or closely resemble some of the US races 5, 6 and 11. As shown in Table 1, they show similar reaction patterns on the Japanese differential varieties with susceptible reaction on only two or three varieties, namely, Usen, Ishikarishiroke and Aichiasahi. They are characterized by nonpathogenicity to all varieties of Japonica type except Aichiasahi and Caloro, thus indicating a striking contrast to the reaction observed with races in the Japanese area; and nonpathogenicity to the so-called Chinese type varieties, that is, Chokoto, Yakeiko, Kanto 51, C.I. 5309 and Dular, also indicating a striking contrast to the reaction of races in the Indian area. Among the varieties of Indica type, these races are all pathogenic to Usen, and some of them are pathogenic to Raminad Str. 3 and Wag wag.

Major races found in India, Ceylon, and West Pakistan, and some races in Indonesia, are identical or show close similarity to the US race 8 or 9. As shown in Table 1, all the major races in this area are nonpathogenic to most of the varieties of Japonica type, and in this respect are similar to the races in the Philippine area. However, they are pathogenic to most varieties of the Chinese type such as Chokoto, Yakeiko, Kanto 51, C.I. 5309 and Dular, thus indicating a striking contrast to the reaction of the races in the Philippine area. They also differ in that they are pathogenic to NP-125, which is resistant to most isolates from other areas.

According to the results reported by United States workers, the most prevalent races in the American area were US race 3 and 6 followed by US race 16.

Galvez (1968) reported that US race 6, which corresponded to ID-13, was the most prevalent one in Colombia, and was followed by races II-1, IB-7, IA-1 and IB-38 in the proposed international race number by IRR1. Of these,

race II-1 showed pathogenic reactions to Aichiasahi and Bluebonnet 50, which were used as supplemental varieties in contrast with the isolates quoted in previous papers which were suspected as cultural variants or other species of *Pyricularia*. On the other hand, races with a distinct pathogenicity to Zenith were collected from most of this area, namely the United States, Mexico, El Salvador, Nicaragua, Costa Rica, Colombia, Venezuela and Brazil, and the wider distribution of these kinds of races could be shown to be characteristic of this area. Zenith and Gulfrose are cultivated in this area.

Among areas not included in the above, isolates from Hong Kong did not show different patterns to Philippine isolates but did show distinct pathogenicity to Dular, which is nonpathogenic to almost all the Philippine races. It is suspected that the isolates from Hong Kong indicate characteristics of isolates of mainland China, especially southern China, because their pathogenicity differs from those of neighboring countries such as Taiwan, the Philippines and Vietnam.

Isolates from Guinea in West Africa were similar to Indian races and showed a wider spectrum of reaction on the differential varieties. However, it would be better to omit them from the grouping, because tested isolates were few and no further information is available on the races of this area.

Isolates from Hungary, Egypt and Australia showed only narrower spectrums of reaction on the differential varieties, and tested isolates were too few to group them into areas.

#### **Predominant Races in Several Countries**

Research on predominant or common races of the fungus suggests the global distribution of the races. Predominant races in a certain country can be presumed from the existing data, though the data do not always aim at systematic sampling to know the actual distribution of the races.

The frequency of races differentiated in Japan in 1961 (Table 2) did not result from systematic sampling during the year-long study throughout the country. Japanese race N-2 was the most prevalent race, followed by race N-1. Because the full-scale breakdown of resistance of the so-called Chinese type varieties started two or three years later, C-1 and C-2 were collected in only limited areas in 1961.

Results of last year's isolates (Table 2) were insufficient because data of four prefectural experimental stations were simply accumulated without any

Table 2. Japanese races of rice blast fungus.

Variety	Group T			Group C									Group N					
	1	2	3	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6
Te-tep	±																	
Tadukan	±	±																
Usen	+	+	+															
Chokoto	+			+	±		+		+		+							
Yakeiko	+			+	±	+		+		+	+	+						
Kanto 51	+			+	+	+	+	+	+	+	+	+						
Ishikarishiroke	+		+	+	+		+	+	+			+						
Homarenishiki	+	+	+	+	+		+	+		+	+	+	+				+	+
Ginga	+	+	+	+	+	+	+	+		+	+	+	+	+				
Norin 22	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	
Aichiasahi	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Norin 20	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
No. of isolates* in 1961	1	1	3	52	30	0	1	1	9	10	1	2	51	113	6	14	3	6
No. of isolates** in 1970	0	33	0	129	0	17	0	0	0	0	220	0	137	220	20	2	3	0
International Race Group	IC 1	ID 15	ID 13	IE 1 or IF 1	IE 1	IF 3	IE 1	IE 1	IE 1	IF 3	IF 3	IF 1	IG 1	IH 1	IH 1	IH 1	IG 1	?

International race numbers are based on the proposed number by IRRI.  
(\* Goto, K. et al. 1964, \*\* Aichi, Hokkaido, Nagano, and Ooita Agr. Exp. Sta. 1971)

statistical consideration. Considerable numbers of N-2 and N-1 were collected throughout other countries, though there was some increase or decrease in limited areas. C-8 increased strikingly in number and over a large area from one isolate in 1961 to 220 in 1970, while none of C-2 was collected; C-1 still had considerable numbers but did not show as wide distributions as C-8. The increase of C group races can be explained by the increase in cultivation of the varieties which were originated from the so-called Chinese-type varieties, though there is inadequate explanation for the striking increase of C-8, which overwhelms other races. It is interesting that predominant races in T, C, and N groups, which are T-2, C-8, and N-2, respectively, show the same reaction on the N-group differentials in Japanese differential varieties compared with those of other races.

Yamada, who studied systematic sampling of the pathogenic races of the fungus, pointed out the pathogenic strength of C-8 as the reason for dominating other races. Though inexact, "pathogenic strength" means aggressiveness or horizontal pathogenicity, which is quantitative as well as horizontal resistance.

As the composition of pathogenic races in Japan is simpler than those in other countries such as the Philippines, a relationship between the predominant races and the races which were derived from them appears clearer in Japan than the other countries. From the viewpoint of the pathogenic gene, serial changing of the major races in Japan might be explained as follows:

N-2, which corresponds to international race IH-1, turns into N-1 by obtaining pathogenic gene from Ishikarishiroke, which is named Av-i<sup>+</sup> by Kiyosawa, and those two races, N-2 and N-1, turn into C-8 and C-1 respectively by obtaining pathogenic gene, Av-k<sup>+</sup>, from Kanto 51.

Table 3 shows the unpublished results of my work in Ceylon from 1967 to 1969. In addition to the set of international differential varieties, seven important varieties in Ceylon at the time were tested as supplemental varieties, which divided the international races into subraces.

From the result, the races corresponding to international races IE-1, ID-13 and IC-17, occupied major parts of Ceylonese races. Among them there seems to be serial passages of changing from one subrace to another by the addition of pathogenicity to a certain variety. For example, the IE-1 group appears to be varied from subrace No. 26 to No. 22 one after another by the addition of pathogenicity to Aichiasahi, Podiwee a-8, H-4, and IR-8-68, successively. IE-1 and IC-17 seem predominant races because of their frequencies. However, ID-13 is still doubtful, because it did not include the subrace pathogenic to H-4, which at that time was the most widely distributed variety.

Table 3. Pathogenic races of rice blast fungus in Ceylon (1967-1969)

Subrace	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	0	
Variety																													
1. Raminad str. 3	+	+																											
2. Zenith			±													±													
3. NP 125	+	+	+	+	+	+	+	+	+	+	+	+	+																
4. Usen			+	+	+										+	+	+	+	+	+	+	+							
5. Dular	+	+	+	+	+	+	+	+	+	+	+	+	+	+									+	+	+	+	+		
6. Kanto 51	+	+	+	+	+	+	+	+	+	+	+	+	+	+									+	+	+	+	+		
7. Sha-tiao-tsao S	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	
8. Caloro	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	
-----																													
9. M-302			+	+		+																							
10. IR-8-68			±					+	+						+								+						
11. H-4	+		+	+		+	+		+						+								+	+					
12. Ptb 16			+	+	+											+	+		+										
13. Podiwee a-8	+		+	+	+	+	+	+	+	+					+	+	+	+		+			+	+	+				
14. Aichiasahi	+	+	+	+	+	+	+	+	+	+	+				+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15. P. Perumal	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
International race:	A	A	C	C	C	C	C	C	C	C	C	C	C	D	D	D	D	D	D	D	D	D	E	E	E	E	E	E	G
Number of isolates:	3	1	1	3	2	2	10	2	26	14	11	35	7	1	5	2	23	6	7	2	2	10	16	18	4	13	4	21	

International race numbers are based on the proposed number by IRRI.

Table 4 shows the results of the race differentiation in India by S.Y. Padmanabhan et al. (1970). IC-17 was the most predominant race and IE-1 also showed a larger frequency in Ceylon. On the other hand, ID-1 and IA-number races which were infrequent in Ceylon were commonly distributed in India. Of these, most of the IA-number races from India showed pathogenicity to Dular and Kanto 51, while Philippine IA-number races are mostly nonpathogenic to both varieties. Generally speaking, India and Ceylon can be included in the same race composition group (pages 1-5), and the predominant races of both countries also can be the same or close to each other.

Table 4. Reaction of international rice differentials to pathogenic races of *Pyricularia oryzae* in India

International race group	International race	Raminad Str. 3	Reaction per differential variety							No. isolates	
			Zenith	NP 125	Usen	Dular	Kanto 51	C.I. 8970 (S)	Caloro		
IA	1* (97)**	+			+	+	+	+	+	3	
	4 (113)	+				+	+	+	+	1	
	5 (101)	+			+	+	+	+	+	1	
	6 (65)	+		+	+	+	+	+	+	1	
	7 (114)	+				+	+	+		1	
	8 (116)	+				+	+	+		1	
	9 (122)	+				+	+	+		1	
	10 (121)	+				+	+	+	+	1	
	11 (61)	+		+		+	+	+	+	1	
	IC	1 (1)			+	+	+	+	+	+	12
		3 (17)			+		+	+	+	+	1
4 (19)				+		+	+	+	+	1	
6 (18)				+		+	+	+	+	1	
7 (20)				+		+	+	+	+	1	
8 (24)				+		+	+	+	+	1	
ID	1 (1)				+	+	+	+	+	19	
	3 (5)				+	+	+	+	+	1	
	10 (15)				+	+	+	+	+	1	
IE	1 (1)					+	+	+	+	1	
	2 (3)					+	+	+	+	1	
	3 (6)					+	+	+	+	1	
	4 (4)					+	+	+	+	1	
	5 (2)					+	+	+	+	1	
	6 (5)					+	+	+	+	1	
	7 (5)					+	+	+	+	1	
	8 (8)					+	+	+	+	1	
IF	1 (1)						+	+	+	1	
	3 (2)						+	+	+	1	
IJ(II)**	1 (1)									1	
	4 (4)									1	

blank: Resistant  
 + : susceptible  
 \* : international race number in original paper  
 \*\* : international race number proposed by IRRJ  
 (S.Y. Padmanabhan et al. 1970)

**Table 5. Races of *Fyricularia oryzae* in the Philippines in international numbers (1967)**

Variety	International Race No.												
	IA-45	IA-46	IA-65	IA-109	IA-110	IA-111	IA-112	IA-126	IB-45	IC-1a	ID-13	ID-14	ID-16
Raminad str. 3	+	+	+	+	+	+	+	+					
Zenith	+	+							+				
NP 125			+							+			
Usen	+	+	+	+	+	+	+		+	+	+	+	+
Dular			+							+			
Kanto 51			+							+			
Sha-tiao-tsao S	+	+	+	+	+			+	+	+	+	+	
Caloro	+		+	+		+			+	+	+		
<b>No. of isolates</b>	<b>28</b>	<b>28</b>	<b>25</b>	<b>230</b>	<b>81</b>	<b>8</b>	<b>29</b>	<b>5</b>	<b>6</b>	<b>14</b>	<b>21</b>	<b>7</b>	<b>6</b>

(from IRRI Annual Report, 1967)

Table 5 was taken from the Annual Report of the International Rice Research Institute (1967), from which races possessing less than four isolates were omitted. IA-group races, especially IA-109, which adds the pathogenicity of Raminad Str. 3 to ID-3, occupied greater parts of the isolates tested. In the same report, (IRRI 1967, pp. 82-89) the results of differentiation of Philippine races by Philippine differentials, together with the number of isolates, were also reported. It was impossible from the data of the annual report to directly relate international races to Philippine races, because corresponding tables were not available. Among the Philippine races, P 8, P 15, P 12, and P 30, respectively, showed larger frequencies and they were presumed to be derived from IA-109 or adjacent races from their reaction to Philippine differentials. From those results IA-109, instead of ID-13, could be a predominant race. Unfortunately, the annual reports of following years at IRRI did not give cumulative numbers of isolates of reported races but only the number of isolates of new races in 1968 and the reaction patterns of newly discovered Philippine races. Therefore, the changing trend in frequencies of races could not be obtained although increases of races pathogenic to so-called Chinese varieties could be known.

## DISCUSSION

With the advance of the differentiation study of the races, many new races which were started with a limited number of specimens have been discovered in many countries. But in general view of geographical distribution of the races and their predominant race might not be influenced that much. If this is so, the following hypothesis arises: The actual process of obtaining pathogenicity from a new variety or a new resistant gene is still unknown. But is presumed that a new pathogenic race to a new variety or a new resistant gene occurs from the predominant race at a certain place in that time. For instance, in Japan the occurrences of C-3, C-8, T-2 and also races pathogenic to Fukunishiki, which has a resistant gene from Zenith, could be good examples of this case; and in Ceylon the races pathogenic to H-4 and IR-8-68 also could be presumed to be derived from predominant races at the time. Considering this hypothesis, it would be interesting to know the predominant races of a certain place by using differential varieties which include several indigenous varieties, and to check the race of the varieties on which it is desirable to have a special resistant gene grown in blast nurseries in all important rice-growing areas throughout the world.

The race of blast fungus corresponding to "race O" of *Phytophthora infestans* has not yet been discovered, i.e., there may be no rice variety to be susceptible to all the isolates of *Pyricularia oryzae* in terms of vertical resistance. Caloro, Usen, P. Perumal and some other varieties showed susceptibility to all the isolates obtained from a certain country, but they still might not be susceptible to all of those from other countries. In this regard, a special affinity of the predominant or common races in a certain place to the variety which is indigenous or widely cultivated in the same place could be one of the reasons. In this, evaluation of the resistance of the variety to the blast fungus should be stressed, especially where the variety which aims to be widely applied at every place has the possibility of being cultivated.

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