

UNCLASSIFIED

AD NUMBER

AD362021

CLASSIFICATION CHANGES

TO: **unclassified**

FROM: **confidential**

LIMITATION CHANGES

TO:

**Approved for public release, distribution
unlimited**

FROM:

**Distribution authorized to U.S. Gov't.
agencies and their contractors;
Administrative/Operational Use; JUN 1965.
Other requests shall be referred to
Commanding Officer, Department of the
Army, Fort Detrick, MD 21701.**

AUTHORITY

**30 Jun 1977, DoDD 5200.10; OUSD ltr, 29
Nov 1979**

THIS PAGE IS UNCLASSIFIED

SECURITY MARKING

The classified or limited status of this report applies
to each page, unless otherwise marked.
Separate page printouts MUST be marked accordingly.

"This document contains information affecting the National Defense of the United States within the meaning of the Espionage Laws, Title 18, U. S. C., Section 793 and 794. Its transmission or the revelation of its contents in any manner to an unauthorized person is prohibited by law."

DOWNGRADED AT 3 YEAR INTERVALS:
DECLASSIFIED AFTER 12 YEARS
DOD DIR 5200.10

"ONE HALF ORIGINAL SIZE"

attachment A

**Best
Available
Copy**

CONFIDENTIAL

AD

TECHNICAL REPORT 60

RICE BLAST EPIPHYTOLOGY (U)

Thomas H. Barksdale
Marian W. Jones

JUNE 1965

This publication contains information affecting the National Defense of the United States within the meaning of the Espionage Laws, Title 18, U.S.C., Sections 793 and 794. The transmission or the revelation of its contents in any manner to an unauthorized person is prohibited by law.

**UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK**

GROUP 3
Downgraded at 12 year intervals
Not automatically declassified

CONFIDENTIAL

Re oduction of this publication in whole or part
is prohibited except with permission of Commanding
Officer, U.S. Army Biological Laboratories, ATTN:
Technical Releases Branch, Technical Information
Division, Fort Detrick, Frederick, Maryland, 21701.
However, DDC is authorized to reproduce the publi-
cation for United States Government purposes.

Qualified requestors may obtain copies of this
publicatio from DDC.

Foreign announcement and dissemination of this
publication by DDC is not authorized.

Release or announcement to the public is not
authorized.

Destroy this publication when it is no longer
needed. Do not return it to the originator.

CONFIDENTIAL

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL REPORT 60

RICE BLAST EPIPHYTOLOGY (U)

Thomas H. Barksdale

Marian W. Jones

Crops Division
DIRECTORATE OF BIOLOGICAL RESEARCH
and
Biomathematics Division
DIRECTORATE OF TECHNICAL SERVICES

Project 1C522301A06102

June 1965

CONFIDENTIAL

(U) FOREWORD

(U) Over a period of years, various workers in Crops Division have performed experiments that relate to the epiphytology of the rice blast disease. No document has been prepared recently with data compiled from the many experiments, although an evaluation of the broader scope of the work done prior to 1958 is available. This present report is intended to show where knowledge is lacking about behavior of the disease in the field as well as to present what is known. It is not in any sense a literature review. Field experiments are emphasized, but laboratory studies are also included where they have thrown light on what is likely to happen in the field.

(U) The authors express their appreciation to the following persons for reading and offering helpful suggestions during preparation of this report: Dr. C.H. Kingsolver, Dr. C.G. Schmitt, and Mrs. Vesta Z. Mattie, Crops Division, Fort Detrick; Dr. G.C. Kent, consultant to Crops Division and Head of the Department of Plant Pathology, Cornell University, Ithaca, New York; and Dr. K.S. Quisenberry, consultant to Crops Division and formerly with the U.S. Department of Agriculture.

(U) The data of many scientists working now or in past years in Crops Division have been referred to in preparing this report. Their published and unpublished data are acknowledged throughout the text.

(U) The junior author, Mrs. Marian W. Jones, Biomathematics Division, performed the statistical analysis discussed in the text and is included as the Appendix. She also provided guidance in experimental field design and analyzed data for other Crops Division personnel whose work is reviewed here.

(U) ABSTRACT

(U) Data on rice blast disease from the field and laboratory experiments of many workers are reviewed and related to epiphytotics of the disease. Principal topics discussed are (i) sources of inoculum, (ii) spore dispersal, (iii) meteorological and other conditions required for establishment of infection and disease buildup, (iv) spread, (v) yield reduction, (vi) control measures, and (vii) the present ability to predict disease outbreak, buildup, and yield losses. A theoretical curve describing minimum requirements of dew period and temperature for infection was developed from laboratory data, and appears to fit two sets of field data. This curve was used to find the percentage of days favorable for disease development during 24 epiphytotics, and regression equations were derived relating this parameter, along with other parameters derived from disease increase data, to yield loss caused by epiphytotics.

CONFIDENTIAL

5

(C) DIGEST

(C) In nature, the sources of primary inoculum for rice blast, caused by Piricularia oryzae, are rice straw refuse in the temperate zones and refuse or infected plants in the tropics. The source of inoculum for secondary disease cycles in both zones is infected rice plants. Weed hosts of Piricularia species, rice seed, and soil are not considered sources of inoculum at present. Various types of hand dusters and sprayers can be sources of inoculum for inducing epiphytotics in small experimental fields.

(U) Sporulation on diseased leaves occurs when relative humidity approaches 100% within temperature ranges of about 60 to 90 F. Laboratory measurements indicate that individual lesions are capable of producing several thousand spores; sporulation increases with the length of time 100% relative humidity prevails and with temperature, but decreases as lesions age. Sporulation on leaf surfaces has not been directly measured in the field.

(U) The latent period varies inversely with temperature, but is 6 days at the temperatures usually found in the field.

(U) Spore dispersal is primarily the result of air currents and wind. Release from conidiophores occurs chiefly at night, and peak concentrations usually are observed between midnight and 6 AM. The traditional slide spore collectors trap spores inefficiently and are unsuitable for predicting outbreaks of disease; the rotorod sampler, however, shows promise of being useful for prediction. Spore concentrations of several thousand spores in a cubic meter of air during one hour have been found over infected rice fields at the height of epiphytotics. Spore concentration decreases vertically during the quiescent conditions of the early morning hours before sunrise at a rate of about 31% per foot.

(U) Rain occurring after experimental inoculations results in a lower lesion count than if no rain had fallen.

(C) Field and laboratory experiments to date have been unsuccessful in defining the minimum amount of inoculum that can initiate blast epiphytotics. Releases of from 0.5 to about 15 grams of viable dry spore preparations, resulting in average dosages ranging anywhere from 2 to 237 particle minutes per liter, have been used in 1-acre rice fields to initiate experimental epiphytotics resulting in serious yield losses. Many, if not most, of the spores released, however, were carried by the wind far beyond the boundaries of the field. A rate of 0.1 gram of viable spores per acre has resulted in some lesion production when applied to small 10-square-foot plots under settling conditions. Recently, plants with a total of 17 lesions were placed in the center of a one-acre field; disease spread throughout the field and a serious epiphytotic occurred.

CONFIDENTIAL

(U) An equation,

$$\frac{1}{D} = 0.265 - \frac{12.26}{T}$$

where D = hours of dew and T = temperature (degrees Fahrenheit), was derived from laboratory data to describe the minimum conditions of dew and temperature required for infection. Field data from two experiments indicate that this equation could be used to predict when conditions would be favorable for lesion formation on about 80% of the nights when experimental inoculations are to be made. With temperatures below 60 F or dew periods less than eight hours, lesions are not likely to occur in the field. An analysis of 24 epiphytotics showed that the rate of disease buildup was greater with an increased percentage of nights that had a combination of dew and temperature more favorable than described by the above minimum equation.

(U) Requirements for panicle infections are thought to be similar to those for leaf infections, but no detailed quantitative laboratory data are available. Although yields are reduced by severe outbreaks of blast on the leaves, the percentage of panicle infections is correlated in a highly significant way with yield reduction.

(U) Nitrogen fertilization has a marked effect on disease development, since nitrogen increases plant susceptibility. Rates above 80 to 120 pounds of elemental nitrogen per acre are likely to contribute to serious blast damage, especially if applied in a split application. However, rates above 100 pounds for Japonica varieties and above 40 pounds for Indica varieties are not widely used, and probably will not be used until varieties are developed that withstand detrimental agronomic characteristics, such as lodging, that accompany the use of these higher rates.

(U) Yield reductions ranging from none to 100% have been recorded for epiphytotics initiated experimentally. Low yield reductions can result from an unsuitable race-variety combination, weather unfavorable for disease development or plant growth, and low levels of nitrogen fertilization. High yield reductions occur when pathogenic races are used, weather is favorable and high levels of nitrogen fertilizer are applied. Equations for estimating per cent yield loss were developed from an analysis of 24 epiphytotics. An equation combining three variables was computed:

$$y = -50.94 + 0.08426x_2 + 1.2343x_3$$

where y = per cent yield loss, x_2 = the highest lesion count during exponential disease increase on the leaves, and x_3 = the percentage of days with weather favorable for leaf infections.

(U) The most widely used control measure is the growing of varieties resistant to endemic races of the pathogen. There is no known rice variety resistant to all races, just as there is no known race able to attack all varieties. Fungicides can be used for control, but this is done economically and on a large scale only in Japan.

(U) CONTENTS

Foreword
Abstract
Digest	5
I. INTRODUCTION	9
A. Causal Organism	9
B. Rice, The Host Plant	10
C. Rice Blast Epiphytotic	16
II. SOURCES OF INOCULUM	17
A. Sources for Primary Infection	17
1. Rice Straw Refuse	17
2. Weed Hosts	18
3. Seed	19
4. Soil	19
5. Rice at a Distant Location	19
6. Artificial Sources	21
B. Sources for Secondary Infection	22
1. Number of Spores Produced on Infected Tissue	22
2. Environmental Conditions Affecting Sporulation on Lesions	23
3. Latent Period	24
III. DISPERSAL	26
A. Dispersal by Air	26
1. Release	26
2. Flight	27
3. Landing	34
B. Dispersal by Water	37
C. Mammals, Birds, Insects, and Man	37
IV. INCUBATION	39
A. Infectivity of Inoculum	39
B. Intensity of Inoculum in Infection Courts	40
C. Conditions Required for Incubation	42
V. INFECTION	57
A. General Description of Infection Process	57
B. Secondary Cycles	57
C. Number of Lesions Needed for Damage	63
VI. FACTORS INFLUENCING EPIPHYTOTIC DEVELOPMENT	68
A. Race of Pathogen	68
B. Meteorological Conditions	69

C. Agronomic Practices	72
VII. SPREAD	86
A. Within a Field	86
B. Over Long Distances	87
VIII. YIELD REDUCTIONS	90
IX. PREDICTIONS AND CONTROL	93
Literature Cited	101
Distribution List	125

(U) APPENDIX

Bases for Prediction of Rice Blast Epiphytotics	107
---	-----

(U) FIGURES

1. Minimum Length of Dew Period Required for Infection at Various Temperatures	45
2. Minimum Length of Dew Period Required for Infection, as a Function of Temperature	49
3. Number of Lesions per 100 Square Centimeters Obtained from Daily Inoculations from Unpublished Field Data of Kulik, Placed on Points Indicating the Average Night Temperature (2000 to 0600 hours) and Dew Period During Incubation and Against a Background Indicating the Minimal Conditions Found in Laboratory Experiments	50
4. Pictorial Chart for Estimating Severity of Rice Blast on Leaves of Plants in the Field	67
5. Reduction in Yield with Distance Downwind	89

(U) TABLES

1. Growth of Rice Varieties Belle Patna, Nato, Bluebonnet, and Rexoro	14
2. Cross-Inoculation Studies with Isolates of <u>Piricularia</u> from Several Hosts	20

3. Comparison of the Rotorod Spore Sampler and the Stationary Slide Spore Collector	28
4. Concentrations of <i>P. oryzae</i> Spores in the Air During 30-Day Periods of Highest Concentrations Over Four Fields with Rice Blast Epiphytotics	31
5. Dosages of <i>P. oryzae</i> Spores Measured at about One Meter Above the Ground During Artificial Inoculations of Rice Fields	35
6. Lesions Per Plant Resulting from Inoculations, Beaumont, Texas, 1959	41
7. Data Relating to Epiphytotics of Rice Blast	52
8. Reduction of Infection by Drying for One Hour after Short Dew Periods	55
9. Change of Resistance of Necks of Panicles with Age	59
10. Panicle Infections on Variety Vegold as Influenced by Stage of Exsertion at Time of Inoculation	61
11. Per Cent of Panicle Blast on Two Rice Varieties at Progressive Dates after Heading	61
12. Per Cent of Neck Blast Occurring Seven Days after Heading and at Harvest	62
13. Amount of Yield Loss Caused by Leaf Disease	65
14. Races of <i>Piricularia oryzae</i> Recovered from Field Plots Following Artificial Inoculation	70
15. Date of Seeding and Nitrogen Level in Relation to Development of Rice Blast in Thailand	80

I. (U) INTRODUCTION

(U) In 1958, Minarik and Latterell¹ summarized the experiments on epiphytology of rice blast prior to that date. This report covers subsequent work on that subject.

(U) Background information is provided that will be helpful to those studying data on rice blast epiphytology. Brief descriptions of the causal fungus, the host plant, and the course of a typical epiphytotic are described before the data are reviewed.

A. (U) CAUSAL ORGANISM

(U) The fungus, Piricularia oryzae, causing rice blast disease was described as a new species by Cavara in 1891.² It is synonymous with Dactylaria oryzae (Cav.) Sawada, and from time to time has been referred to by that name, by Piricularia grisea (Cooke) Saccardo, or by Dactylaria grisea (Cooke) Shirai, especially in the older literature. The best entry into the literature on the taxonomy of the fungus is given by Padwick.³

(U) Because of the morphological similarity of the fungus spores to those found on other grasses, some workers prefer the name Piricularia grisea (Cke) Sacc., which has precedence for the fungus on these grasses. However, other workers have had difficulty in obtaining infections on rice or on other hosts with any fungus isolate except the particular one(s) that came originally from the given host. This indicates the existence of physiological specialization within P. grisea, and because of a predominant host specialization for any one isolate, some workers prefer to call the fungus that attacks rice P. grisea f. sp. oryzae.⁴ The name P. oryzae, however, is widely used today and has been for many years. The fungus has no known sexual stage and is accordingly a member of the Fungi Imperfecti; it propagates by the formation of asexual spores or conidia. The conidia are hyaline (or nearly so), pear-shaped, 2-septate, and measure 20 to 22 by 10 to 12 microns (μ), although the variation in size may be from 14 to 40 by 6 to 13 μ .³ Another source lists the averages in size as 19.2 to 27.3 by 8.1 to 10.3 μ .⁵

(U) P. oryzae is a facultative saprophyte. It will grow and produce spores on media of various types and can sometimes be isolated from refuse piles of rice straw; in nature, however, it is found chiefly on living rice plants. Information on the production of spores for experimental purposes, including the artificial initiation of epiphytotics, is beyond the scope of this review, but can be found elsewhere.⁶

* Pages 9 through 22.

(U) Isolates of the fungus from a particular rice variety may not infect some other varieties to the same degree. Dr. F.M. Latterell and her co-workers^{4,7} have worked on the separation of these isolates and their subsequent characterization as races of P. oryzae. Races are distinguished on the basis of their ability to infect a standard set of differential rice varieties. Cooperation to establish an international set of differential varieties was stimulated by a paper given by Dr. Latterell at a symposium on the rice blast disease at the International Rice Research Institute in the Philippines.^{6,7} That a number of other investigators, especially those in Asia, are also aware of the existence of races of P. oryzae can be seen in some of the other symposium papers. Although it is not the intent here to review the race problem, certain aspects of it are of interest with respect to epiphytology.

(U) For example, a rice variety grown in one location may be free of blast and considered resistant for several years until suddenly an outbreak of the disease occurs. Obviously, a race of the fungus not present before (or at least not established) has appeared. Where did it come from? Is this an example of long-distance spread of the particular race involved, or did the race arise locally from some as-yet-unexplained ability of the fungus to vary, or was the race selected from the population of spores by the host varieties grown? There are examples of isolates of the fungus varying with respect to pathogenicity in the laboratory, but as yet the genetic mechanism of this variation is unknown. When the source of variation is known, it might affect our thinking about what could happen in either natural or experimentally induced epiphytotics.

(U) Isolations from a large field of one or more varieties infected with a mixture of races have not been made at the end of the epiphytotic to discover the distribution pattern of races causing damage in the field. Would only one race predominate at the end of the epiphytotic, or would several? Would this predominance be caused by the selectivity of the rice varieties growing in the field, or by some attribute of the individual races, such as sporulating ability? Could this predominance be caused by some environmental or climatic factor that was more favorable to one race than to another in a given year? Would the mixture of races interbreed somehow and produce a new race?

B. (U) RICE, THE HOST PLANT

(U) Rice is probably the most varied of any commercial crop, and the world collection of varieties at the International Rice Research Institute contains more than 10,000 entries (as of 1964) and continues to grow. The wide variations observed among rice varieties growing in the field have some important consequences for rice blast epiphytology.

(U) Rice is a grass. The principal cultivated species and the one in which we are interested is Oryza sativa L. Other species of Oryza exist some annual and some perennial. The cultivated species is usually grown as an annual, except in some areas where a ratoon crop is obtained, although under suitable conditions it can persist for several years. O. sativa is sometimes divided into subspecies; the two most common divisions are Indica and Japonica. Although the morphological bases for separating these two forms are often obscure because of wide variation within each group and the existence of hybrid forms, the separation can be useful. A list of contrasting characteristics follows:

<u>Japonica types</u>	<u>Indica types</u>
Short grained (length to width ratio 1.4:1 to 2.9:1)	Long grained (length to width ratio 3.1:1 to 3.5:1)
Widely grown in northern areas of Asia	Widely grown in southern areas of Asia
Flourish under long photoperiods but generally respond to short day lengths of tropics by maturing so rapidly as to become worthless	Not so sensitive to photoperiod
Leaves dark green	Leaves pale green
Yields respond well to heavy applications of nitrogen	Yields do not respond as well to heavy applications of nitrogen
Seeds usually have no dormant period	Seed dormancy is more common

(U) Furthermore, although both types have 24 chromosomes that seem to be morphologically the same, crosses between the two types are difficult and some genetic incompatibility is indicated. American rice varieties generally have a mixed parentage, with most having a larger share of Indica genes. The pearl rices grown in California, however, are entirely Japonicas.

(U) An example of how a difference between the two types of rice may influence blast epiphytology is given here. Susceptibility of the rice plant to blast increases as rates of nitrogen fertilizers are increased (Section VI, C, 4). Since yields of Japonicas also increase with increased nitrogen applications, blast is likely to become a problem whenever high yields are sought; as a rule, the situation is not as clear with Indicas because they usually fail to make yield responses to nitrogen applications above modest levels.⁸

(U) Information on the growth and development of the rice plant can be obtained from many sources, but three useful summaries in English are suggested.⁹⁻¹¹ Upon germination, which occurs in about 48 hours, the radicle emerges and a primary root system with short branching fibrous roots appears. This system is relatively short-lived, however, and is replaced by roots developing from the first node of the plumule and other underground nodes. Root development is primarily horizontal and most roots are found in the upper few inches of soil. Root development can be influenced by soil texture, cultivation methods, available nutrients, and by the system of transplanting.

(U) The stem or culm of the plant is at first enclosed in the coleoptile, but after two to three days the first leaf emerges through a split in the coleoptile; the culm at this time is rudimentary. As the culm develops it is smooth, cylindrical, and hollow except at the nodes, where there is a septum. Leaves arise at the nodes (one leaf per node) and buds in the leaf axils may give rise to new culms called tillers. As a tiller emerges it pushes aside the leaf from whose axil it came and this leaf eventually dies and falls off. After some time, a tiller may develop tillers from the axils of its own leaves, and so on. Although tillers are produced during several weeks, the panicles generally emerge and ripen along with the panicle of the main stem. Under controlled conditions the number of tillers produced is a varietal characteristic, but it can be influenced by cultural practices. Although some varieties produce as many as 50 tillers per plant, the normal for transplanted rice ranges from 15 to 20 and for drilled rice from 5 to 8. The last few tillers produced are nonbearing and are called "invalid" tillers. The culm elongates by internodal growth from the base, and as this growth progresses differences in the heights of varieties become apparent. Depending on the variety, ultimate plant height is from 2 to 6 feet. The terminal internode of the culm ends at a node, called the neck, above which is the panicle.

(U) Leaves normally have four parts — blade, sheath, auricles, and ligule — although the first leaf to break through the coleoptile may have practically no blade and some varieties lack auricles. Leaves are alternate and are borne in two ranks. The sheaths envelope the stem and overlap one another. As the sheaths are oval in cross section they tend to give the tillers a flattened appearance, although the true stem that is inside the sheaths is round.

(U) The auricles and ligule are small projections at the place where the sheath and blade meet. They play no role in blast epiphytology.

(U) The leaf blade is long and slender and varies in dimensions with variety from about 10 to 20 inches long and $\frac{1}{2}$ to 1 inch wide at its broadest portion. It has a conspicuous midrib. The blade is usually pubescent or hispid. As the stems grow and new leaves emerge, the older leaves turn yellow and die, so that at any one time only four to seven leaves on any given tiller are living. The last leaf to emerge is the boot or flag leaf, and its sheath encloses the panicle just prior to

emergence. The flag leaf is usually different in appearance from other leaves in that it is shorter and broader. The flag leaf is at first nearly parallel to the axis of the panicle as it emerges, but later assumes an angle with the axis that is characteristic for the variety. Complete emergence generally takes place within a week.

(U) The panicle consists of a central axis that is divided into a number of nodes and internodes. Branching occurs at these nodes either singly or in whorls, and these grain-bearing branches may be either single or branched. Branches bear flowers called spikelets. Each spikelet is a complete flower bearing six stamens and two long, feathery stigmas. The spikelet is enclosed by two glumes. As the ovary develops it fills the space between these glumes and the developed ovary together with its covering glumes comprises the grain. The number of spikelets per panicle is a varietal characteristic that may vary from about 50 to as many as 300.

(U) Rice is essentially self-fertile, but a small percentage, generally less than one per cent, of natural crossing can occur. Pollination occurs just before or just as the flowers open; the flowers remain open for a period of a few minutes to more than an hour. The peak time of flowering is in the forenoon, from 10 AM to noon. Flower opening proceeds from the tip of the panicle downward and begins on the day of panicle emergence or the day after. The most favorable temperatures for opening and for germination of the pollen are between 80 and 90 F. It may take several days for all the spikelets in a panicle to flower, and the period from flowering to ripening, depending on variety, may be from two weeks to two months, but is usually around 40 days. The total number of days from planting to maturity ranges from 90 to 260.

(U) A study of the growth and development of rice is underway in this country and a preliminary report has been published.¹² In this study, four varieties of different maturity dates were studied: Belle Patna, 110 days; Nato, 130 days; Bluebonnet, 140 days; and Rexoro, 170 days. Table 1 is essentially a reproduction of the table used to summarize the data. Normally, one might assume that varieties that stand in the field for long periods of time would be subject to more damage by disease than those varieties maturing earlier, since the plants would be exposed to the pathogen longer and there would be more time for the increase of an epiphytotic. This is not the case with rice blast, however, since before panicle emergence the leaves of the rice plant are most susceptible in the tillering stage of growth. As can be seen from the table, the length of this tillering stage is essentially the same whether a variety matures in 107 or 164 days. The big difference in the length of growth periods among these varieties is between the end of tillering and the beginning of panicle emergence, a time when internode elongation occurs, panicle initiation begins, and the leaves of the rice plant become resistant to Piricularia. Data on this resistance phenomenon are presented in Section VI, C, 1. Detailed data in English on growth and development of individual far-eastern varieties are difficult to locate, but from the writers' observations in the Far East the same general growth patterns hold.

TABLE 1. (U) GROWTH OF RICE VARIETIES BELLE PATNA, NATO, BLUEBONNET, AND REXERO^{a/} (U)

Growth Stages	Number of Days from Seedling Emergence to Growth Stage Indicated			
	Belle Patna	Nato	Bluebonnet	Rexero
First tiller	24	24	24	24
Final panicle-bearing tiller	55	55	55	64
Final tiller	61	61	67	64
Internode elongation	53	56	67	69
Panicle initiation	56	60	73	97
First heading	83	85	100	127
Complete panicle insertion	85	88	103	132
Maturity (moist 20%)	107	118	132	164

a. Data of Em Wilson.¹²

(U) All parts of the rice plant can serve as infection courts for the fungus -- roots, stems, leaves, and panicles.

(U) Roots are only weakly attacked by the fungus, and evidence for the ability of the fungus to infect roots has been gathered principally from laboratory experiments.¹³ There is a little evidence that roots can be attacked in the field, but only to a very minor extent if the soil is kept moist or flooded.¹⁴ There is apparently no evidence that root infections play any role in epiphytotic development of blast.

(U) Stems can be infected, but lesions are usually found only on exposed parts. Near maturity and after stem elongation the nodes on many varieties are exposed and sometimes become infected under field conditions. The exposed portion of the last internode beneath the neck of the panicle is frequently found to be infected when any appreciable amount of leaf blast is found in the field. Occasionally, even the portion of this last internode that is covered by an apparently healthy sheath of the flag leaf is found to be infected, and here the mechanism of infection is not understood. Infections of the stem usually occur at the end of the growing season and, hence, at the end of the epiphytotic.

(U) The leaves provide the vast majority of infection courts during development of the disease. Although sheaths are probably as susceptible as the blades and sheath infections have been useful in laboratory studies, sheaths in the field are usually not involved to the extent that the blades are. This may be because of their vertical orientation or some other factor related to klendusity; anyhow, the observation that sheaths are not often severely infected in the field could bear further study and explanation. Much of the information that follows in later sections refers to infections on the leaf blades and, unless specific reference is made to sheaths, the reader should interpret the word leaf to mean leaf blade.

(U) Any part of the panicle from the neck node, the rachis and its branches, and even the glumes can be infection courts for the pathogen. Infections of the neck, or of the internodal portion just above or below it, are frequently damaging because a lesion in this location can cause failure of the grain to fill in the entire panicle. It is the senior author's opinion that in an epiphytotic, lesions on the panicles are probably terminal, i.e., by the time lesions on a given panicle could produce spores, other panicles in the field probably would be mature enough to escape damage even though infected by these spores; also, resistance of panicles tends to increase with age after emergence (Section V, B, 2).

C. (U) RICE BLAST EPIPHYTOTIC

(U) The typical epiphytotic of rice blast is usually recognized only after it is well underway, as is true with most other plant diseases. Opportunities to observe early stages of disease progress in the field are, therefore, rare under natural conditions. By artificially inoculating a field early in its growing season these early stages of disease progress can be observed. About 6 days after artificial inoculation, a few widely scattered lesions can be found on the leaves; occasionally groups of lesions will appear. These lesions increase in size, and spores formed on their surfaces can cause new infections that begin to appear in about a week. At this point the rate of increase of the disease is compounded, and it is here that the casual observer first notices the presence of disease. Disease severity may increase rapidly for 2 to 4 weeks and then seem to decline somewhat after the end of the tillering stage. By the time panicles begin to emerge it may even be difficult to find leaf lesions, the older infected leaves having fallen off and decayed, although some leaf lesions usually are found. In fields attacked during the tillering stage, a certain percentage of neck and panicle blast (concomitant with yield reduction) is almost always present.

(U) The severity of disease buildup on the leaves and the amount of infection on the panicles is dependent on many factors. These factors include race-variety considerations, the effect of various cultural practices on buildup, and the meteorological conditions. It is of some importance here to note that infections of P. oryzae occur at night, so that it is inaccurate to speak of meteorological conditions that occur during a "day" or on a given date. When thinking of a given period of spore incubation in the field, one should consider the conditions occurring before, during, and following a night.

CONFIDENTIAL

17

II. (C) SOURCES OF INOCULUM

(U) Our information on the source(s) of inoculum in nature is not satisfactory. A number of workers have studied the problem from time to time and, at best, have come up with "leads," i.e., possible or probable sources. Field studies on blast often rely on what will be called an artificial source of inoculum, although lack of information as to natural sources has sometimes hampered these studies. Occasionally, it has been impossible to know how much of the disease in experimental fields was caused by artificial inoculum and how much was caused by inoculum from some endemic source.

A. (C) SOURCES FOR PRIMARY INFECTION

(U) *F. oryzae* seems to be very elusive during winter months in the temperate regions and during dry seasons in the tropics when rice is not grown. (Rice is sometimes grown during dry seasons in the tropics in those areas where irrigation facilities are well-developed. Here, holdover of the disease as secondary infections on rice would be possible and sources of primary inoculum might not need to be considered.)

1. (U) Rice Straw Refuse

(U) One possible way the fungus overwinters (or overseasons) is as mycelia inside rice straw refuse left in piles or scattered about fields after harvest. Such mycelia, when moistened at temperatures from 18 to 32 C, can produce viable conidia. In one series of experiments, mycelia in tissue kept dry remained viable for about three years, but in moist tissue had lost their viability by April the next spring (about 6 months). Conidia that remain dry could be the overseasoning inoculum in the tropics, although conidia produced by mycelia held over in dry plant debris might be the more likely primary inoculum.^{3,5}

(U) In his discussion of data relating to the prevalence of rice blast, Hoshioka considered that the areas of disease occurrence, with respect to overwintering or overseasoning, could be grouped into zones roughly bounded by latitude:

CONFIDENTIAL

CONFIDENTIAL

<u>Source of inoculum</u>	<u>Temperate (36°-44°N)</u>	<u>Subtemperate (28°-36°N)</u>	<u>Subtropical (15°-28°N)</u>	<u>Tropical (15°N-25°S)</u>
Mycelia outdoors	Impossible (except in piled straw)	Possible	Possible	Possible
Conidia outdoors	Impossible	Possible	Possible	Possible
Living leaves	Impossible	Impossible	Maybe	Possible

Hashioka's book also provides an entrance into the literature on this subject.¹⁵

(U) Work by Andersen et al. has substantiated the earlier Japanese work indicating that spores remain viable longest if kept at low temperatures and low relative humidities.¹⁶ In these experiments, conidia kept at 8°C and 20% relative humidity (RH) were 75% viable at the end of a year. More recent unpublished observations indicate that spores can remain viable for at least eight years, but artificial storage phenomena are beyond the scope of this review.

2. (U) Weed Hosts

(U) As stated in Section I, A, there are a number of grasses that can be infected by fungi fitting the description of *P. oryzae*. The extent to which these fungi may be related has not been investigated satisfactorily, although, occasionally, someone will collect specimens and attempt inoculations to rice or cross-inoculation studies. Several such studies are mentioned in the literature^{3,4} but the most complete compilation is in a paper by Asuyama.^{5*} Two studies of a preliminary nature have been made at Fort Detrick.

(U) Latterell isolated *Piricularia* from 14 grasses and one sedge. These were not pathogenic to any of eight rice varieties. Several isolates from rice, however, did cause typical lesions on two strains of St. Augustine grass and resistant pinpoint-type lesions on sugarcane.⁴ She has occasionally obtained isolates from grasses since publication of these findings, but thus far, has found few that will infect rice.^{4*}

* Pages 9 through 22.

** Personal communication.

CONFIDENTIAL

CONFIDENTIAL

(U) G.N. Asai* collected isolates from six wild grasses and performed cross-inoculation tests with them and with isolate 7:0 and the rice variety C.I. 8970S (Table 2). All isolates could cause infection on the host species from which they were isolated. Five isolates not from St. Augustine grass caused infection on that grass, although these infections were not as severe as that caused by the St. Augustine grass isolate itself. The isolate from Digitaria ischaemum infected two other grasses. Grass isolates did not cause infection on the rice variety tested; in further research, these isolates should be tried on more than one rice variety.

3. (U) Seed

(U) Some of the early Japanese workers considered seed a source; it is not a likely source in the writers' opinion. There is no doubt that spores can overwinter on the seed or that mycelia can sometimes be found growing in the tissues of infected seed.^{3,5**} However, when the cultural methods used to germinate the seed in the field and the aerobic requirements of the fungus are considered, it is difficult to understand just how a spore lodged on a seed coat or spores from mycelia supporting spore production (where -- in the mud?) could reach a suitable infection court. Hot water seed treatments do not control the disease,³ and chemical seed treatment, which is a common practice in Japan,^{5***} Taiwan, and the United States, has no apparent effect on the occurrence of the disease in those countries.

4. (U) Soil

(U) Apparently, soil has not been considered as a source of primary inoculum.

5. (U) Rice at a Distant Location

(U) So little is known about the spread of Piricularia spores through the air over long distances that it is difficult to know how much importance to attach to the possibility that infected rice in one country may be a source of inoculum for the disease in another. Spore traps set out to catch P. oryzae spores in Japan and Taiwan, as part of a disease forecasting system, usually collect spores after a few lesions can be found in the field locally by careful observation. Dr. ... Ou**** collected Piricularia spores on glass slides excised from the windows of aircraft flying at 4000 to 7000 feet altitude over parts of Thailand when he served as an FAO Agricultural Officer there; however, the source of the spores is not known.

* USABL Notebook CD 3814. UNCLASSIFIED.

** Pages 9 through 22.

*** Pages 399 through 408.

**** Personal communication.

CONFIDENTIAL

CONFIDENTIALTABLE 2. (C) CROSS-INOCULATION STUDIES WITH ISOLATES OF PIRICULARIA FROM SEVERAL HOSTS^a/ (U)

		Results of Inoculation to Grass Host Species and Original Host Locale			
Source of Isolate	Isolate	Panicum repens (Okinawa)	Leersia japonica (Okinawa)	Digitaria ischaemum (Maryland)	Stenotaphrum sanguinalis (Maryland)
Panicum repens	+	++b/ -c/	-	-	++
Leersia japonica	-	+++	-	-	-
Digitaria ischaemum	-	-	+++	+++	+
Digitaria sanguinalis	-	-	-	+++	+
Stenotaphrum secundatum	-	-	-	-	++
Setaria viridis	-	-	-	-	++
Oryza sativa	-	-	-	-	+++

- a. Unpublished data of J.N. Asai.
 b. Plus signs indicate degree of susceptibility.
 c. No lesions resulting from tests.
 d. Geographic source not known.

CONFIDENTIAL

CONFIDENTIAL

21

6. (C) Artificial Sources

(U) Ultimately, the source to which we refer here is a laboratory where a spore preparation is made by one of several methods. From the standpoint of plants in the experimental field, the source is some sort of atomizer, duster, pistol, etc. Presumably, once the velocity of release from these artificial sources has been dissipated, spore travel is governed by the same laws that govern spore travel from natural sources, except that sometimes the artificial source may be so close to the plants that spores are put onto plant surfaces by the force of discharge. Then, too, an artificial source may provide spores that are wetter or drier — or in some other way modified — than are spores arriving from natural sources.

(C) Panzer et al. used a small duster inserted into a tent that covered the area to be inoculated.¹⁷ Another of their methods relied on the explosive force of a 2-inch firecracker to release a dry spore powder from a waterproof bag at predetermined time intervals.¹⁸

(C) Sprayers or atomizers dispensing inoculum suspensions have been used in three field studies. Rorie used an atomizer powered by compressed nitrogen to disperse spores into a tent placed over a confined area.¹⁹ Allison^{a/} sprayed an area of approximately one acre with a liquid spore suspension with a tractor-mounted sprayer. Marchetti^{b/} used a spray gun powered by compressed carbon dioxide to inoculate small confined areas in rice plantings.

(C) Dusters or dust sources of various kinds have also been used for the inoculation of either confined or unconfined areas. Barksdale²⁰ in 1960, Allison^{c/} in 1961, and Dahlke^{d/} in 1963 inoculated one- to two-acre fields in Florida by releasing dry inoculum along a line with a blower developed by Rorie and Asai. Barksdale^{e/} used a midget duster to release inoculum alongside fields in Okinawa and Taiwan; Willis^{f/} used this kind of

-
- a. Allison, W.H., and Dahlke, G.R. Unpublished data and the associated Analysis 5744 (16 Apr 1963), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.
 - b. Marchetti, M.A. Unpublished data and the associated Analysis 6269 (15 July 1964), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.
 - c. Allison, W.H. Unpublished data and the associated Analyses 5219 (28 Mar 1962) and 5312 (17 May 1962), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.
 - d. Dahlke, G.R. Unpublished data and the associated Analysis 6433 (5 Aug 1964), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.
 - e. Barksdale, T.H. Unpublished data, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.
 - f. Willis, G.M. Unpublished data and the associated Analysis 6144 (19 Feb 1964), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

CONFIDENTIAL

duster to aim inoculum at specific but unconfined areas in experiments on Taiwan. Kulik* released a cloud of spores from a modified carbon dioxide pistol into a portable settling tower in his field studies in Texas.

(C) The choice of any particular inoculation method for field studies is based on the kind of experiment and the kind of spore preparation available to the investigator. Small plots can be inoculated quantitatively with either dry spore dusts or with wet spore suspensions. Since spore suspensions obtained by washing some surface like a sporulating lesion or agar plates must be evaluated each time the suspension is made, it is more convenient to take a standard sample from a uniform dry spore preparation that can be stored for a period of time. It probably makes little difference whether the dry sample is released as a dust into a settling tower or is mixed with a liquid and released into a tent. If the spores remain wet very long, however, they will begin to germinate before the beginning of the natural dew period; this may or may not be desirable, depending on the experiment. The carbon dioxide - powered hand atomizer that Marchetti used is undoubtedly more convenient than Rorie's cumbersome nitrogen cylinder in the field, although for quantitative work the hand atomizer would have to be calibrated in some way. The merit of the firecracker method was the timing device, but if the investigator chooses he can make inoculations at night by some other, and probably better, method. For inoculation of a field, if the worker wants to observe a gradient of infection associated with a gradient of dosage, release from a line or point source is indicated; if a uniform inoculation is desired, perhaps a tractor- or aircraft-mounted sprayer or duster could be used. The senior author prefers the use of a liquid spore suspension for quantitative small-plot inoculation.

B. (U) SOURCES FOR SECONDARY INFECTION

(U) The chief source of inoculum for secondary infections is infected rice leaves, especially the infected leaf blades. It is possible that diseased leaf sheaths, nodes, and panicles could provide inoculum for secondary disease cycles, but it is doubtful that these sources ever provide more than a negligible percentage of secondary inoculum during most epiphytotics. Lesions on sheaths do occur in the field, although in the writers' experience they are seldom numerous except in rare instances when plants are killed by the disease during the leafy growth stages. Node and panicle infections occur near or at the end of epiphytotics.

1. (U) Number of Spores Produced on Infected Tissue

(U) It has been estimated that theoretically approximately 8 million spores per square centimeter could be borne on the surface of infected leaves.¹ We are not aware of any measurement made in the field; however, actual numbers of spores measured under laboratory conditions generally do not approach the theoretical figure and the numbers vary widely.

* Kulik, M.M. Unpublished data and the associated Analyses 4451 (5 Dec 1960) and 4447 (20 Sept 1960), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

(U) Measurements generally are made by one of two techniques: either lesions on detached leaves are incubated under a given set of conditions for a fixed length of time and the spores are mechanically harvested by some washing procedure,^{21*} or lesions on attached leaves are incubated for fixed times or at intervals and the spores are allowed to fall naturally onto some suitable collecting surface, such as water agar.²² The latter procedure allows spore production on the same lesion to be examined over a period of days or even weeks, and field conditions can be mimicked to a certain extent. Both procedures seem to be more useful in defining the conditions under which spores are formed on lesions than in determining the number of spores produced. Some of these conditions are age of lesion, age of host tissue when infected, the race-variety combination, and various meteorological conditions.

(U) As the age of lesions increases from 7 to 35 days, their potential to support sporulation decreases.²¹ The greatest number of spores produced by any one lesion is found on about the 9th day after inoculation, while the lesion is still actively increasing in size.²²

(U) The age of a leaf at the time of infection makes no appreciable difference on the number of spores produced, according to one study.²¹ Since plants and leaves tend to increase in resistance with age, however, one would expect "type 3" and "resistant type" lesions (in the sense that Latterell uses these terms⁷) to support less sporulation.

2. (U) Environmental Conditions Affecting Sporulation on Lesions

(U) The most important and essential meteorological factor needed for sporulation is moisture approaching 100% RH; i.e., other factors such as light and temperature influence the relative numbers produced but without high RH there would be no sporulation. The lower limit at which spores can be formed is about 89% RH, but maximum numbers are produced at humidities near saturation.^{15,23} The minimum time for sporulation after a lesion has been placed in a suitable environment is approximately 8 to 9 hours,²¹ although there is some indication that if the lesion has already produced spores during a previous period when it was in a suitable environment, this time may be shortened to about 6 hours.²² Latterell has observed the production of conidiophores on panicle lesions and found a few conidia that formed within 5 to 6 hours.** Of course, as the length of time that a lesion is kept moist increases, the conidial production also increases.²¹

* See also U.S. Army Biological Laboratories Notebook CD 3814. UNCLASSIFIED.
** Personal communication.

(U) In 24 hours of continuous dew, the numbers of conidia produced per square centimeter of infected tissue may range from 100 at 60 F to 20,000 and 30,000 at 80 to 85 F. Numbers in the hundreds of thousands are occasionally observed. Conidia increase with length of time that lesions are exposed to dew. In one test, the number of conidia increased with increasing temperatures from 60 to 85 F, with a slight decrease at 90 F during dew periods 24, 48, and 72 hours long.²¹

(U) Light of as much as 1500 foot-candles (ft-c) has been shown to stimulate sporulation of the fungus in culture, with maximum sporulation occurring in the range of 200 to 500 ft-c.²⁴ Experiments on sporulation using detached leaves have shown that light at 150 ft-c stimulates sporulation at temperatures of 70 and 80 F.* A summary of one such experiment follows:

<u>Temperature, F</u>	<u>Light</u>	<u>Time, hr</u>	<u>Number of Spores per Lesion (Average of 4 Replicates)</u>
70	Dark	24	9,000
70	Light	24	20,000
80	Dark	24	26,500
80	Light	24	77,000
70	Dark	48	15,000
70	Light	48	35,500
80	Dark	48	80,500
80	Light	48	113,000

(U) Not all isolates of P. oryzae show this response to light. The importance of light on sporulation in nature has not been assessed, but since RH conditions are most favorable at night, it seems that light would be an effective stimulant only on misty or foggy days. Perhaps moonlight could have some role in stimulating sporulation on clear nights.

3. (U) Latent Period

(U) The latent period is defined as the length of time between the initial establishment of infection in tissue and the time when that tissue itself becomes infectious, i.e., supports sporulation. With rice blast, new lesions can usually be seen a day or two before they will support spore production. The most important factor influencing the speed at which lesions develop is temperature:²⁵

* USABL Notebook CD 3814. UNCLASSIFIED.

<u>Temperature, C</u>	<u>Days for Lesion Appearance</u>
9-11	13-18
17-18	7-9
24-25	5-6
26-28	4-5

(U) At temperature ranges normally found in the field during rice blast epiphytotes, it is about 6 days before sporulation begins on a lesion,²⁶ and maximum sporulation usually occurs a day or so later.²²

CONFIDENTIALIII. (C) DISPERSAL

(U) In the preceding section, the sources of inoculum were examined and various facts were related to the production of inoculum on diseased tissue. In this section, data on how inoculum travels from its source to the infection courts are discussed. Dispersal of inoculum by air and wind currents has been documented for many years, and there is no question that this means of dispersal operates in the buildup of epiphytotics within local areas. Other means of dispersal — by water, mammals, birds, man, and insects — are plausible, especially as they might be involved in transferring the inoculum that initiates the primary disease cycle in a particular field. These other means, however, are only slightly documented.

A. (C) DISPERSAL BY AIR

(U) The information on dispersal by air can be usefully considered under three headings: release, flight, and landing of spores.

1. (U) Release

(U) No one to our knowledge has ever seen a spore leave the conidiophore on which it was produced. The little bump that appears on the basal cell of the spore is part of a tiny stalk cell that joins the spore to the conidiophore. When release occurs, this stalk cell ruptures, leaving part of the cell on the conidiophore and part on the spore.* The mechanism of rupture is not known.

(U) Under quiescent conditions, the spores fall downward under the influence of gravity, which indicates that release is not very violent, at least that it is not violent in some direction other than downward. When lesions caused by some isolates are placed in moist chambers at temperatures of 70 to 80 F and at 100% RH, spores are released only in the dark. After exposure to light, these lesions cease to release spores, although spores continue to be produced. This diurnal pattern of release coincides with the diurnal pattern of spore concentrations found in the air (the highest numbers of conidia are usually found above an infected field between midnight and 6 AM). When sporulating lesions on attached leaves are taken from a dry environment and placed in a moist chamber, a condition simulating the start of a dew period, spore release begins within 6 to 8 hours. Occasionally a few spores, presumably those already matured or nearly so during the previous "dew" period, are released within the first couple of hours after a lesion is returned to an environment of 100% RH.²²

* Personal communications from Drs. F.M. Latterell and C.T. Ingold.

CONFIDENTIAL

CONFIDENTIAL

27

(U) The above laboratory studies emphasize a still, quiescent environment, a situation that is common in nature at night in rice-growing areas, especially within a canopy of rice plants. Any wind or other mechanical disturbance can alter the release pattern, however, and some Japanese work has shown that the numbers of conidia separated from their conidiophores is proportional to wind velocity.^{5*}

2. (C) Flight

a. (C) Measurement of Spore Load

(U) The early work on measurement of spore load in the air relied almost entirely on some type of stationary glass slide spore collector. This type is inefficient and an investigator runs a large risk of drawing inaccurate conclusions if he considers data from it as quantitative. Japanese workers up to now have relied almost entirely on the slide spore collector, and they have developed a considerable body of useful qualitative data on the spore load, especially on the time of day that the greatest number of spores are in the air.^{5,15}

(U) In 1957, Panzer et al. working at Fort Detrick, improved the slide collector so that it was more nearly quantitative.²⁷ Use of their 24-hour slide spore collector enabled them to confirm the diurnal fluctuation of Piricularia spores that had been discovered by the Japanese. Before their collector could be used to contribute to further information on P. oryzae, the very efficient rotorod sampler²⁸ was developed and workers at Fort Detrick have used it since 1960.

(U) Recently, the rotorod has been tried in Japan, and, apparently, the workers there are pleased with it. Because of difficulties in using the slide spore collector to detect small numbers of spores in the air, spore trapping there in past years to help predict the initial outbreak of blast has been largely unsuccessful because lesions can be found in the field before the slide traps will collect spores. Ono's group^{5**} compared the two collectors in the field; their data are shown in Table 3. The units of area or volume that the spores occupied are not given, so that it is difficult to compare the data with those from other sources, but the table does indicate that it would be much better to use the rotorod for predictions. It is interesting that their rotorod picked up spores 7 days before infections were seen in the field.

* Pages 153 through 162.

** Pages 173 through 194.

CONFIDENTIAL

TABLE 3. (U) COMPARISON OF THE ROTOROD SPORE SAMPLER AND THE STATIONARY SLIDE SPORE COLLECTOR^{a/} (U)

Date	Sampling Period		Number of Spores Caught			Remarks
	Starting Time, AM	Length, hours	Rotary Sampler		Stationary Sampler	
			130 cm, high	10 cm high	130 cm high	
June 19	5	3	0	0	0	
20	5	3	0	0	0	
21	4	4	0	0	0	
22	4	4	0	0	0	
23	4	4	0	0	0	
24	6	4	1.0	0	0	
25	8	1.5	2.0	0	0	
26	5	3.5	6.5	0	0	
27	5	3.5	2.5	0	0	
28	8	1	1.5	0	0	
29	0	1	0	0	0	
30	8	1	0.5	0	0	Initial outbreak of leaf blast
July 1	6	0.5	0	0	0	
2	5	2	2.5	0	0	
3	8	1	0	3	0	
4	4:30	3	65.5	0	0	
5	5	3	82.5	6	0	
6	7:30	1.5	25.5	6	0	
7	5	2	5.0	4	0	
8	5:30	1	67.5	2	0	
9	7:30	1	0	0	0	
10	12 noon	2	0	0	0	Showing many lesions
11	8	1	0.5	1	0	
12	7	1	171.5	12	5	
13	6	2	1425.5	154	4	

a. Data of Ono, pages 173 through 194.³

CONFIDENTIAL

29

(C) A word of caution about measurement of the spore load is in order, regardless of what sampling device is used. The investigator should be certain the spores he traps are related in some way to the rice field where he is studying blast. For example, the senior author used the sequential rotorod sampler to measure spores in the air near a field that he had inoculated in Okinawa in 1961.* Eventually, it became clear that a grass, Panicum repens, growing abundantly along the dikes between small rice fields had sustained a heavy infection of a Fusicularia species. Latterell made several isolations from this grass and was not able to obtain infections on rice except in one case (her isolate 238), in which only a few lesions were obtained.** On the rotorod, spores from the two sources could not be distinguished from one another and, therefore, the data from the sequential sampler in this instance are meaningless.

b. (U) Patterns of Air Movement

(U) A considerable volume of information on the patterns of movement in air carrying various allergens, fungus spores, smog particles, etc., is available. Little information is available that specifically relates to P. oryzae, although there is no reason to believe that it moves any differently than other particles in its size range.

(U) In an effort to study movement of air currents in a rice field and its surrounding area, Van Arsdel et al.³⁹ released colored smokes from standard U.S. Army smoke grenades. In daytime winds, smoke released at zero and at 12 feet above ground moved downwind at the general level of release and with a spiraling movement. Their photographs and observations showed a very complex structure of air currents within air currents. The helical movement of the smoke caused the direction of movement at the top of a loop to be different from that at the bottom, and they commented that spores carried in such a pattern could be deposited in isolated spots. In sunlight, updrafts occurred over locally warmed areas such as levees, bare soil, and sunny sides of earthen mounds. Two-foot-wide openings in four-foot rice had downdrafts, and smoke went into these openings and then slowly diffused through the stand of rice. Apparently, only limited observations were made with these smokes at night, possibly because of the difficulty in making observations on these movements, although observations at night would certainly be of interest because P. oryzae spores are usually present in higher concentrations then.

* Barksdale, T.H. Unpublished data, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

** Personal communication.

CONFIDENTIAL

(U) Higher concentrations of spores are more likely to be found near the ground than at distances above it. Marchetti* used a rotorod sampler at intervals of zero, 2, and 5 feet above plant height during an epiphytotic and found on 28 nights spread throughout the growing season that the greatest concentrations were nearest the rice. The exponential decrease rate was estimated as 30.69% per foot; 95% confidence limits were 6.65 and 54.73% per foot.

(U) Using a stationary slide spore collector, Ono and co-workers^{**} found spores distributed as high as 24 meters above an infected rice field. Their measurements were made over a period of 4 weeks and included some generalized weather data. They found many spores at the lower levels and a rapid decrease in numbers collected as the height increased. Strong winds were thought to lessen the vertical spore gradient.

c. (U) Concentrations Found in the Air

(U) The highest concentrations of spores in the air are found at night, usually between the hours of midnight and dawn.^{5,15,20,27,*,**} The exact hour during which peak spore load occurs varies from day to day, as will the numbers of spores collected; causes of this variation are discussed below. Significant numbers of spores are sometimes found during daylight, especially at the height of an epiphytotic.²⁰ Data are available from four experiments in which the sequential rotorod sampler was placed in a field during an epiphytotic.^{20,*,**} That month (30-day period) during which highest concentrations were found was selected and data associated with these high concentrations are shown in Table 4. In all instances, the 30-day period occurred during the mid- to post-tillering stage of plant growth. Yield reductions in three of the fields were high. Data from only one field showed low yield reductions, but it is probable that lower spore concentrations would have been measured under circumstances that resulted in higher yields.

* Marchetti, M.A. Unpublished data and the associated Analyses 6171 (13 Feb 1964) and 6269 (15 July 1964), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

** Pages 173 through 194.

*** Allison, W.H. Unpublished data and the associated Analyses 5219 (28 Mar 1962) and 5312 (17 May 1962), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

TABLE 4. (U) CONCENTRATIONS OF P. ORYZAE SPORES IN THE AIR DURING 30-DAY PERIODS OF HIGHEST CONCENTRATIONS OVER FOUR FIELDS WITH RICE BLAST EPIPHYTOPHICS (U)

Worker end Location	Dates	Race of Fungus and Rice Variety	Yield Reduction, %	Spores Per Cubic Meter of Air		
				During One Hour		Mean
				Day of Highest Observations	Day of Lowest Observations	
Barksdale ^{a/} Avon Park, Fla.	10 June-10 July 1960	Race 8 (770) ^{b/} Colusa	95	1814.3	24.9- 5712.1	2.1
Allison ^{a/} Avon Park, Fla.	21 June-21 July 1961	Field 4, Race 1 Gulfrose Arkrose	21.5 ^{c/} 0	77.1	3.3- 346.7	7.7
		Colusa	0			
		Field 6, Race 3 Gulfrose Arkrose	39.5 ^{c/} 47.5	84.5	10.0- 503.3	5.9
		Colusa	26.0			
Marchetti ^{a/} Crowley, La.	1963	Race 1 (429) Gulfrose	55 ^{c/}	2097	67- 9580	0.235

a. Unpublished data, Crop Division, U.S. Army Biological Laboratories, Frederick, Maryland.

b. Indicated race used as inoculum but race 19 isolated from the field.

c. Yield reductions calculated from workers' yield data using average commercial¹³⁰ and experimental station³¹ yields as controls.

d. (U) Effect of Meteorological Conditions on Concentration

(U) The effect of several conditions on sporulation has already been noted, and it might be expected that these same conditions — temperature, light, and RH — plus others like wind and rain will affect spore concentrations observed in the air. In the field, however, it is very difficult to separate the amount of influence that each of these factors exerts on the observed concentrations, since they all interact. For example, it is not possible to hold temperature, RH, wind and frequency of rain constant for a week in order to measure the effect of light on spore concentration. Even if it were possible, the amount of diseased tissue and the age of sporulating lesions would change. An observer may see a factor that he thinks is exerting a major influence on concentration on a particular day, but his control observation is never as sound as he would like.

(U) Despite this handicap, it has now become apparent that periods of rainfall do exert an influence on spore concentration. Panzer et al.* found that more spores were collected on rainy days than on dry days, and that afternoon rains were associated with a peak spore load occurring before two o'clock the next morning. This peak load occurred earlier than the peak load on dry days. The senior author²⁰ found that on rainy days, especially on days when showers occurred in the late afternoon so as to effectively lengthen the dew period, there were higher concentrations than on days without rain. This was so when three parameters were considered: the maximum spore concentration during a one-hour period associated with the dew period, the average hourly concentration during the dew period, and the average concentration during the early hours of the dew period that preceded the last eight hours.

(U) Wind also seems to exert an influence on concentration. There are sometimes sharp peaks in the spore load during daylight hours, although these are well below peak concentrations found during the subsequent night. The writer associated some of these daytime peaks with the high winds of thundershower activity.²⁰

(U) Allison subjected his spore concentration data to statistical analysis. In 1961, eight variates (maximum temperature, minimum temperature, length of dew period, intensity of dew, rainfall, barometric pressure, light intensity, and an 8-day moving average of rainfall) for observations made the day of the count together with eight variates for averages of observations made 7 and 8 days prior to the count were used in the analysis. The latter variates were used because Allison believed that 7 to 8 days was the lag time from inoculation to the time that coridria would be produced on new lesions. It was necessary for purposes of the analysis to describe a hypothetical trend for spore counts in order to find the deviation of observations from the trend.

* Panzer, J.D.; Tullis, E.C.; and Van Andel, E.P. "Epiphytology of rice blast, Piricularia oryzae," Unpublished manuscript on work performed 1955 through 1957, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

33

Allison suggested that, concurrently with the growth of new rice tissue, conidia counts ought to increase with increase of disease on the leaves, fall off somewhat as fewer new lesions were produced between the end of tillering and the heading stage, then rise again as neck lesions sporulated, and finally decrease. Mrs. Jones examined his data and found that the sum of two Pearson's Type III functions seemed best to fit the experimenter's notion of the expected trend and she superimposed two equations of this type on data from each of the two fields. None of the factors measured was shown to be associated in a statistically significant way with deviations from the trend.* Allison himself, however, felt that the lack of rainfall early in the season inhibited abundant sporulation and thereby lessened the numbers of conidia found in the air then. In 1962, his conidia counts were compared with both rainfall and a degree-hour term for the day of the observation and for 8 days prior to the observation. The degree-hour term was a multiple of the minimum temperature times the length of the dew period, with the term equaling zero if either temperature was less than 65 F or the dew period was less than 8 hours. The simple correlation ($r = -0.26$) between conidia count and the degree-hour term on the day of observation was significant at about the 95% level, but the other correlations were not. The writers cannot interpret the minus sign on the correlation coefficient.

e. (C) Distances Traveled in Flight

(U) Several articles on dispersal in the Japanese literature have been reviewed by Hashioka.^{6**} These papers indicate that the disease in a particular field extends downwind, that conidia are blown by wind to the leeward of inoculum sources (piled straw in the case cited), and that dissemination toward the wind is restricted. The numbers of conidia separated from conidiophores are proportionate to wind velocity, and the distribution of conidia is also affected by wind velocity because the greater the velocity the wider the extent of vertical distribution of spores. Unfortunately, no specific data are given.

(U) Kawai³² placed slide spore collectors in the field at varying distances from a pile of rice straw obtained from blast-diseased plants. He found 596, 275, and 12 conidia per 54 square millimeters at distances of 0.39, 1.98, and 35.64 meters, respectively.

* Allison, W.H. Unpublished data and the associated Analyses 5219 (28 Mar 1962), 5312 (17 May 1962), and 5744 (16 Apr 1963), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

** Pages 153 through 162.

CONFIDENTIAL

CONFIDENTIAL

(U) In preliminary trials, W.B. Johnson* released a dry mixture of spores of Puccinia graminis var. tritici and Piricularia oryzae and found the latter at several distances up to 760 feet away from his release line. The numbers of both kinds of spores collected seemed to decrease at about the same rate.

(C) Several disseminations of spores have been made by Fort Detrick personnel in order to inoculate small rice fields. The general procedure used was to release inoculum along a line so that it would form a cloud at distances ranging from zero to 100 feet from the nearest edge of the field. The object in all cases was to inoculate the field as uniformly as possible, so that the greater distance was preferable; local conditions, such as the presence of other rice fields, sometimes precluded this. Rotobars were placed in (occasionally outside) the fields to measure the concentration of the spore cloud drifting over the field. A summary of these data is shown in Table 5. In no case was the original concentration of the cloud at the release line measured. In some fields, the difference in dosages from place to place seemed not to follow any pattern, but in most there was an obvious decline in dosage with distance from the release line. Although the data do not allow accurate quantitation of the distances to which spores will travel beyond a few hundred feet, they do indicate that tests set up to measure spore clouds over longer distances would probably yield a family of curves.

(U) As far as we are aware, Johnson's simple test described above is the only one that shows that a P. oryzae spore will travel 760 feet from a known source. Undoubtedly, they do travel much farther.

3. (U) Landing

(U) After traveling through the air, spores land; we are interested in those that land on a suitable infection court. Although accurate measurement of spores traveling through air is possible, as we have just seen, accurate measurements of spores landing on leaves usually are not made and are technically difficult (see Kahn's work described below in Section IV, B). The Japanese have approached the problem by putting removable strips of sticky tape on leaves of plants growing in the field. There are no data on the relationship between the numbers of spores traveling in the air and the numbers landing on leaves.

(U) Observations of spores collected on rice leaves in the field have shown that the numbers of spores collected on the upper and lower surfaces of the youngest leaf were about equal, but that on the lower leaves the number deposited on the upper surface was much greater than on the lower surface.
^{5**}

* Unpublished data, 1962, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

** Pages 173 through 194.

CONFIDENTIAL

CONFIDENTIAL

35

TABLE 5. (C) DOSAGES OF P. ORYZAE SPORES MEASURED AT ABOUT ONE METER ABOVE THE GROUND DURING ARTIFICIAL INOCULATIONS OF RICE FIELDS (3)

Worker and Location	Field and Date	Wind Speed, mph	Distances from Release Line at Which Dosages Were Measured, ft ^a /		Dosages in Particle Minutes Per Liter Range ^a	
					Mear	
Barksdale, ^b / Avon Park, Florida	Paddy 6 18 May 60	3	20-240		4.6-24.3	13.2
	Paddy 7 24 May 60	5	140-620		3.0-40.6	15.2
	Paddy 3 31 May 60	9	100-275		5.5-14.8	9.9
	Paddy 8 3 June 60	variable	60-200		1.3-48.4	14.6
Allison, ^b / Avon Park, Florida	Paddy 4 30 May 61		135-275 ^c /		26.2-58.4	38.27
	Paddy 7 5 June 61		135-275 ^c /		0.6-6.2	2.04
	Paddy 5 8 June 61		135-275 ^c /		0.4-11.8	5.71
	Paddy 6 13 June 63		135-275 ^c /		21.2-42.6	33.16
Barksdale, ^b / Okinawa	Hillside 24 Apr 61		100-150		25.4-80.4	51.7
Dahlke, ^b / Avon Park, Florida	Paddy 5 30 Apr 63	4.4	45-260		29.2-144.8	67.5
	6 May 63	4.4	45-185		185.8-307.2	277.2
	14 May 63	2.1	75-215		38.4-225.8	84.3
	24 May 63	2.4	45-185		(0)16.8-249.6	96.2
	Paddy 3 7 Jun 63	2.8	30-170		24.4-101.2	58.9

a. Highest dosages usually found near the release line.

b. Unpublished data, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

c. Distances assumed by the writers from the procedure described.

CONFIDENTIAL

(U) Given the same spore load in the air, not all rice varieties will collect equal numbers of spores on their leaves. Ono divides rice varieties into groups based on the angle that the leaf blade makes with the stem and on the blade's drooping habit. If blades droop, only the middle portion of the blade, i.e., the portion that is nearly horizontal, receives a high spore deposit. Varieties whose leaves neither droop severely nor have a sharp angle with the stem receive the largest deposits, probably because of their essentially horizontal orientation. Varieties having leaves that do not droop and that have a sharp angle to the stem, i.e., a stiff growth habit, will trap very few spores.* In Section I, B we stated that variations in horticultural characteristics among the more than 8,000 rice varieties would affect epiphytology. The information above, when related to that in Section IV, B on the intensity of inoculum in the infection court, provides a case in point.

(U) There has been some thought given to the tenacity with which spores stay on leaves once they land. Kahn did some laboratory studies and concluded that spores were not removed in numbers sufficient to significantly reduce infection by experimental procedures simulating strong wind and rain.³³ Somewhat different data and observations have been obtained by others. When Hartman inoculated potted plants in either a wind tunnel or a settling tower, he obtained fewer lesions on plants subjected to $\frac{1}{2}$ or $2\frac{1}{2}$ hours of simulated rain after inoculation and before receiving an 18-hour dew than on plants receiving the 18-hour dew period without the intervening rain.** In field studies by Panzer et al.,*** plants of three varieties were inoculated before and after a rain on the same day and the number of lesions that developed on plants inoculated before rain was less than the number on plants inoculated after rain. In field studies, made by Rorie¹⁹ to quantitate the factors influencing initial infection, he found that ". . . generally, in those cases when there was a hard or relatively heavy rain shortly after inoculation, some lesions were produced but not at the level that was expected with a heavy dew having a duration of comparable length; . . . there was an insufficient number of cases when inoculations were preceded by rain to establish any trends of the effects of this rainfall on lesion production." The workers who did the last three studies all suggested that the low number of lesions observed on plants inoculated before rain was due to physical washing of the spores off the leaves. This is the most obvious suggestion, but since there are no experiments in which spore counts on leaves were actually made before and after rain, it seems to need verification. The important point is that the effect of rain is real; whether rain actually removes spores or not, some factor related to rains occurring after artificial inoculations causes a reduced lesion count.

* Pages 173 through 194.

** USABL Notebook CD 3814.

*** Panzer, J.D.; Tullis, E.C.; and Van Arsdel, E.P. "Epiphytology of rice blast, Piricularia oryzae," Unpublished manuscript on work performed 1955 through 1957, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

(J) In Rorie's work,¹⁹ the other natural factor that might influence tenacity, wind, apparently did not do so between the ranges of zero and 24 miles per hour. At least there was no statistical correlation between wind speed at inoculation and the number of lesions produced.

B. (U) DISPERSAL BY WATER

(U) Since rice is usually grown in flooded fields and since irrigation water in many places flows from field to field, or from a common source into several fields, it is intriguing to suppose that *P. oryzae* spores might be carried by irrigation water and that such water is a possible means of dispersal, especially for the primary disease cycle.

(U) Sueda²⁴ states that conidia remain viable for "pretty long" (time not given) when floating on the surface of irrigation water in the rice field, but that they die within two weeks after they sink under the surface. Andersen et al.²⁶ concluded that conidia would not survive in irrigation water for more than 24 hours. If we consider how a spore on or in irrigation water might arrive at a suitable infection court, the problem is further complicated; splashing from rain drops or the dipping of leaves into the water under pressure from the wind are two means within the realm of possibility. Dispersal by water has not been documented and seems unlikely to account for any but a very low number of inoculations.

C. (U) MAMMALS, BIRDS, INSECTS, AND MAN

(U) Any animal could conceivably be an agent of inoculation. The writer has seen large mammals in rice fields, but water buffaloes and horses are usually found in fields before planting rather than afterward. Birds are very frequent visitors, but usually only near harvest time. Moving insects such as leaf hoppers are common during the growing season and could brush spores from a lesion and transport them, but insects have never been tested as possible carriers.

(U) Man remains an unknown quantity. In the Orient it is common to see people transplanting, weeding, spreading fertilizer, and doing other farming operations in which their hands, bodies and clothing comes in contact with plants. In 1963, Dahlke* had a sprinkler irrigation system moved from a heavily infected field to a healthy and younger field. It is possible that the men moving the equipment spread the disease to the younger field. At the same place, a small planting of the variety C.I. 8970P, known to be susceptible to races of the fungus infecting that variety

* Dahlke, C.R. Unpublished data on 1963 work, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

in three other fields up wind of the planting and several miles away, remained free of the disease. In th' planting, care was taken so that men worked in it early in the m^{orn}ing with clean clothes, and before they had visited any other field.

CONFIDENTIAL

39

IV. (C) INCUBATION

(U) After spores arrive at the infection courts, several conditions must be met before infection is established. The inoculum must be viable, and several factors affect how long it will remain viable after reaching the infection court. Not every viable spore that reaches an infection court will cause an infection. Even under presumably ideal conditions a certain intensity of inoculum is required. Various conditions including moisture, temperature, and the time required for the establishment of infection have been studied in the laboratory and related to what happens in the field. These conditions have also been studied directly in the field.

A. (U) INFECTIVITY OF INOCULUM

(U) Literature that deals with this problem is lacking. It seems generally assumed that spores produced on lesions are nearly 100% viable if placed in a suitable environment for germination soon after they are formed. The range of environmental conditions suitable for spore formation is similar to that for germination, and spores occasionally germinate while still attached to their conidiophore.

(U) Although viability usually is measured by germination tests, and infectivity is inferred, in our opinion a more reliable index would be the percentage of spores forming appressoria. H. Suzuki* has a number of isolates that are characterized by either their lack of or the kind of appressorial formation following germination. Pathogenicity of his isolates usually increases as the elaborateness of the appressoria increases, and those isolates whose spores germinate but fail to form appressoria do not infect. (He maintains his cultures by serial transfer to agar, and not by repeated inoculation to and re-isolation from plants.)

(U) With artificially, mass-produced spores, germination is less than 100% and usually ranges from 60 to 80%. The writer has the impression from the few of these isolates with which he has worked that they vary with respect to the percentage of those spores germinating that form appressoria. In the past, quantitative work with this kind of spore preparation has depended upon adjusting the amount used to allow for the germination percentage. The percentage of appressorial formation in water drops on glass slides or on agar plates might prove to be a better standard for adjusting the number of spores used in an experiment if the investigator wants a measure of the infectivity of inoculum. A great deal more work on appressorial formation per se, and on the conditions influencing it, needs to be done before making this assumption.

* Personal communication.

CONFIDENTIAL

B. (U) INTENSITY OF INOCULUM IN INFECTION COURTS

(U) Using a settling tower to make quantitative inoculations, Kahn²¹ found that it took, on the average, 25 spores to form four appressoria to produce one lesion. All counts were made per square centimeter of leaf tissue.

(U) Experiments in the field using different rates of inoculum have been performed by several workers. Although the precise intensity of inoculum in the infection courts was not measured, one can infer from the number of lesions resulting that the number of spores deposited on leaves was greater at the higher rates of inoculum.

(U) Panzer et al.* inoculated four varieties at daily intervals with inoculum calculated to deposit spores on small, confined plots at rates of 0.2, 2, 20, and 90 grams per acre. The percentage of inoculations that resulted in at least one lesion per foot of row was always higher at the 90-gram rate than at the other three rates, but the differences in the latter three were not consistent. In the early part of the growing season when some environmental condition(s) may have been close to limiting, two of the four varieties became infected at the high rate. A possible interpretation is that under a given set of conditions one "susceptible" variety may require a greater intensity of inoculum than another "susceptible" variety in order that both varieties may sustain an equal number of infections. Another interpretation is that with higher dosages of inoculum there is a greater likelihood of finding the few spots in the field or plot where moisture remains long enough to permit infection when conditions are marginal.

(U) Rorie¹⁹ also performed a series of daily inoculations. Plots of one variety were inoculated at rates of 10, 1.0, 0.1, and zero grams of viable spores per acre. The rate of inoculum used was always highly significant with respect to the number of lesions produced, and as shown by brief summary of his data (Table 6), the number of lesions increased roughly in proportion to the amount of inoculum landing on infection courts.

(U) From the standpoint of initiating epiphytotics in the field, the minimum intensity of inoculum needed under specified conditions is not known. Allison in 1961** inoculated comparable fields at different rates and measured yield reductions at the end of the resulting epiphytotics. He inoculated four fields from a line source, two with isolate 640 and two with isolate 844a. Either 0.5 or 5 grams of each

* Panzer, J.D.; Tullis, E.C.; and Van Arsdel, E.P. "Epiphytology of rice blast, Piricularia oryzae," Unpublished manuscript on work performed 1955 through 1957, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

** Allison, W.H. Unpublished data and the associated Analyses 5219 (28 Mar 1962), 5312 (17 May 1962), and 5744 (16 Apr 1963). Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

41

TABLE 6. (U) LESIONS PER PLANT RESULTING FROM INOCULATIONS,
BEAUMONT, TEXAS, 1959^a/ (U)

Rates of Inoculum, Grams Per Acre Viable Spores	Average Number of Lesions Per Plant					
	Plot I (N=30) ^c /	Plot II (N=30)	Plot III (N=26)	Plot IV (N=24)	Plot V (N=24)	Plot VI (N=20)
0.0 ^b /	0.009	0.077	0.429	0.045	0.402	0.151
0.1	0.116	0.137	0.456	0.072	0.488	0.164
1.0	0.682	0.699	0.757	0.157	1.116	0.440
10.0	7.034	7.310	4.542	1.448	5.900	2.737

a. After data of Rorie and Asai.¹⁹

b. Lesions resulting from this rate are due to natural infection.

c. N equals average number of subsubplots involved.

isolate were released and dosages over each field were measured. With isolate 640 there was no effect of dosage at initial inoculation on yield, and with isolate 844a the lower yield occurred in the field inoculated at the lower dosage level. Assuming no differences among fields, this result indicates that dosage at the initial inoculation influences the number of lesions that appear at the beginning of the epiphytotic, but that its influence is negligible after a few disease cycles during which the number of spores produced within the field have a far greater effect on the rate of buildup than do the comparatively few spores involved at the time of inoculation. Analysis of Allison's data indicates that there were differences among fields, but the above idea about the lack of relationship between initial dosage and yield reduction may be valid anyway.

(U) The problem of experimentally initiating epiphytotics reverts to the minimum amount of inoculum required to give the concentration of spores in the air that will result in the intensity in the infection court needed to produce lesions. Both Allison and the senior author attempted to measure this minimum amount by using a portable wind tunnel that could be set over small plots of rice in the field. Measured amounts of inoculum were released in front of the tunnel and a fan then pulled the spores over the rice before the inoculum was removed from the air by a filter placed in back of the tunnel. Rotobars set at several locations inside the tunnel showed that dosages varied widely from place to place and lesion counts were very erratic. Neither set of data was amenable to analysis.

CONFIDENTIAL

CONFIDENTIAL

C. (C) CONDITIONS REQUIRED FOR INCUBATION

(U) This is one of the most extensively investigated areas in rice blast research.

(U) Weintraub et al.³⁵ found that germination begins about one hour after spores are seeded on agar and that the contents of the agar may affect the rate of germination after that time. Rice polish, for example, apparently contains a substance(s) that stimulates germination. A similar substance, or at any rate a stimulator, is also present in the dew and guttation water that collects on rice leaves.³⁵ Kawamura and Ono (cited by Kozaka^{5*}) found that dew contained a stimulator of both germination and appressorial development. They further observed that dew on the surfaces of plants receiving high amounts of nitrogen fertilizer contained more of the stimulator.

(U) Since the spores have three cells, it is of academic interest to know from which cell the germ tube comes. Any of the three cells can germinate but the middle cell rarely does.** Germ tubes are commonly seen coming from either the basal or apical cells, but sometimes both cells will germinate and both will form appressoria. Theoretically, then, it would be possible for one spore to cause two infections more or less simultaneously, but the infections would be so close that they would result in the appearance of one lesion. Many people have counted germination on a percentage basis as a means of getting data on the influence of some nutrient, fungicide, or condition on the fungus, but few workers seem to have recorded from which end(s) the germ tube(s) grew.

(U) The environmental conditions to which spores are subjected prior to being placed in an environment suitable for germination may affect the rate or even final percentage of germination. For example, in one of Asai's** experiments he allowed one set of sporulating lesions to dry and another set to remain in a moist chamber under light so that germination would be inhibited. After 24 hours, the lesion surfaces were pressed onto water agar and percentage germination was observed with time. Data from three replicates are averaged below:

Pre-germination Treatment	Per Cent of Germination at Hour Indicated					
	Start	½	1	2	3	5
Dry	0	2	8	42	61	85
Wet	0	71	92	98	98	

* Pages 421 through 440.

** USABL Notebook CD 3814.

CONFIDENTIAL

CONFIDENTIAL

These data are presented to point out a situation that may be relevant to field studies. Many artificial inoculations in the field have used dry spore material. If this dry material germinates over a longer period of time than it takes for fresh material the conditions of environment needed to establish primary infection will have to occur for a longer time than would be needed to establish infection in the secondary disease cycles. Means of artificially disseminating spores in the field may also influence germination, e.g., the use of dusters can lower, very slightly, the percentage germination.*

(U) In Sections II, B, 2 and III, A, 1 we stated that light stimulates sporulation and inhibits the release of spores from their conidiophores. Light also affects germination. Hashioka^{5**} states that direct sunlight suppresses germination and diffuse light reduces the germination percentage by about one-half. Light also inhibits germ tube elongation. Asai* recorded that germ tubes had a negative phototropic response; there was an indication that this response was caused by light of blue wave lengths. Kaha²¹ found that light inhibited the establishment of infection if it was present either during the first six hours or the final ten hours of a 16-hour dew period. As light increased from five to 100 foot candles, infection decreased. In his experiments, light for the 24 hours following a dark 16-hour dew period had no effect on lesion production.

(U) It seems definite that light does inhibit germination and perhaps other phases of the incubation process as well, but the limits of intensity, quality, and time frame within which light exerts its effects in the field are not established. It is certainly not possible to say with any precision how light influences the course of disease buildup in the field.

(U) There is a dearth of literature in English on appressorial development, penetration of the leaf surface by infection hyphae, and development of the fungus within the leaf tissue. It may not be necessary to know the details of these processes for the purposes of epiphytology. Kahn²¹ found that appressoria formed in six hours at 80 F, but the additional length of time required to establish infection after this was apparently two to four hours. He also found that as many appressoria formed on resistant as on susceptible varieties. Sadasivan^{5***} found that fewer of the spores (60%) germinating on plant grown at high night temperature formed appressoria than did spores (85%) germinating on plants grown at low night temperatures. His temperatures are not specifically stated, but from the context of his data they were probably 20 and 30 C. Sadasivan's findings are somewhat divergent from Kahn's because a susceptible variety grown at high night temperature is apparently less susceptible than if it is grown at lower temperatures.

* USABL Notebook CD 3814.

** Pages 153 through 162.

*** Pages 163 through 172.

CONFIDENTIAL

CONFIDENTIAL

(U) A great deal of information about the conditions under which incubation and infection will occur has been obtained in the laboratory and field. This information has been obtained without knowledge or concern for the details of germination, appressorial formation, and penetration. The assumption in these experiments is that if lesions appear then conditions were favorable and the incubation period was sufficiently long. Furthermore, it is assumed that the more lesions that appear, the more favorable was some factor(s) of time and/or environment during the incubation.

(U) The three factors during an incubation period that seem to have the most important bearing on the number of lesions resulting are temperature, dew, and time. The last two variables are usually combined and are thought of as a dew period. From laboratory experiments, the number of lesions resulting from exposure to a given dew period increases with temperature ranging from 60 to 95 F, although a maximum number of infections usually occur between 80 and 85 F.³³ In later work, Kahn³¹ found that although infections decrease above 85 F, the number of appressoria formed increase. This indicated to him that there was an inhibitory action of the higher temperatures on the infection hyphae. On the other hand, if temperature within this range is kept constant, more infections will result as the length of the dew period is increased between 8 and 24 hours.

(U) From experiments reported by Kahn and by others, it is possible to construct a curve indicating approximately the set of conditions of temperature and dew period that would be minimal for the establishment of infection (Figure 1). The analysis* that enabled this curve to be derived is contained in the Appendix. Every point in this figure comes from data indicating the conditions pertaining to the establishment of the least number of infections in any one experiment. These points are, of course, dependent on the ability of the investigator to detect lesions. A more accurate curve could have been computed if a person could see a fractional lesion and if more plants and more spores had been used as well as shorter intervals of time and temperature. As it stands, Figure 1 merely approaches the minimum conditions. Curves indicating the 95% confidence limits of the minimum curve are also shown.

* Jones, M.W. Analysis 6143, Biomathematics Division, U.S. Army Biological Laboratories, Frederick, Maryland. 10 April 1964.

CONFIDENTIAL

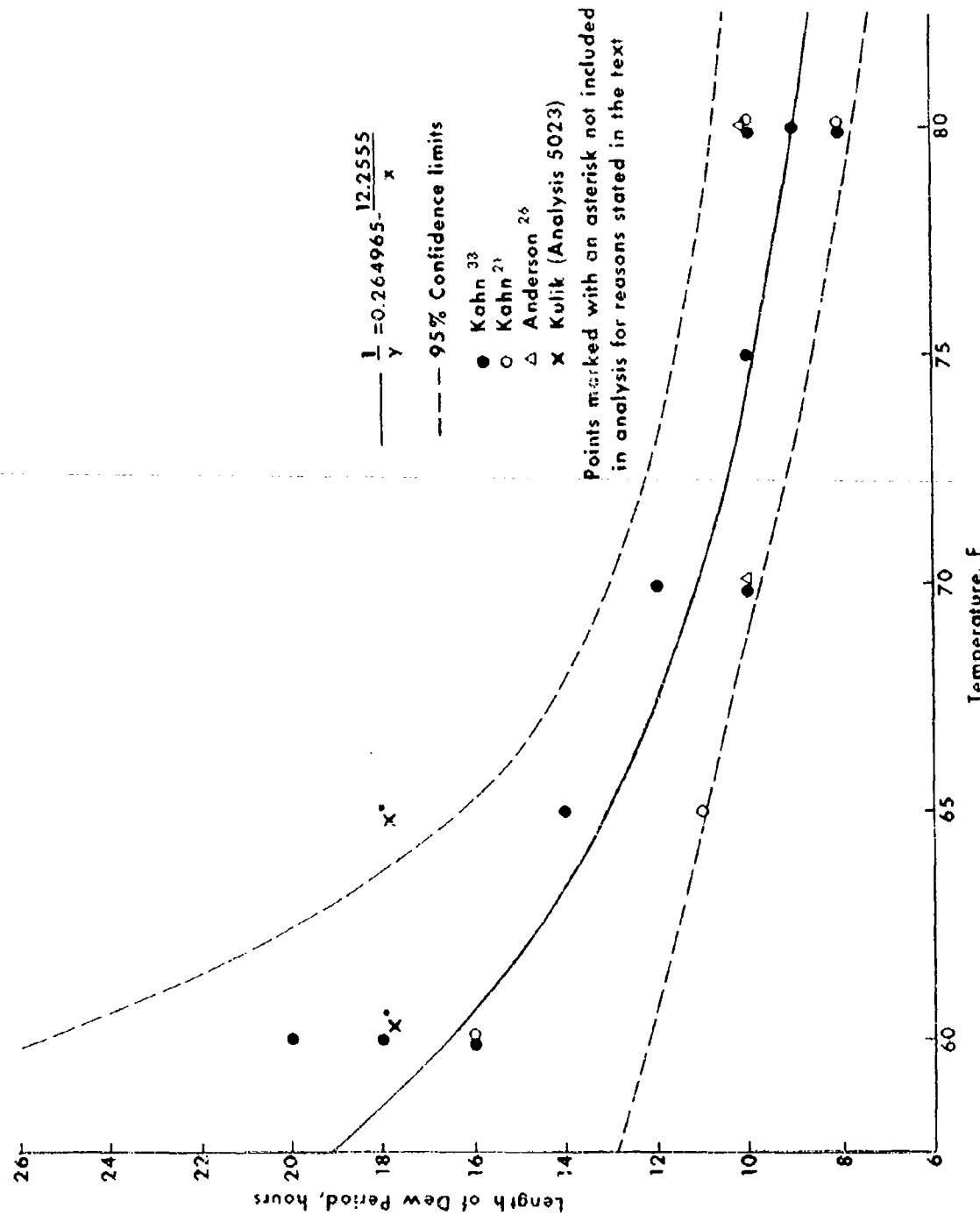


Figure 1. (U) Minimum Length of Dew Period Required for Infection at Various Temperatures. (Ü)

CONFIDENTIAL

(U) Comments about some of the points in Figure 1 are needed. The point at 55 F and 20 hours represents one lesion and is apparently a rare event. This observation was made during some of the early work by Kahn. Later he found that a few panicle infections could be produced at this temperature and stated that no leaf infections could be formed at 55 F. The observation at 55 F was so rare that he had forgotten it. This point was not included in deriving the curve. Some of Kulik's experiments* were done between temperatures of 60 and 65 F. Because so few people have done as many inoculations and held plants at these temperatures, his points are plotted as a matter of interest, but were not used for deriving the curve.

(U) Minimum conditions of temperature and length of dew period (limits) at which infection can occur appear to exist. Theoretically (Appendix), these are 46.25 F regardless of length of dew period and 3.78 hours of dew regardless of temperature. In practice, as 60 F is approached from a higher temperature, the likelihood of infection becomes less until below 60 F the probability of obtaining infections becomes low, in both laboratory and field, no matter what the length of dew period; the 16- to 20-hour dew periods required at this temperature are seldom found in the field. As an 8-hour dew period is approached from longer periods the probability of infection becomes less and in periods shorter than 8 hours the probability of infection becomes very slight, regardless of temperature. In our view, in epiphytology we ought not become preoccupied with rare events and with events whose probability of occurrence is low. Epiphytotics occur when conditions are such that events, especially infections, occur in the millions!

(U) Several races and rice varieties were used in the tests from which the curve in Figure 1 was computed. This may explain some of the variation in the value of either parameter when the other is held constant. Indeed, from Kulik's data* it seems that pathogenicity of isolates, on the basis of the geometric mean number of lesions, depended on the length of dew period. "For the 12-hour dew period . . . races 1, 7, 8, 9, and 13 were the most pathogenic, races 5 and 6 the least pathogenic, while other races were intermediate. . . . At the 18-hour dew period, races 1, 3, and 8 were the most pathogenic, race 4 the least pathogenic, with other races being intermediate." Kulik's inoculations were made on 24 varieties and the analysis was performed on data from incubation temperatures held at two ranges, 75 to 80 F and 85 to 90 F.

* Kulik, M.M. Unpublished data and the associated Analysis 5023 (10 Jan 1962), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

CONFIDENTIAL

47

(U) The curve in Figure 1 suggests a hypothesis concerning the epiphytology of the rice blast disease: The number of infections caused by a given amount of inoculum and occurring in the field on a given night will be greater if the conditions during incubation are described by some point above the curve and lesser when the point is below the curve. There is a corollary: The rate of disease buildup of an epiphytic will be greater the higher the percentage of nights (incubation periods) during buildup that have conditions of temperature and dew described by some point that lies above the curve.

(C) Field data are available for testing this hypothesis and its corollary. The points used to derive the curve were obtained from experiments in which temperatures were constant. So, to test the hypothesis it is necessary to assume that the fluctuation in night temperature is not great (which is generally true in the semitropical and tropical rice-growing areas) and to use the average temperature either during the known dew period or during the time when dew is most likely to occur. In those instances where only minimum temperatures were recorded it would be reasonable to use a value several degrees higher, depending on experience in the geographic location concerned. In the senior author's experience, and on the basis of detailed hygrothermograph records from Florida and Okinawa, the average night temperature during rice-growing seasons between the hours of 2000 and 0800 was 2 F higher than the minimum temperature. Actual measurements of the difference ranged from 0 to 6 F, but were usually from 1 to 3 F. From Kulik's experience in Texas during the spring and early summer, the temperature difference between 2000 and 0600 hours was 3.7 F with a range of 0 to 10.5 F, although most differences were in the range of 1 to 5 F; the correlation ($r = +0.96$) between minimum and average night temperatures is excellent. Marchetti observed that the difference between average night temperature and the minimum in Louisiana was 4 ± 3 F when minimums were in the 60's, but that as the weather warmed up and the minimums went into the 70's the difference was 2.2 ± 1 F.

(U) Three sets of field data*, **,¹⁹ are available for artificial inoculations made on small plots during a series of nights. The resulting lesions were counted soon after they appeared, and their numbers were taken as an indication of how favorable the conditions had been during the night following inoculation. These data should give some indication of the validity of the hypothesis stated above.

(U) Unfortunately, Parzer's data^{*} are incomplete. Although daily records of infection and temperature are available, daily records of moisture have been lost over the years. This provides a strong argument for either keeping permanent notebooks in the field or of writing reports that contain detailed appendixes.

* Panzer, J.D., Tullis, E.C., and Var Arnsel, E.P. "Epiphytology of rice blast, Piricularia oryzae," Unpublished manuscript 1955 through 1957, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

** Kulik, M.M. Unpublished data and the associated Analyses 4451 (5 Dec 1960) and 4447 (20 Sept 1960), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

CONFIDENTIAL

(U) Rorie¹⁹ made a series of 77 daily field inoculations at Beaumont, Texas, to discover how various weather conditions and other factors influenced the establishment of infection. Plots were inoculated with a spore suspension at rates of 10, 1, 0.1, and 0 grams of viable spores per acre and there were two replications. The length and intensity of the dew period and the amount of leaf area at the time of inoculation significantly affected the number of lesions resulting; estimating equations derived from analysis of the data are given in Section IX. If the curve in Figure 1 had been used to predict whether infection would have occurred on any given night with the highest rate of inoculum, the yes-or-no prediction would have been correct for 77% of the nights (Figure 2). No lesions were formed on eight nights when conditions of temperature and dew were those described by some point above the lower 95% confidence limit of the curve; lesions did form on 10 nights described by some point below the lower limit of the curve. To prepare Figure 2, lesion counts for the two plots inoculated at the highest rate were averaged and the number was rounded off to the nearest whole number. Lesion counts less than 0.5 were usually put on the Figure as zero, especially if they appeared not to differ from count on the uninoculated checks. In instances where there was an apparent difference between inoculated and check plots, yet very low counts, a question mark is placed beside the number.

(U) Kulik's data* were also obtained from daily inoculation studies at Beaumont, Texas. Kulik used inoculum at the rate of ten grams per acre applied as a dust to two rice varieties. The Colusa variety was apparently less susceptible than C.I. 8970. It became infected on fewer evenings and to a lesser degree than C.I. 8970. The length of dew period, period of 100% RH, amount of daily rainfall, and leaf area at the time of inoculation affected the number of lesions resulting from his inoculations. Estimating equations are given in Section IX. When the number of lesions resulting from a given inoculation of C.I. 8970 are placed on a plot of mean night temperature (2000 to 0600) versus length of dew period (Figure 3), infection is shown to have occurred on only two nights when points fell below the lower 95% confidence limit of the curve (Figure 1). There were seven evenings when no infections occurred even though points describing conditions on these evenings fell above the lower limit of the curve. For the total of 50 nights, a yes-or-no prediction of infection would have been correct 82% of the time.

* Kulik, M.M. Unpublished data and the associated Analyses 4451 (5 Dec 1960) and 4447 (20 Sept 1960), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

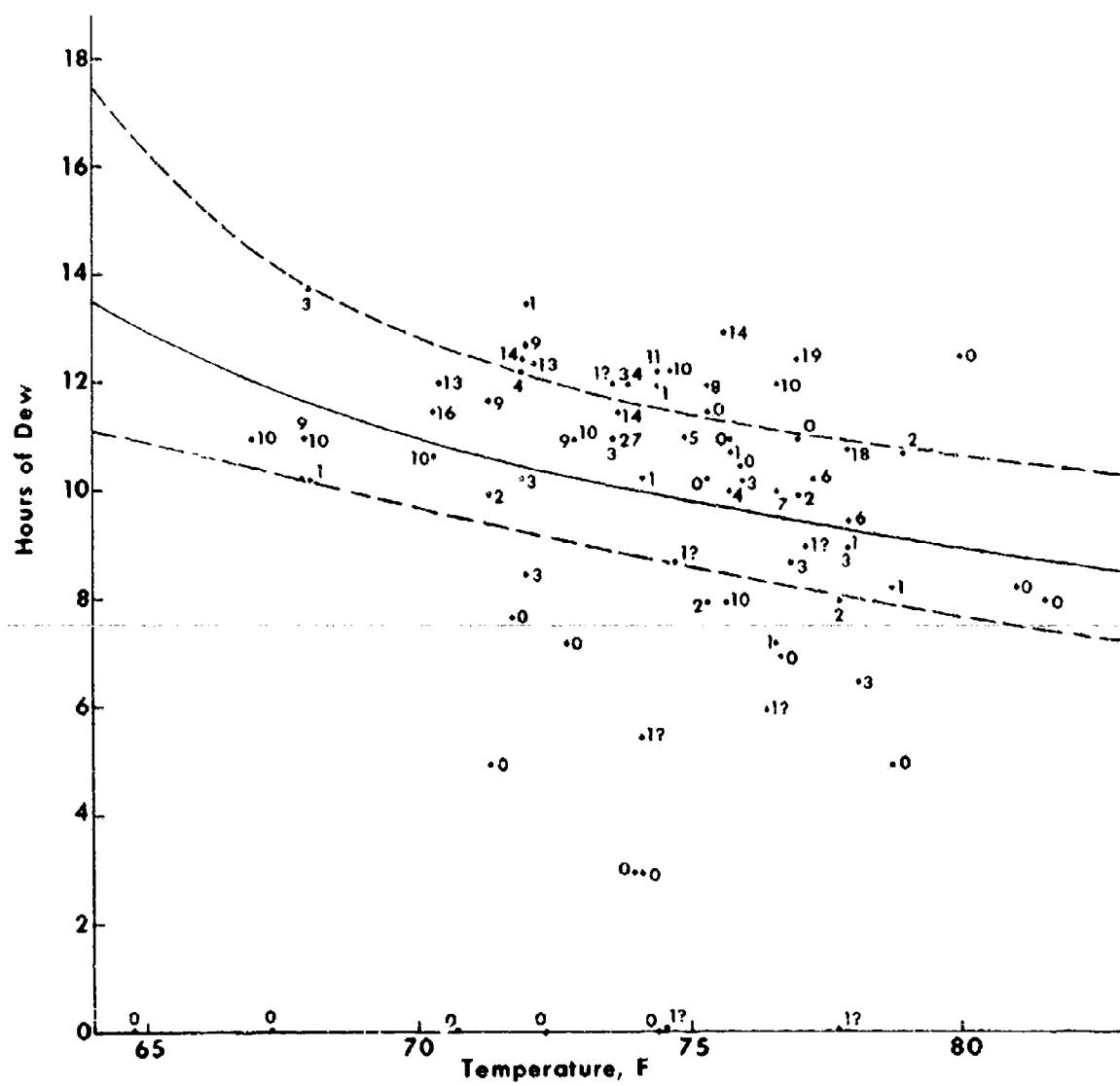


Figure 2. (U) Minimum Length of Dew Period Required for Infection, as a Function of Temperature. (U)

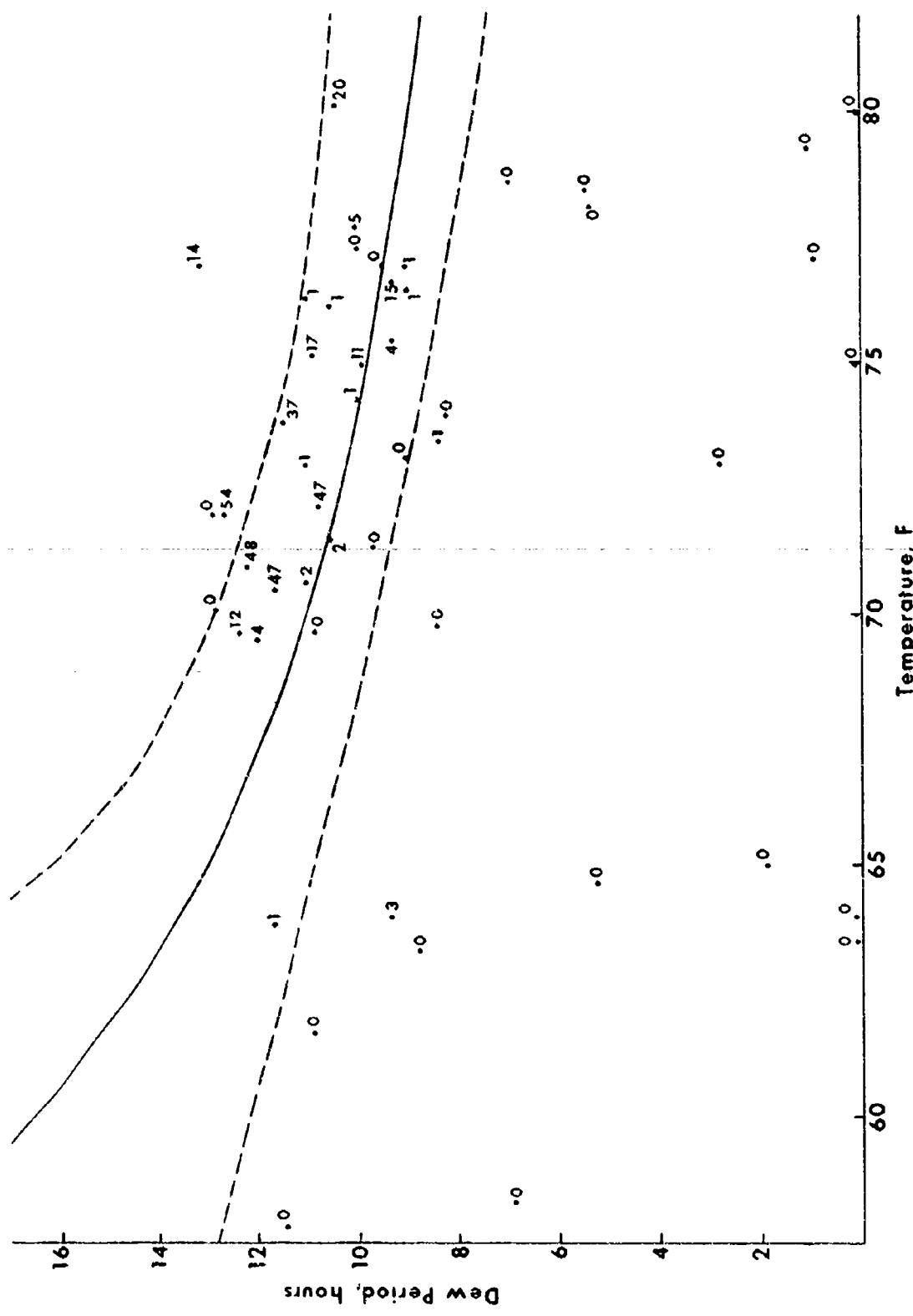


Figure 3. (U) Number of Lesions per 100 Square Centimeters Obtained from Daily Inoculations from Unpublished Field Data of Kulik. Placed on Points Indicating the Average Night Temperature (2000 to 0600 hours) and Dew Period During Incubation and Against a Background Indicating the Minimal Conditions Found in Laboratory Experiments. (U)

CONFIDENTIAL

51

(U) Statistical analysis of Rerie's and Kulik's data failed to show a significant effect of temperature, the most likely reason being that where they worked night temperatures during the rice-growing season did not vary widely and were generally between 65 and 80 F.

(U) There is an over-all trend for the data in Figures 2 and 3 to fit the hypothesis suggested. Lesion counts tend to be higher when conditions of temperature and dew are described by points above the curve rather than below it. The use of Figure 1 by itself for prediction purposes is certainly not justified. Many other factors besides temperature and dew would have to be considered by the forecaster, e.g., the age and growth stages of the plants, the nitrogen fertilization practices, and the amount of inoculum in the air. We believe, however, that Figure 1 can be a useful aid in forecasting the likelihood of infection occurring on a given night.

(U) Now let us examine the corollary that the rate of disease buildup of an epiphytotic will be greater the higher the percentage of nights (incubation periods) during buildup that have conditions of temperature and dew described by some point that lies above the curve in Figure 1. For this purpose, the data from 24 epiphytotics, most of which the senior author observed personally, were examined. The dates during which there was a rapid climb in lesion numbers were determined for each epiphytotic and six days (the latent period under temperature ranges usually found in the field) were subtracted from each end of the period to get the probable dates of incubation. These probable incubation periods were then examined by using Figure 1 with respect to average night temperature and dew period. Lesion counts (per foot of row or per hill) were used to determine several measures of disease increase. The "amount of disease increase" was obtained by finding the multiple of the first lesion count that would give the final or highest lesion count on the straight-line portion (on a log scale) of the curve describing disease increase. The "average daily increment of disease increase" was found by dividing the "amount of disease increase" by the number of days. The rate of disease increase was calculated by fitting the equation $C_t = C_0 e^{kt}$ to the lesion count data. These measures of disease increase, together with the highest lesion count observed, the lesion count 18 days after the date of the probable first incubation periods, and the yield loss at the end of the epiphytotic were analyzed (Table 7 and Appendix).

(U) The reader should note several things about the data in Table 7. Weather conditions during incubation are not sufficient to tell the whole story, especially if some other factor(s) is limiting. As an example, the data from Paddy 8 in Avon Park refer to three sub-paddies planted at different dates. Sub-padd. 8-1 was the oldest at the time of inoculation and 8-3 was the youngest. Buildup and yield reduc-

CONFIDENTIAL

CONFIDENTIAL

TABLE 7. (C) DATA RELATING TO EPIPHYTOPSIS OF RICE BLAST (U)

Worker and Location		Epihytoptic Disease	Increase on the Leaves	Highest Lesion Count ^{a/}	Percentage of Nights with Weather Favorable for Incubation ^{b/}	Yield loss, per cent ^{c/}	Epihytoptic Reference Number Used in Appendix
Barksdale							
Avon Park, Florida	1960						
Paddy 6-1	18 May-9 Jun	39.9	478.5	69.2		95	1
Paddy 7-1	24 May-15 Jun	36.5	288.6	69.2		67	2
Paddy 7-2	31 May-15 Jun	37.7	234.1	70.0		91	3
Paddy 7-3	31 May-15 Jun	54.5	829.3	70.0		96	4
Paddy 8-1	31 May-21 Jun	30.0	56.0	73.3		84	5
Paddy 8-2	7 Jun-21 Jun	-	-	70.0		43	6
Paddy 8-3	14 Jun-28 Jun	50.1	238.6	72.7		46	7
Paddy 8-4	17 Jun-1 Jul	18.7	30.6	85.7		68	8
Paddy 8-5	17 Jun-1 Jul	35.2	462.6	85.7		95	9
Paddy 8-6	17 Jun-1 Jul	65.6	555.6	85.7		95	10
Allison ^{d/}							
Avon Park, Florida	1961						
Paddy 6, Gulfrose	20 Jun-17 Jul	10.9	70.0	68.0		18	11
Arkrose	20 Jun-17 Jul	34.2	60.0	68.0		47	12
Calusa	20 Jun-17 Jul	-	-	68.0		47	24
Barksdale							
Okinawa	1961						
1st Crop, Shuri	7-28 May	0	0	42.9		0	20
Nago	7-28 May	0	0	55.6		0	21
2nd Crop, Shuri	13-27 Sept	0	0	40.0		0	22
Nago ^{e/}	12-26 Sept	36.3	57.5	46.7		20	13
Ishikawa	10-20 Sept	29.3	28.0	27.2		0	14
Barksdale							
Okinawa	1962						
1st Crop, Shuri ^{f/}	14-29 May	27.7	48.0	56.3		20	15
Nago	17-31 May	0	0	50.0		0	23
Ishikawa	11-31 May	31.5	43.0	35.0		0	16
2nd Crop, Shuri ^{g/}	11-27 Sept	36.1	31.3	75.0		11	17
Nago ^{h/}	20 Aug-18 Sept	26.5	21.0	63.6		18	18
Ishikawai ^{i/}	7-24 Sept	24.6	188.7	61.1		1	19

a. Usually taken as dates when the log number of lesions fell on a straight-line curve, but where little or no disease increase occurred these dates were considered the most likely period for disease to have occurred.

b. "k" is the slope of the plot log number of lesions versus time.

c. Made on the following basis: for Barksdale, lesions per foot of row or per hill; for Allison, lesions per square meter.

d. Probable incubation periods, found by subtracting 6 days from each end of the period of observed exponential disease increase. The average night temperature and dew period for each night was then compared with the curve in Figure 1 and if the point fell above the lower limits (95% confidence level) of the curve, the night was considered to have been favorable for incubation and the establishment of infection.

e. Yield loss usually considered the percentage of panicle blast.

f. Middle planting date.

g. Fujisaka 5 with high nitrogen.

h. Caloto with high nitrogen.

i. Nagomusari with high nitrogen.

CONFIDENTIAL

CONFIDENTIAL

53

tion were greatest in the youngest planting and least in the oldest, in spite of the fact that conditions during incubation for primary and secondary disease cycles were essentially the same in all three plantings. Here, plant age limited susceptibility and its effect was superimposed upon any limitations caused by the weather. Allison's data for 1961 were difficult to use for the analysis, although a sample is included. Allison took lesion counts only three times on the assumption that three points were enough to establish a line or curve describing buildup. Unfortunately, for any one variety and planting date, all three of these points seldom fall on the steep, straight-line portion of the generalized sigmoid growth curve. From the data on buildup in Okinawa, examples were selected chiefly from the most susceptible variety grown at a given location and fertilized with a high rate of nitrogen fertilizer in order to remove, as far as possible, the limits imposed on buildup by varietal resistance and low levels of nitrogen.

(U) In spite of the various limitations of the data, the analysis showed significant correlations (Appendix). The percentage of days with conditions favorable for incubation was correlated with the exponential rate of disease increase ($r = +0.484$) and with the highest lesion count observed ($r = +0.499$). These coefficients were significant at the 95% level, and indicate that the corollary stated previously is valid: disease buildup is greater as the percentage of incubation periods favorable for infection increases.

(U) Other correlations, significant at the 99% level as found by the analysis, associated the per cent yield loss with: (i) the exponential rate of disease increase, $r = +0.705$; (ii) the highest lesion count observed, $r = +0.769$; and (iii) the percentage of days with weather conditions favorable for incubation, $r = +0.749$. Using these three parameters, regression equations were calculated that may be useful for predicting yield loss (Section IX and Appendix). It is interesting to note here, however, that measures of disease on the leaves are correlated with yield loss percentage determined in most of the epiphytotics studied by the percentage of panicles blasted. The percentage of panicle blast is a conservative estimate of yield loss (Section VIII). This may be somewhat puzzling because disease buildups on leaves and on panicles are separated in time by several weeks. What apparently happens is that as panicles emerge they are inoculated with spores produced on old leaf lesions, many of which may be located on dead leaves by the time of panicle emergence. Considerations of this sort digress rather far from the topic of incubation, however.

CONFIDENTIAL

CONFIDENTIAL

(U) There is some question about the ability of spores that land on an infection court to remain viable longer than one day, especially if they are exposed to short dew periods and begin to germinate. From laboratory experiments of Andersen et al.²⁶ it appears that a dew of 8 hours or less followed by drying conditions simulating daytime will reduce by 30 to 50% the number of infections resulting from a suitably long dew period the next evening. Drying for as short a time as one hour during a dew period of nearly optimum length can also reduce the number of infections (Table 8) according to Kahn's data as quoted by Panzer.* Kahn³³ also found in the laboratory that spores on inoculated plants apparently decreased in viability if the plants were not put in an environment suitable for infection to occur, and even if the plants were kept dry for several days. The only field data are from the work of Panzer et al.,* in which there was an indication that if conditions (in this case, temperature) were not favorable on the night of inoculation, the number of infections resulting under favorable conditions the next night would be reduced. This problem of hold-over from one night to the next (and succeeding nights) should receive further study in the field. It is our opinion that hold-over is probably of little or no significance during buildup of an epiphytotic, but it could be of great importance in the initial establishment of disease in a field, especially when artificial inoculations are made.

(U) In order to insure good "take" of artificial inoculations, it seems reasonable that the fields should be inoculated late enough in the afternoon so that (i) the spores will escape any deleterious effect from strong sunlight or any other daytime environment factor, and (ii) the spores will be in the infection courts at the start of the dew period. The only field data available on the most suitable time of day for artificial inoculations come from the work of Panzer et al.* They made inoculations at hourly intervals on four days from 6 PM to 8 AM under conditions where dew remained on plants until about 11 AM. Few if any lesions resulted from inoculations made after 2 AM. This type of experiment should be repeated under a wider range of environmental conditions and especially at that time of year when there are some evenings with limiting conditions.

* Panzer, J.D.; Tullis, E.C.; and Van Arsdel, E.P. "Epiphytology of rice blast, Piricularia oryzae," Unpublished manuscript on work performed 1955 through 1957, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

TABLE 8. (J) REDUCTION OF INFECTION BY DRYING FOR ONE HOUR
AFTER SHORT DEW PERIODS^{a/} (1)

Duration of Initial Dew Exposures, hr	Blast Lesions Per Square Centimeter
½	12.9
1	11.8
2	8.7
3	4.2
4	2.4
5	3.2
Control A (25½ hours continuous dew)	24.1
Control B (19½ hours continuous dew)	23.5

- a. Unpublished data of Kahn and Otten cited in Panzer, J.D.; Tullis, E.C.; and Van Arsdel, E.P. "Epiphytology of rice blast, Piricularia oryzae," Unpublished manuscript on work performed 1955 through 1957, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

(J) Dews in the field vary in intensity and a question arises as to whether a light dew is as favorable as a heavy dew for infection. In laboratory experiments, Kahn³³ found that relative amounts of dew did not influence the number of lesions. He even obtained infection with dew so light that it could not be seen without magnification. Data from the field are conflicting. Rorie¹⁹ found the intensity of dew (on an arbitrary scale of 1 to 8 determined by looking at the width of the trace on a Taylor dew meter plate) highly significant in influencing lesion numbers at all rates of inoculum. In Kulik's work* the following year, intensity of dew (on an arbitrary scale of 1 to 3) did not contribute to the estimate of lesions.

* Kulik, M.M. Unpublished data and the associated Analyses 4451 (5 Dec 1960) and 4447 (20 Sept 1960), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

(U) In work on another disease³⁶ it was found that light occurring near the end of an incubation period would hasten the establishment of infection. It is not known whether this same phenomenon occurs with rice blast. Should it prove true, it would tend to favor epiphytic development of blast in semitropical areas, because dew in those places usually dries off in the mid-morning hours.

(U) In this section, an attempt has been made to examine conditions required for the successful completion of incubation and the establishment of infection in the field. Much of the information has come from laboratory experiments instead of the field. Nevertheless, these laboratory data help to interpret what the worker observes in the field and may help to predict what will happen in the field. As a case in point, field data did fit to some extent the hypothesis and its corollary drawn from laboratory data concerning the conditions of temperature and dew period needed for the completion of incubation and the establishment of infection.

V. (U) INFECTION

A. (U) GENERAL DESCRIPTION OF INFECTION PROCESS

(U) Most detailed information about the infection process at the cellular level within the leaf is in the Japanese literature. A thorough review of this information (even if it were available in English) would probably not increase our understanding of epiphytotics. Briefly, after the fungus enters the leaf, it ramifies until at the end of five to seven days symptoms can be seen. If plants are kept at a constant temperature of 80 F, symptoms appear in four days at 60 F, in eight days. The temperature or relative humidity during this period does not affect the number of lesions that eventually appear. Temperature does affect the growth of lesions; when plants are kept at 80 F the lesions will become about twice as long at the end of one week as those on plants kept at 60 F.²¹

(U) The end of a particular disease cycle occurs with the death of the leaf bearing a lesion. The leaf may die because of the number or placement of lesions on it, or simply because of the natural attrition that occurs with leaves that have had tillers formed in their axils. That portion of the dead leaf tissue that contains mycelia can still support a small amount of sporulation, however, and the senior author has observed that these portions form a few spores as long as two months after death of the leaf. (As pointed out earlier, refuse piles of infected plants serve as overwintering reservoirs of the fungus in Japan.) Spores derived from dead leaves probably contribute very little to the buildup phase of an epiphytotic, since their numbers are so few by comparison with numbers of spores formed on fresh lesions. They might play a highly significant role as the inoculum for panicle infections, however, especially in those varieties that have a lapse of several weeks between their tillering stage and panicle emergence.

B. (U) SECONDARY CYCLES

1. (U) Buildup

(U) The number of secondary cycles that occur on leaves during an epiphytotic is phenomenal. Information on the amount of buildup has already been presented for a number of examples (Table 7), and more recent examples are available from the data of Dahlke* and the senior author.** Van der Plank³⁷ has pointed out that a useful calculation about an epiphytotic is the rate of disease increase prior to the onset of the epiphytotic (arbitrarily set at the one or five per cent

* Dahlke, W.R. Unpublished data and the associated Analysis 6433 (5 Aug 1964), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

** Barksdale, E.H. Unpublished data from spread studies, 1963-1964, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

level of disease severity). For most field studies to date, this calculation is difficult if not impossible, sometimes for lack of data on the very early stages and, in many instances, because the disease becomes severe so quickly that only one observation was possible before disease severity became greater than one or five per cent. It may be that in some of the field studies involving artificial inoculations, natural or endemic inoculum is contributing to early increase more than the observer is able to detect.

2. (U) Panicle Infections

(U) Panicle infections provide a rather special kind of secondary cycle in that they are more than likely the terminal disease cycles of the epiphytotic. Furthermore, this kind of infection is of great importance because one neck lesion can effectively eliminate the yield from a tiller, although the number of leaf lesions required to destroy a tiller would be in the hundreds if not thousands. The spores causing panicle infections probably come from leaf lesions, and in many Japanese prefectures leaf blast is used as an index of the outbreak of neck blast. The correlation ($r = 0.90$) in Miyazaki Prefecture is very close between leaf blast during the last ten days of July and neck blast occurrence. In two other Prefectures, leaf blast on the flag leaf is considered the important index.^{5*}

(U) From experiments of Ou,** it appears that panicle resistance or susceptibility is the same as that determined on leaves of the same variety for a given race of the pathogen. Willis** has pointed out some possible exceptions to us. With respect to what may happen in the field it is important that this be resolved.

(U) The minimum moisture period necessary for infection of panicles is about nine to ten hours. With dew periods of 16 to 18 hours, panicle infections will occur at all temperatures between 55 and 85° F.²¹

(U) Resistance of panicles tends to increase with age, i.e., with time after emergence, according to Hashioka.¹⁵ His data (Table 9) do show this trend, but we cannot understand why percentages of infected panicles were not higher when inoculations were made just before or just after panicle emergence.

* Pages 173 through 194.

** Personal communication.

TABLE 9. (U) CHANGE OF RESISTANCE OF NECKS OF PANICLES WITH AGE^{1*} (U)

Days After Heading	Per Cent Infection Resulting From Incubation		
	Experiment 1a/	Experiment 2b/	Experiment 3c/
-3d/		38.0	68.0
-2	55.6	59.0	45.0
-1	56.4	36.0	72.0
0	25.8	40.0	38.5
1	11.5	34.0	19.4
2	7.8	15.0	13.2
3	8.5	40.0	25.1
4	14.1	18.0	8.0
5	12.9	22.0	15.3
6	12.5	10.0	7.4
7	11.9	8.0	14.0
8	11.1	18.0	14.8
9	11.9	22.1	15.4
10	16.4	10.8	4.0
11	10.8	5.0	9.0
12	15.8	8.0	0
13	7.8	2.0	10.3
14	9.0	0	3.5
15	5.0	0	0
16	0	7.0	4.0

a. Inoculation, May 24, 1943; observation, June 8.

b. Inoculation, Aug 9, 1943; observation, Aug 24.

c. Inoculation, Aug 20, 1943; observation, Sept 5.

d. Minus sign indicates that panicle was still inside sheath; inoculations were made by unfolding the sheath to expose the necks.

(U) Some field data indicate that panicles may be more susceptible when high levels of nitrogen are used than when low levels are used.^{1, **, 38} This association is not proved, however, because in the field, plants grown with high levels of nitrogen usually have high numbers of leaf lesions and there is the possibility that the greater number of observed panicle infections is simply due to a locally high inoculum potential in the vicinity of these panicles.

* Barksdale, T.H. Unpublished data for 1961 and 1962, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

** Willis, C.M. Unpublished data and the associated Analysis 6144 (19 Feb 1964), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

(U) Ono and Suzuki³⁹ attempted to discover when panicles became naturally infected in the field by covering tillers in various stages of development with paper bags. If tillers were covered before panicle emergence no neck blast developed, but if they were covered one day after heading, then 70 to 90% neck blast occurred. Symptoms of neck blast on the three varieties they studied appeared six to ten days after heading in the field and the number of infected panicles increased only slightly thereafter. In the absence of controls, we cannot be sure that paper bags applied after heading did not act like moist chambers in view of the very high percentage of neck blast developing. Their test does give some indication about the time of natural inoculation, however.

(U) Marchetti* made artificial inoculations in the field at different heading stages and found, as did Ono and Suzuki, that panicles are more likely to be inoculated after they emerge than before (Table 10). The 1.5% infection resulting from inoculating tillers whose panicles were still in the boot may be an indication of a low level of natural inoculum.

(U) Allison** made observations on the per cent of neck blast in two varieties on three or four dates after heading (Table 11). Because his samples for each observation were small and selected at random, an anomalous figure occasionally appears, but there is a definite trend for the observable percentage of neck blast to be greater as time elapses after panicle emergence. Similar data for 12 varieties were obtained by Dahlke,*** but he made only two observations in time (Table 12). His data show that a substantial portion of the neck blast seen at harvest is already present 7 days after the average heading date for a given variety.

* Marchetti, M.A. Unpublished data and the associated Analyses 6171 (13 Feb 1964) and 6269 (15 July 1964), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

** Allison, W.H. Unpublished data and the associated Analysis 5744 (16 Apr 1964), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

*** Dahlke, G.R. Unpublished data and the associated Analysis 6433 (5 Aug 1964), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

TABLE 10. PANICLE INFECTION^{a/} ON VARIETY VEGOLD AS INFLUENCED BY STAGE OF EXSERTION AT TIME OF INOCULATION (U)

Growth Stage	Number of Panicles	Per cent of Panicles Infected
Panicles fully exserted	76	65.8
Neck of panicle $\frac{1}{2}$ inch above to $\frac{1}{2}$ inch below junction of flag leaf sheath and blade.	63	55.6
Flag leaf sheath split and a small portion of panicle exserted.	198	12.1
Panicles completely within boot.	272	1.5

a. Inoculation made on 20 September and final lesion counts made in October 1963.

* Marchetti, M.A. Unpublished data for 1963, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

TABLE 11. (U) PER CENT OF PANICLE BLAST ON TWO RICE VARIETIES AT PROGRESSIVE DATES AFTER HEADING* (U)

Number of Days After Average Heading Date	Planting of 14 May			Planting of 16 May			Planting of 31 May		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Calusa									
0	- ^{a/}	7.7	15.8	-	-	31.8	-	5.3	33.3
3	5.0	-	-	12.5	21.0	-	24.3	-	-
8	-	17.1	10.0	-	-	29.0	-	23.1	38.2
10	-	-	-	-	-	-	26.6	-	-
11	16.7	-	-	21.4	26.6	-	-	-	-
15	-	5	19.0	-	-	41.4	24.2	32.0	45.7
18	20.0	-	-	24.2	22.2	-	-	-	-
20	-	27.3	25.0	-	-	37.5	-	35.4	25.6
23	25.0	-	-	22.2	33.3	-	-	-	-
Arkansas									
2	-	-	-	-	-	-	0	5.0	0
4	0	4.5	4.8	0	0	0	-	-	-
8	-	-	-	-	-	-	21.4	29.4	21.4
9	15.8	25.0	18.2	24.1	8.3	22.7	-	-	-
14	-	-	-	-	-	-	20.0	14.3	11.1
15	25.0	27.8	31.8	26.1	26.7	30.0	-	-	-
21	26.6	18.7	40.0	31.2	23.5	17.6	-	-	-

a. No reading.

* Allison W.F. Unpublished data for 1961, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

TABLE 12. (U) PER CENT OF NECK BLAST OCCURRING SEVEN DAYS AFTER HEADING AND AT HARVEST* (U)

Variety	Per Cent Neck Blast ^{a/}		Average Number of Days Between Observations
	7 Days After Heading	At Harvest	
Colusa	45	61	14
Taichung Native 1 ^{b/}	0	0	12
Toro	54	71	16
Toro + fungicide	25	16	15
Arkrose	38	67	21
C.I. 8970	29	70	16
Culfrose	0	0	15
Fujisaka 5 ^{c/}	89	89	19
Yi Kon Pou ^{b/}	3	7	13
Taichu 65 ^{c/}	2	2	20
C.I. 5309	0	1	25
Nato ^{b/}	32	81	16
Rexoro ^{b/}	21	39	5

* Dahlke, G.R. Unpublished data for 1963, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

a. Average of 16 observations (4 replications in each of 4 fields).

b. Average of 12 observations.

c. Average of 8 observations.

Although samples of Dahlke's test were larger than Allison's samples, a few anomalous percentages still appear in Table 12. Another set of data on the percentage of panicle blast was obtained by Willis,* who found that the greatest number of infections usually were found near harvest. The

* Willis, G.M. Unpublished data and the associated Analysis 6144 (13 Feb 1964), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

counts for one plot of the Tschung Special 6 variety, however, were lower near harvest than in the week before because some infected panicles had actually dropped off. The increase in the percentage of panicle blast with time observed by all three of these workers indicates that those who wish to use this percentage as a measure of yield reduction (Section VIII) should collect more than one set of data from a field.

(U) Matsuo¹⁰ presents some data from the work of Cho on the cytohistology of panicle and grain development. Anthesis begins on the day of emergence. Pollination occurs on the day a flower opens, and 7 days later the embryo is completely formed. After about 7 days the grain has reached its maximum length, its near-maximum breadth after 9 days, and its near-maximum thickness after 12 days. Rapid increase in weight occurs up to the 9th or 10th day following anthesis. If infection by Piricularia is to prevent an acceptable, edible grain from being formed, it seems probable from the times stated above that infection should occur in about the first 10 days. Since individual flowers on a panicle may bloom over a period of several days, a neck infection ought to occur within about 2 weeks after emergence to reduce the yielding ability of the entire panicle. The vast majority of panicles of a given variety will emerge within a few days of one another, so we can add another week. Now, a given infection does not stop grain development on the day of inoculation but surely begins to do so before the lesion appears, so we subtract one-half the latent period, probably 3 days. Therefore, on entirely hypothetical grounds, one is left with a period of about 2½ weeks during which panicle infections must occur to appreciably affect yield within a field. This is not a long period of time, especially when weather conditions may not be favorable every night. Of course, this hypothetical length of time varies with variety, but for any one group of similar varieties this period can be determined. In our view, it can be determined with enough accuracy to be useful in predicting yield loss from blast.

C. (U) NUMBER OF LESIONS NEEDED FOR DAMAGE

(C) The choice of a good measurement of disease is difficult. Since a lesion located near the base of a leaf blade can cause its death although it might require several hundred lesions on other portions of the blade to achieve the same result, counts of lesion numbers per leaf, tiller, or plant are usually not satisfactory for small-plot (or pot) studies. A more accurate measurement is the number of lesions per unit area, which is commonly used in quantitative laboratory studies. In the field, however, it is difficult to measure leaf area without removing leaves or plants from the field, and in many kinds of experiments the investigator wants the plants to remain in the field so that he can observe lesion appearance and development with time. Most of the field workers until now, therefore, have measured disease by the number of lesions per foot of row or per hill if the crop was transplanted in the Oriental manner. Since the distances between rows or hills is often known, this measure also gives an indication of the amount of disease in the crop area as well as on the plants.

(U) In Kulik's field data* from Texas, identical conclusions resulted from the statistical analysis whether lesions were reported on the basis of average number per plant or per 100 square centimeters of leaf area.

(U) A serious objection to lesion counts is that in instances of rapid disease increase the "kill effect" makes the counting of lesions impossible, usually within a couple of weeks after rapid increase begins. The Japanese have developed a system for estimating disease damage without relying on lesion counts. A set of pictures showing the same or a similar scheme was acquired by the senior author (Figure 4). This scheme was used by workers from Crops Division in the field for the first time in 1963, and it may be possible to derive a relationship between lesion counts and disease severity.

(U) Severe leaf blast causes declines in the number of ripe panicles, plant height, weight of 1000 grains of brown rice, and the weight of brown rice per hill. Severely affected hills also are delayed in growth, which results in some delay in heading. As a general rule, when leaf disease breaks out early, the yield losses are greater. These relationships^{5**} have been found by the Japanese workers and some are shown in Table 13.

(U) There is an example of the relationship between lesion counts and yield in Allison's data.*** Although the correlation between yields and lesion counts on two dates was generally poor for the Gulfrose and Colusa varieties (although with Colusa it was significant at the 0.10 level), it was real (significant at the 0.01 level) for the Arkrose variety, where double the number of lesions decreased yield approximately 18 bushels per acre.

* Kulik, M.M. Unpublished data and the associated Analyses 4451 (5 Dec 1960) and 4447 (20 Sept 1964), Crops and Biomathematics Division, U.S. Army Biological Laboratories, Frederick, Maryland.

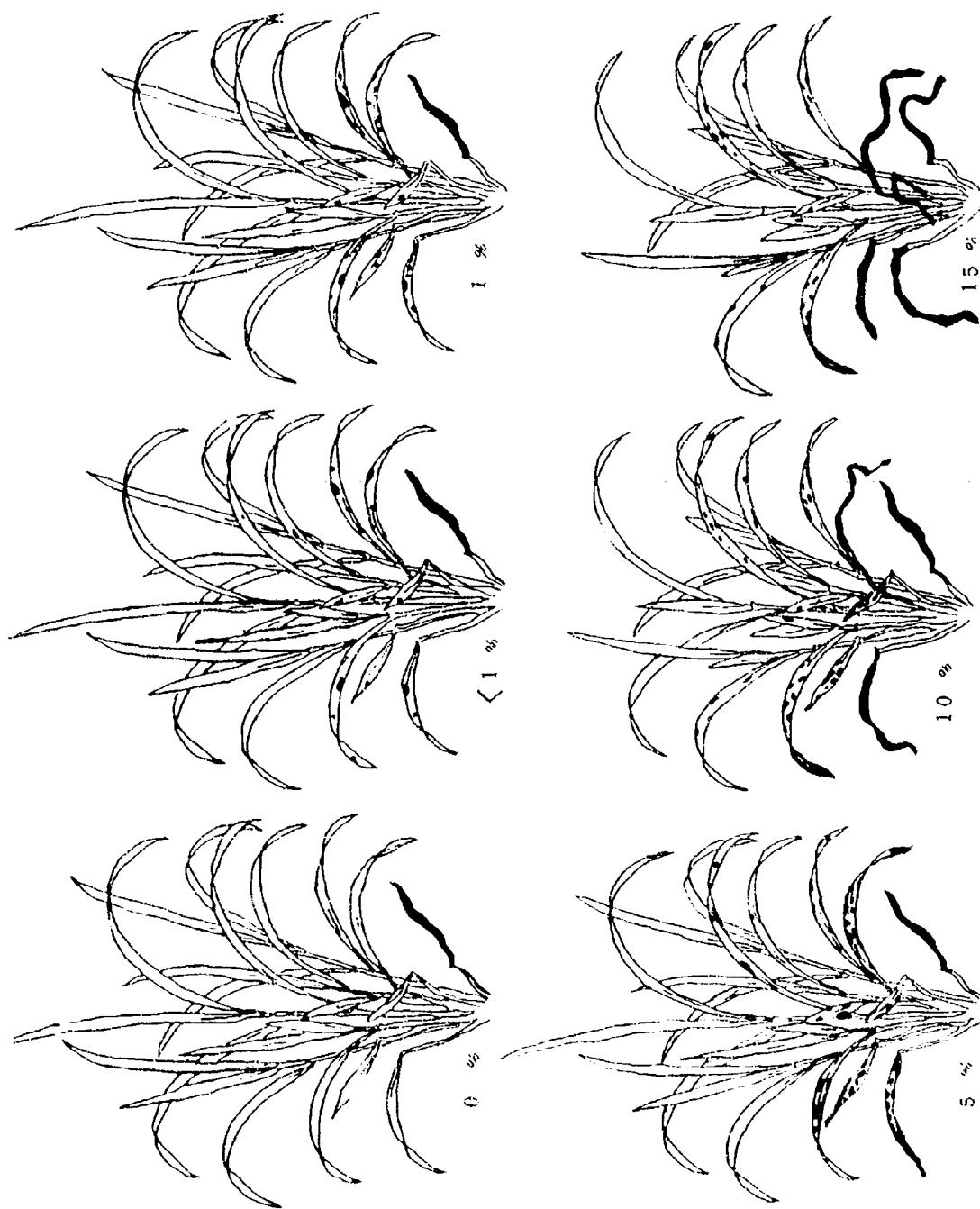
** Pages 195 through 202.

*** Allison, W.H. Unpublished data and the associated Analyses 5219 (28 Mar 1962) and 5312 (17 May 1962), Biomathematics Division, U.S. Army Biological Laboratories, Frederick, Maryland.

TABLE 13. (3) AMOUNT OF YIELD LOSS CAUSED BY LEAF DISEASE.

Per cent Severity Rating on leaves 30 Days Before Heading ^a /	Per Cent Reduction in Height of Plants	Yield Loss caused by Leaf Disease, %
5	None	0
10	10	5
20	20	15
25	30	25
30	35	30
35	40	35
40	50	40

* After data of Goto,⁵ pages 195 through 202.
 a. See Figure 4.



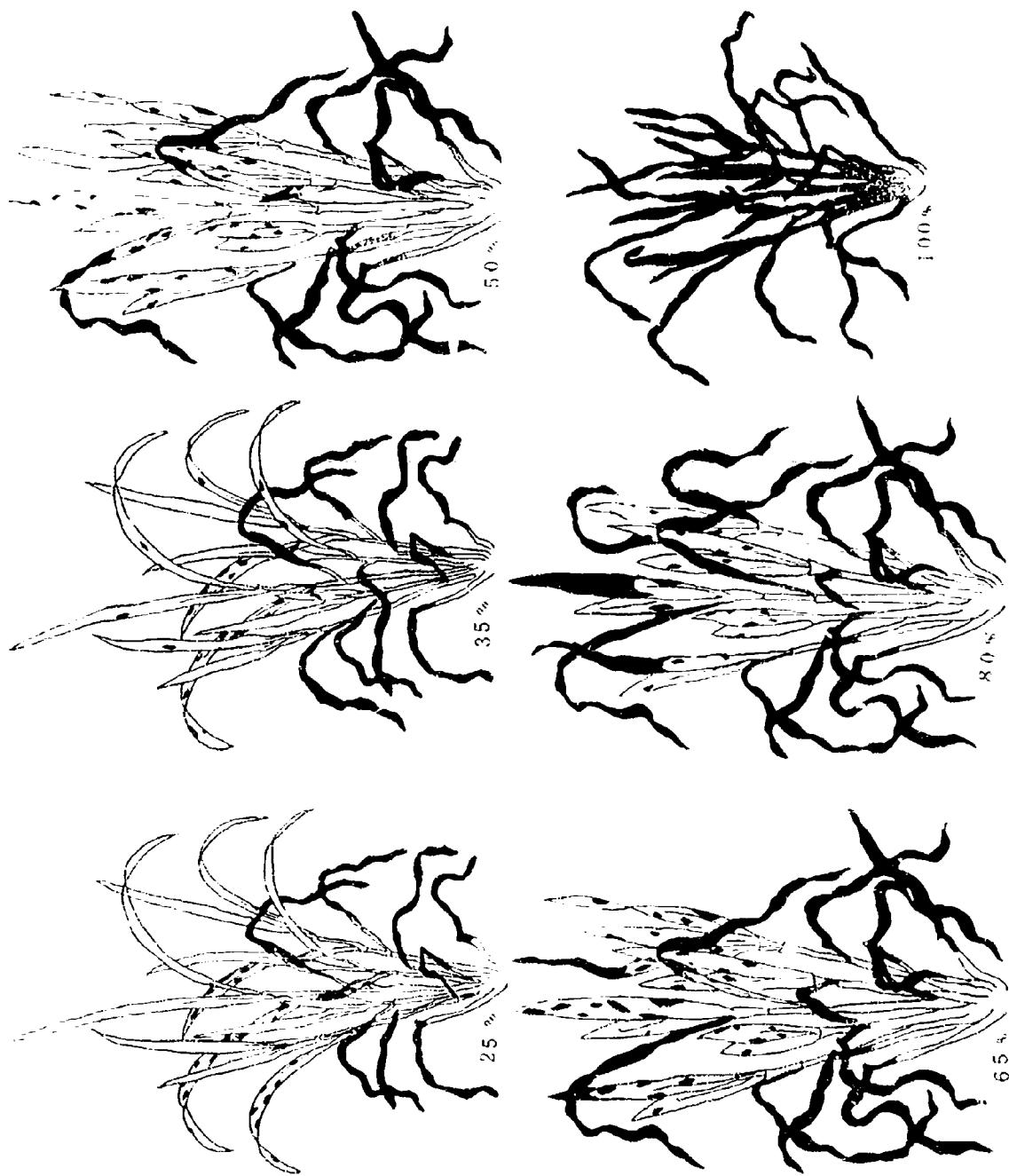


Figure 4. (U) Pictorial Chart for Estimating Severity of Rice Blast on Leaves of Plants in the Field. (U)

CONFIDENTIALVI. (C) FACTORS INFLUENCING EPIPHYTIC DEVELOPMENT

(U) A number of factors that influence epiphytic development have been discussed or referred to above if they were known to exert some influence on a specific portion of the disease cycle. In this Section, some of the factors that influence a sequence of disease cycles, i.e., an epiphytic, are discussed.

A. (U) RACE OF PATHOGEN

(U) One would not expect a race to which a given variety was resistant in the laboratory to cause any trouble on that variety in the field. The opposite result in the laboratory does not permit such a simple statement, however, about what may be expected in the field. For example, in working on sporulation of rice, several workers^{21,22,33} have found that different race-variety combinations giving susceptible reactions will produce different numbers of spores under the same environmental conditions. We might speculate that in the field under the same conditions these combinations would have different rates of disease increase because of different numbers of spores produced and that this would perhaps result in different amounts of yield reduction at the end of the epiphytic. This has not been tested in the field. One approach would be to install spore samplers over a number of small plots of different rice varieties in a field where only one race was causing disease increase, because the data of Marchetti* indicate that differences in spore concentrations can be detected over small plots of a single variety planted close together but with different rates of buildup caused by agronomic practices.

(U) The number of lesions, an index of susceptibility, may also differ among varieties considered susceptible to a given race because of laboratory studies. In studies conducted at Beaumont, Texas, in 1960, the two varieties C.I. 8970 and Colusa showed marked differences in susceptibility to race 8 in a series of nightly inoculations.** There is also an excellent example of different numbers of lesions being formed over a period of time (an epiphytic situation) on ten varieties attacked by race 6 (probably race 31) and grown under the same field conditions in Florida.***

* Marchetti, M.A. Unpublished data and the associated Analyses 6171 (13 Feb 1964) and 6269 (15 July 1964), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

** Kulik, M.M. Unpublished data and the associated Analyses 4451 (5 Dec 1960) and 4447 (20 Sept 1960), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

*** Dahlke, C.R. Unpublished data and the associated Analysis 6433 (5 Aug 1964), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

CONFIDENTIAL

69

(U) In the laboratory, susceptibility is likely to be defined under a standard set of conditions that are nearly optimal for infection to occur and for lesions to develop. In the field, conditions are never standard and usually are optimal for only a certain percentage of incubation periods. This would lead one to suspect that a given race-variety combination might cause serious disease under one set of conditions in the field, although a different race-variety combination might not cause much disease under the same set of conditions. From laboratory data obtained by Kulik* on 25 varieties, 12 races, two lengths of dew period, and three temperature ranges, pathogenicity (based on the number of lesions produced) among races depended on length of dew period as well as on variety; resistance to blast among varieties also depended on dew period as well as on race.

(U) Isolations made from fields inoculated with material prepared in the laboratory sometimes result in finding a different race at the end of the epiphytotic (Table 14). In most instances, lesions were collected from the field during or at the end of the epiphytotic; Willis's first collections, those reported in Table 14, were made soon after inoculation and in this one set of data the agreement between race used and race collected is perfect. The disappointing thing about these data is not the results (which are extremely interesting) but their scarcity. From no field have enough race determinations been made to know whether they are representative of the race(s) that caused the epiphytotic in that field. Race identification programs everywhere^s seem to be designed to discover new races or variants of old ones rather than to provide a service to those who are interested in what happens in the field.

B. (C) METEOROLOGICAL CONDITIONS

1. (C) Dew

(U) The length of dew period can influence the amount of sporulation and the number of lesions produced. Intensity of dew probably plays a role, also. Drying or an interruption of dew period is detrimental to the establishment of infection on a particular night, but on the whole field records indicate that this is a relatively rare phenomenon. Drying between dew periods during the day is probably much more a factor in limiting the number of infections that occur, since undoubtedly the drying of germinated spores before they can enter the tissue kills many of them. As an example of the great waste in nature(or conservation, depending on your viewpoint) the situation is one that defies quantitation at present.

* Kulik, M.M. Unpublished data and the associated Analysis 5023 (10 Jan 1962), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

CONFIDENTIALTABLE 14. (C) RACES OF PIRICULARIA ORYZAE RECOVERED FROM FIELD PLOTS FOLLOWING ARTIFICIAL INOCULATION* (U)

Source of Specimen, Location, and Year	Field Designation and Variety	Race Used For Inoculation ^{a/}	Race Recovered ^{a/}
Barksdale Avon Park, Florida, 1960	Paddy 3: Colusa	3 (640)	19 (92)
	Colusa	3 (640)	8 or 19 (111)
	Colusa	3 (640)	10 (149)
	Paddy 6: Colusa	8 (770) & 3 (640)	8 or 19 (112)
	Paddy 7: Colusa	8 (770) & 3 (640)	19 (122)
	Paddy 8: Colusa	8 (770) & 3 (640)	8 or 19 (123)
Barksdale Okinawa, 1961	Nago: Caloro	2 (167)	4 (296)
Allison Avon Park, Florida, 1962	Paddy 3: 5309	2 (226)	2 (335)
	Colusa ?	2 (226)	2 (395)
	Paddy 4: Colusa	not inoculated	19 (337)
	5309	not inoculated	19 (338)
Barksdale Okinawa, 1962	Nago: Taichu 65	2 (189)	2 (342)
	Fujisaka 5	2 (189)	4 (343)
	Caloro	2 (189)	4 (346)
	Fujisaka 5	2 (189)	4 (348)
	Taichu 65	2 (189)	2 (354)
Taiwan, 1962	Ta-Yah: Taichu 65	{ mixture of 2 (226), Taichu 65 21 (243), & 20 (244)	2 or 4 (355) 4 (437)
Willis Taiwan, 1963	Chiayi:		
	Taichung Special 6	6 (747)	6 (456)
	Wu Ker	6 (774)	6 (457)
	Taichung Native 1	6 (747)	6 (458)
	Taichung 65	21 (243)	21 (459)
	Chianung 242	21 (243)	21 (460)
Dahlke Avon Park, Florida, 1963	Paddy 5: Colusa	6 (747)	31 (478)
	8970S & P	6 (747)	6 (479)
	Taichu 65	6 (747)	31 (480)
	Arkrose	6 (747)	31 (481)
	Paddy 6: Toro	6 (747)	31 (461)
	Paddy 4: Toro	not inoculated	31 (477)

a. Culture numbers in parentheses.

* Latterell, F.M.; and Marchetti, M.A. Unpublished data, 1960-1964 Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

CONFIDENTIAL

71

(C) One of the unanswered questions about dew concerns the frequency and sequence of nights with dew periods of suitable lengths for infection. The senior author drew some frequency bar graphs for dew periods occurring at two sites on Okinawa in 1962.* In every month, from one-half to three-fourths of the nights had no dew, but on those nights when dew did form it was usually of a length considered favorable for infection. These conditions did not alternate every other day but occurred in blocks of several nights each, and disease buildup was slight to moderate. If favorable conditions had alternated with unfavorable ones every other night, or if a long block of favorable nights had occurred during the proper plant growth stage, would buildup have been more severe? Any particular epiphytotic lacks a suitable check, but the hope is that someday enough field examples will have been observed to establish the trend. In the meantime, workers would do well to report data more fully than did Ackins,⁴⁰ for example, when he reported severe outbreaks of blast in Texas and Louisiana. He listed the mean temperature and number of dew periods by the month. Detailed records of nightly dew periods, temperatures, and several mathematical descriptions of disease severity in time would help develop information useful for predictions.

2. (U) Rain

(U) The humidity and surface moisture associated with rain influence sporulation, and whether rain occurs before or after an artificial inoculation may influence the number of resulting lesions. These two effects relate primarily to the number of infections that occur on a given night, but there is little information on how rain influences development of disease in the field over a period of time. Frequent showers (how frequent and how intense?) might wash some spores from the air and thereby reduce the inoculum potential. A rainy period might also exercise some predisposing factor on plant susceptibility.

(J) The role(s) of rain with respect to panicle infections is unknown. In addition to increasing sporulation and extending dew periods, rain could conceivably help wash spores into the boot leaf sheath when it splits open as panicles emerge. According to the analyses of Allison's field data** for 1961, the combined effect on yields of a degree-hour term (found by multiplying the minimum temperature by length of dew period) and rainfall, both averaged over the period 30 through 16 days prior to harvest, did approach significance. However, neither factor was shown

* Barksdale, T.H. Unpublished data, 1962, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

** Allison, W.H. Unpublished data and the associated Analyses 5219 (28 Mar 1962) and 5312 (17 May 1962), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

CONFIDENTIAL

to be an effect in itself. The thought here was that panicle lesions causing yield reduction are likely to occur 30 to 16 days prior to harvest for the varieties in his test, and that weather conditions during that period may affect yield by influencing the number of panicle lesions that occur. The following equation was estimated:

$$Y = 90.31 + 0.1403X_1 - 39.83X_2$$

where: Y = predicted yield in bushels per acre,

X_1 = average degree-hours per day 30 to 16 days before harvest, and

X_2 = average inches of rainfall per day 30 to 16 days before harvest. The analysis of variance did not show the regression equation to be significant at the customary 95% level, but did indicate significant at a level close to 90%. Rainfall probably contributes in some undefined way to panicle blast and yield reduction.

3. (U) Temperature

(U) Temperatures normally found in the field after the early growing stages of rice in temperate and semitropical regions and during all plant growth stages in the tropics are satisfactory for growth of the fungus. In fact, the warmer temperatures (28 ± 2 C) are generally considered nearly optimum for fungus growth. This makes rather puzzling the observation that blast is not as prevalent in the field during the warm summer months in Taiwan, for example, as it is in either the late spring or fall. Hashioka⁵ was one of the earliest to offer an explanation. In his experiments, plant resistance was increased in proportion to the increase of temperature at which the plants were grown for 25 days prior to inoculation, although this change in resistance was more pronounced in varieties of temperate than of tropical origin. Years later, in summarizing work on this topic, Hashioka^{5*} stated that the effect of temperature in predisposing plants to infection varies with the duration of the temperature treatment, with the combination of temperatures in soil and air, with plant or leaf age, and with the varieties employed. He generalized that temperate varieties, like those grown in Japan, are more likely to be predisposed to infection when grown at low temperatures (18 to 20 C) than are tropical varieties. Warm weather in the tropics (28 C or higher) intensifies host resistance.

(U) Recent work by Sadasivar^{5**} indicates that plants grown with night temperatures of 20 C and alternating with day temperatures of 30 to 35 C are susceptible, but if night temperatures rise above 26 C, then infection seldom occurs on susceptible varieties. He hypothesized that low night temperatures favored the accumulation of soluble nitrogen. Excess nitrogen fertilizer applied to soil and a high level of soluble nitrogen in

* Pages 153 through 162.

** Pages 163 through 172.

CONFIDENTIAL

CONFIDENTIAL

73

plants has long been associated with increased susceptibility (Section VI, C, 4). In one of his experiments, he inoculated plants of a susceptible and a resistant variety that had been grown at three different night temperatures and obtained the following reactions:

<u>Night Temperature, C</u>	<u>Co 29 (Resistant)</u>	<u>Co 13 (Susceptible)</u>
30	0	+
25	0	++
20	0	+++

Even the very resistant variety sustained limited infection at the low night temperature, and the susceptibility of the susceptible variety was markedly enhanced by the lowest temperature studied.

(U) Kulik's work* indicated that with a 12-hour dew period more lesions were obtained in the temperature range of 75 to 80 F than of 85 to 90 F, although the difference was not significant at the usual levels.

(U) Considerable information from Japan shows that soil and water temperatures can predispose plants to infection. Kozaka^b** has reviewed these data. Cool irrigation water of less than 20 C makes leaves more susceptible and cold soil temperatures between 18 and 24 C makes panicles more susceptible. The soil or water temperature usually seems not to be acting entirely by itself, however, and other factors, such as the silicon and nitrogen content of the soil and the length of the treatment period prior to inoculation, influence the degree to which temperature affects susceptibility. In the cooler parts of Japan, various practices are recommended to get or keep the irrigation water warm: using shallow reservoirs, allowing water to flow through a shallow ditch prior to entering a field, and preventing percolation by thorough puddling of the soil.

* Kulik, M.M. Unpublished data and the associated Analysis 5023 (10 Jan 1962), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

** Pages 421 through 440.

CONFIDENTIAL

CONFIDENTIAL

4. (C) Wind

(C) Apart from the role that air currents and wind play in transporting inoculum, probably the most important effect of wind is an indirect one — on dew. The senior author considered the presence of winds in the insular climate of Okinawa to be the primary reason for the infrequent occurrence of dew periods sufficiently long for infection.

(U) Strong winds lasting for a considerable time probably exert some influence on plant susceptibility, but there are no field data on this matter.

(U) In one field study, spores were released and allowed to settle on plants inside plastic tents that were removed 20 minutes later. Wind speed did not influence the number of lesions resulting from these artificial inoculations.¹⁹

5. (U) Light

(U) Light may be an important factor in influencing the development of epiphytotes in certain local situations. Light enhances sporulation of many Piricularia isolates. During cloudy weather with periods of drizzle or light rain that keep plant surfaces wet and relative humidity near 100%, light could very well enhance sporulation during daylight and consequently enhance the epiphytotic. On the other hand, cloudy weather is not always accompanied by long periods of precipitation; there are sometimes only brief periods of rain accompanied by high winds, a situation that leads to intermittent dryness that could retard sporulation, incubation, and epiphytotic development. Cloudy weather, with associated low light intensities, is known to be a factor in lowering rice yields, but whether prolonged periods of low light intensities during daytime has any effect on plant susceptibility to blast is unknown. At least, there are no field data.

C. (C) AGRONOMIC PRACTICES

(U) The most thorough way to examine the influence of agronomic practices would be to list all practices used in rice cultivation and then to indicate what is known about the effect of each on blast development. In our view, however, the important data in this area can be discussed under four headings: the time of planting, transplanting, irrigation, and fertilizer.

CONFIDENTIAL

CONFIDENTIAL

75

1. (U) Time of Planting

(U) Farmers plant crops at specific times for many reasons. If there is a dry season, they may wait for a rainy season not only for enough water to grow the crop but also because the ground is easier to prepare then. This is true of rice cultivation in parts of southern Asia in areas where water is not available for irrigation except in the rainy season. If there is a winter season, they must wait for the absence of frost, as they do in temperate or semitropical regions. Planting dates may be influenced by the desired time of harvest. In Okinawa, two rice crops are grown each season; the first is planted to mature before the onset of major typhoon activity and the second to mature after this period in an effort to reduce loss from lodging and wind-induced sterility. The dates of planting (and transplanting) may have much to do with whether or not blast becomes a problem under natural conditions. Because susceptibility varies with plant age and because periods of weather favorable for disease buildup are often seasonal, plants must be at their most susceptible age during periods of favorable weather for maximum epiphytotic development.

(U) With seedlings that are not transplanted, maximum infection occurs at ages between three and five weeks and infections decrease with plant age up to nine to 11 weeks, after which the leaves of susceptible varieties become resistant except for trace amounts of infection.³³ Increased resistance with an increase in plant age was observed on both greenhouse and field-grown plants grown under flooded or non-flooded conditions.³¹ In work with individual leaves, Volk et al.⁴¹ found that as a rice leaf aged, it became increasingly more resistant. As a leaf emerged, its susceptibility was greatest. With respect to leaves on a single tiller, as each successive leaf emerged its "maximal susceptibility" was less than that of older leaves. Additionally, each successive leaf remained susceptible for a shorter period of time than preceding ones. When a plant is given a single inoculation, the susceptibility pattern as influenced by leaf age and leaf position results in a gradient decreasing from younger upper leaves to older basal leaves. In older plants, this gradient disappears and the plant as a whole becomes resistant. To extrapolate to the field situation, it seems probable that a field will be most susceptible when it has the greatest number of newly emerged leaves on young plants, a time that occurs during the tillering growth stage.

(U) Some of the best early work on the age-resistance problem was done by Hashioka¹⁵ working on Taiwan. He grew plants in pots to correspond with either the first or second cropping season on the island. In one series of experiments, all seeds were sown at the same time and the plants inoculated at intervals; in the other series, pots

CONFIDENTIAL

CONFIDENTIAL

were seeded at different dates and all plants inoculated at the same time. Both procedures have drawbacks that he pointed out, but the data are indicative of the range of ages during which plants are most susceptible. For experiments in the first cropping season, susceptibility (as measured by considering both the number of lesions per plant and the number per unit leaf area) was greatest for plants aged 40 to 60 days by the first procedure and 30 to 60 days by the second procedure. For experiments during the second crop, susceptibility was greatest for plants aged 20 to 35 days by both procedures. The difference of about two weeks between first and second crops is due to more rapid growth of the second crop. It is planted when temperatures are warmer than for the first crop, and the shortening day lengths may also hasten maturity. Of course, the effect of high night temperature in increasing resistance (Section VI, B, 3) would also interact with the effect of age in the second crop.

(U) Some striking examples from the field of the relationship between plant age and susceptibility are available. In Rorie's initial infection studies,¹⁹ the amount of plant leaf area increased with plant age, and the trend was that those plants with more leaf area had greater numbers of lesions. Leaf area at the time of inoculation was statistically correlated with lesion production and was one of the variables included in the equations for estimating lesion production (Section IX). Here we have an example of increasing susceptibility of field plots with increase in the amount of young leaf tissue during the tillering growth stage.

(U) In the senior author's work in Florida,²⁰ the Colusa variety was planted in four fields on three dates spaced two weeks apart. Each field was inoculated once. The typical pattern of disease development showed the greatest amount of increase in the youngest planting, the least in the oldest at the time of inoculation.

(U) The attempt at commercial rice growing in Florida during the mid-1950's was made in order to obtain a summer crop on land ordinarily farmed in the winter. Rice blast was a serious disease every year in many of the plantings, and although no experiments per se on planting dates were made, observation indicated that the early plantings in March and April had little or no disease but later plantings in May and June were frequently blasted.⁴² The effect of early planting was that the rice went through its most susceptible growth stages before temperatures became warm enough to favor serious disease development.

CONFIDENTIAL

CONFIDENTIAL

77

(C) The senior author observed some examples of the effect of planting date on blast development during his work in Taiwan and Okinawa.* In one instance, an area of about one-half acre was planted two weeks late, was severely infected, and sustained a severe yield loss even though the surrounding acreage had little disease. Several factors favoring disease development were operating in the small planting; probably the most important was that the younger plants were undergoing rapid tillering and provided much susceptible tissue to the modest spore load produced by the neighboring but older plants. In a second instance, a somewhat reverse situation was observed on southern Taiwan, where the temperatures became very warm early in the spring. A field had been prepared by experiment station personnel to test a number of varieties for blast resistance during the first crop season. Two plantings, using the same variety for spreader rows, were made about two weeks apart. Otherwise, they were treated identically. In the earlier planting, the spreader variety was severely infected; in the later planting this variety was not damaged. One factor that might possibly have been operating here was temperature-induced resistance affecting the later planting more so than the earlier one.

(U) Whatever the reasons — increased resistance with plant age, the occurrence of favorable weather conditions at certain times of year, temperature-induced resistance, or some other — it is clear from numerous observations that planting date can exert an important if indirect effect on blast epiphytology.

2. (C) Transplanting

(C) Transplanting as opposed to direct seeding is the common method of growing rice in the Far East. On the Japanese island of Hokkaido, where the rice-growing season is short, attempts to extend the season by growing seedlings in cold frames protected by plastic or tarpaper covers are common. Part of Otani's book⁴³ is a comparison between the traditional and cold frame methods of raising seedlings as these methods affected plant susceptibility to rice blast. Although seedlings raised under the warm conditions in the cold frame were more susceptible, seedlings from both environments became resistant after being transplanted to the field. The writer has observed that seedlings transplanted in the first crop on Okinawa or Taiwan seemed to undergo a shock, and because of this and the relatively cool weather did not begin to produce new leaves and tillers for about two or three weeks after transplanting when presumably plants would regain their susceptibility. Transplanting during the warmer weather of the second crop seemed neither to delay plant growth nor reduce susceptibility. These observations seem to support the idea that

* Barksdale, T.H. Unpublished data, 1962, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

CONFIDENTIAL

young tissue is susceptible whereas old tissue is resistant. Kozaka's review⁵ indicates that delayed transplanting after pulling or deep transplanting may increase susceptibility. The important thing for the field researcher to remember is that transplanting practices do influence susceptibility and to plan experiments in the field with this in mind.

3. (U) Irrigation

(U) Rice does not yield well if the soil dries out and, consequently, most of the world's rice is grown under flood irrigation. Rice can be grown successfully by upland farming methods if it rains frequently or if permanent irrigation facilities are available, and if weeds can be controlled. Even in areas where frequent rains are common, flood irrigation is used because it is the cheapest and easiest way to control most weeds. Overhead or other permanent irrigation facilities are an economic luxury in most of the world.

(U) Kahn²¹ found that plants grown under non-flooded culture are more susceptible than those grown in flooded culture. Kozaka^{5*} reviews the literature on this question, and concludes that the current recommendation is to avoid excessive drying in midsummer if the disease is present and to delay drainage until about one week to ten days before harvest when much disease is present. Rice specialists in the United States often recommend that farmers either flood or raise the level of their irrigation water when leaf blast occurs. This seldom causes any harm and it may do some good (nobody is sure why).

4. (C) Fertilizer

(U) The use of excessive amounts of fertilizer, especially nitrogen, is often associated with outbreaks of blast in the field. Nitrogen apparently affects plant susceptibility. As background, some information from laboratory experiments on this effect is presented here first, followed by a discussion of what may be considered an excessive amount and some data on the amounts actually used in rice-growing areas. The other two commonly used fertilizer elements, potassium and phosphorus, seem to have little effect on the disease.^{5*} In the following discussion, rates of nitrogen fertilizer are given as actual nitrogen (N).

* Pages 421 through 440.

CONFIDENTIAL

CONFIDENTIAL

79

(U) The nature of increased susceptibility in plants receiving high amounts of N is not fully understood, but in such plants, the soluble N fractions (amino acids and amides) increase at a rate greater than the protein fractions, and the number of silicated epidermal cells decrease.^{5*},⁴³ For some time, it was thought that resistance found at low N levels might be somewhat mechanical and that the higher number of silicated epidermal cells acted as a physical barrier to prevent fungus penetration.⁴³ More recent work, however, has indicated that resistance was largely caused by less absorption of N, since the degree of resistance to invasion is not always parallel to silica content, but always to a decrease in N content.^{5*} In work in Crops Division, Volk et al.⁴¹ found that silicon content and susceptibility were related inversely; the silicon content and the degree of susceptibility of leaves at any moment were related to the amount of silicate available to the roots and, indeed, the level of N fertilizer seemed to influence the amount of silica absorbed. Whichever chemical influences the absorption and/or action of the other, it seems clear now that any future scientific explanations of resistance at the cellular and biochemical levels will have to account for the effects of both N and silica and probably of an interrelationship between the two.

(U) For most of the world's farmers, the problem is one of increasing production by use of N fertilizer. The increased susceptibility to blast is an annoying side effect, and it is possible that high N applications could be offset by silica applications as far as blast is concerned. Both Kozaka^{5*} and Volk et al.⁴¹ cite several references on increased resistance through silica applications. Kozaka stated that this effect held true under different environmental conditions within a given variety but not among varieties. Furthermore, he reports that in certain types of soil in Japan (especially volcanic and degraded paddy soils) a high supply of silica results in less blast, better plant growth, and higher yields. These results happen even in fields with high N content. Compost is the most popular source of silica among Japanese farmers, but recently a foundry byproduct containing chiefly calcium silicate has been found effective for disease control and is recommended at 2 tons per hectare where silicon content of plants falls below 10%.

(U) In an attempt to understand what is or may be meant by an excessive amount of N fertilization, field data were examined. Ou** combined a study of the date of seeding and the amount of N fertilizer on rice blast development during his work in Thailand. He relied on

* Pages 421 through 440.

** Ou, S.P. "Some information on rice blast disease in Thailand." Mimeoographed report, FAO, 1959.

CONFIDENTIAL

CONFIDENTIAL

natural inoculation of his field plots. His data (Table 15) show that later dates of seeding and higher amounts of N resulted in greater blast development, the effect of seeding date being due to a greater abundance of natural inoculum as the season progressed. His high rate of N, 131 kilograms per hectare,* was clearly excessive when other factors were not limiting disease development.

TABLE 15. (U) DATE OF SEEDING AND NITROGEN LEVEL IN RELATION TO DEVELOPMENT OF RICE BLAST IN THAILAND* (U)

Date of Seeding	Date of Observation	Nitrogen Applied, ^a /kilograms/hectare	Number of Lesions on 100 Seedlings
June 15	July 15	131	7.7
		66	2.5
		0	0.1
June 30	July 30	131	94.3
		66	33.5
		0	0
July 16	Aug. 16	131	1666.1
		66	921.6
		0	0
July 30	Aug. 30	131	5313.0
		66	18.5
		0	1.0

* Unpublished data of S.H. Ou.

a. Nitrogen applied in ammonium sulfate.

* The term kilograms per hectare is roughly equivalent to pounds per acre because one kilogram equals 2.2 pounds and one hectare equals 2.47 acres. The terms are essentially interchangeable for unit comparisons of fertilizer or yield, but not for estimating large quantities.

CONFIDENTIAL

(U) Padmanabhan^{5*} reports on a fertilizer study in India in which plots were treated at rates of 0, 20, 40, 60, or 80 pounds of N per acre with and without compost over a period of several years. In comparing a severe blast year with 2 years of no incidence, he reported yield losses of 200 to 600 pounds per acre without compost and of 250 to 1,000 pounds per acre with compost. He was unable to make any statistical correlations when he considered the data as a whole. A loss of 600 or 1,000 pounds per acre in a country whose 1956 to 1958 average grain yield was only 821 kilograms per hectare⁴⁴ seems to us to be a serious loss. In this instance, it would be conservative to suggest that 80 pounds of N per acre was approaching an excessive amount.

(U) Atkins⁴⁰ reports data from a variety-fertilizer trial in Texas in which rates of 0, 80, 120, and 160 pounds N per acre were used. The big jump from small to significant blast damage was at the 80-pound level with a susceptible variety, C.I. 8970. The jump was apparently at the 120-pound level with a more resistant variety.

(U) The senior author observed experimental plots of several varieties in northeastern Louisiana in the summer of 1963. They had been fertilized at several rates of N, and the highest rate was more than 120 pounds per acre. (Actually, 120 pounds were applied, but there was some carry-over from nitrogen applied the year before.) Only a trace of blast was seen on any variety in the test. Surely, some factor other than lack of N was limiting blast development. It may have been that the hot weather predisposed the host to resist the blast. Blast on Marchetti's plots in southern Louisiana that year was serious on plots given 120 pounds per acre, although part of the yield losses in his tests were due to lodging, a phenomenon also aggravated by high N fertilization.**

(U) Hashioka¹⁵ performed two series of field tests using varieties of wide geographic origins. His N levels were 0, 40, 80, 160, and 240 kilograms per hectare in one test and 0, 80, and 240 in another. Generally speaking, the varieties of the Japonica type responded with increased numbers of leaf and neck lesions when amounts of N were increased, although there were no striking or consistent differences between treatments for the Indica types. The level of N that caused a striking increase of blast on the Japonicas was somewhere between 80 and 160 pounds per acre.

* Pages 203 through 222.

** Marchetti, M.A. Unpublished data and the associated Analysis 6269 (15 July 1964), Crops and Biomathematics Disisions, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

(C) In one of the senior author's tests* on Okinawa in 1962, a level of 120 kilograms of nitrogen per hectare caused 18.1% panicle blast on Caloro; 80 kilograms per hectare caused only 3.8%. The higher rate of N, however, caused a 26% increase in the number of panicles produced; the net result was a greater yield in spite of greater blast.

(U) The Century Patna variety was fertilized with 30, 60, 120, and 240 pounds of N per acre in a Florida test.³⁸ These rates were applied to four different fields, and there were some differences in procedures among fields. In fields 1 and 2, infection was greater with increasing amounts of N, but only the 240-pound amount caused a high level of infection. In the third field, severe infection occurred at all levels of fertilization and all rice in that field was dead within 70 days from planting. A split application of N had been made, the second topdressing being applied just before rapid disease increase occurred. The fourth field received only one treatment, 120 pounds of N per acre at seeding, and disease buildup was severe. The authors suggested that date of planting, time of inoculation, inoculum levels, weather conditions, and plant susceptibility as influenced by plant age were all involved along with N in influencing the severity of blast.

(U) Kozaka^{E**} is the only plant pathologist we have found stating in print the amount of N fertilization that can be safely used without danger of increasing blast severity. He qualifies his educated guess for reasons like those just mentioned, along with comments about the variability of soil types, varieties, etc. He says that 50 to 60 kilograms per hectare is a safe rate, and that farmers who use rates above this are liable to trouble from blast.

(C) Even if specific amounts of N are known to have been applied to a given field, it is not always possible to know whether that is the only amount present and influencing susceptibility. Often the amount of compost added (green manure or otherwise) is unknown, and compost of various compositions is added in many Asian countries.^{S***} Then too, there is the problem of residual nitrogen remaining in the field from one season to the next. The senior author has seen two striking examples of N hold-over, one at Crowley, Louisiana, and one at the International Rice Research Institute. High rates of N applied to land one season can prevent that land from being used for N-rate studies the following year.

* Barksdale, T.H. Unpublished data, 1962, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

** Pages 421 through 440.

*** Pages 399 through 408.

CONFIDENTIAL

CONFIDENTIAL

83

(U) The method of nitrogen application can have a marked effect on the occurrence of blast. Split applications are common in Japan and the United States, where part of the N is mixed with the soil as the ground is being prepared for transplanting or sowing. The remainder is applied as a topdressing three to four weeks after transplanting to encourage tillering, or about four weeks prior to heading to encourage panicle formation (in addition, a nitrogen topdressing at this later time will result in a higher protein content of the grain), or at both times. If the N application is made three to four weeks after transplanting (during the tillering stage) and other conditions are favorable for disease development at this time, a serious blast situation can result. According to Otani,⁴³ effects of adding N can begin to show 2 days after application and can become striking by the 8th day.

(U) Information on the amounts of N recommended and the amounts actually used come from a variety of sources. One recent reference of interest is an FAO publication⁴⁴ because it compares crop yields and levels of fertilizer used in many countries. This report "indicates a curvilinear relationship between grain yields and fertilizer use in 40 countries. Of the 22 using less than 40 kilograms of plant food per hectare, only one, the United States, had average grain yields above 1500 kilograms per hectare." A selection of data from rice-producing countries (U.S. data are not weighted heavily by rice but are included as a matter of interest) follows; it should be noted that fertilizer here means the combined use of N, P₂O₅, and K₂O.

<u>Country</u>	<u>Fertilizer Use, kg/ha of Arable Land, Average 1956 to 1958</u>	<u>Average Grain Yield, 100 kg/ha, 1956-1958</u>
Thailand	0.8	no data given
Pakistan	0.9	11.17
India	1.2	8.21
Indonesia	2.1	14.95
Philippines	3.7	9.44
United States	30.9	21.46
Ceylon	34.4	13.15
Korea, Republic of	103.0	17.43
Taiwan	163.4	28.57
Japan	257.4	36.33

Even if these figures were for N and rice alone, which they are not, it can be inferred that only in the three north-Asian countries of Korea, Taiwan, and Japan is N application likely to be a common factor in increasing the susceptibility of rice to blast except in special situations.

CONFIDENTIAL

CONFIDENTIAL

(U) Dr. Togari* of the University of Tokyo stated in 1961 that current recommendations for obtaining high yields in Japan were to apply ammonium sulfate at 400 to 500 kilograms per hectare on Japonicas and 100 kilograms per hectare on Indicas; this works out to about 80 to 100, and 20 kilograms of elemental N per hectare, respectively. The reason Togari gave for not recommending higher rates was that plants became too susceptible to lodging at higher rates.

(U) Matsuo¹⁰ lists the range of N use in Japan between 60 and 93 pounds per acre (calculated from the Japanese units).

(U) In the 1940's Hashioka¹⁵ considered 80 kilograms of N per acre a normal rate of application for Taiwan. He was working mainly with Japonicas.

(U) De Geus⁴⁵ states that N supply from natural sources — a very low content in rain and irrigation water, liberation of N from decomposition of organic matter, and fixation of atmospheric N by bacteria and algae — is usually quite inadequate, and that in most rice-growing countries, N application is a most effective way to increase yields. He gives the normal rate of application for wetland rice at 30 to 60 kilograms per hectare, with rates above 60 seldom needed to achieve economical yield responses. For Japan, the average rate of N application from commercial fertilizers is 60 kilograms per hectare; an additional amount of about 35 kilograms comes from farm manures. (Japan seems the exception to his general rule.) De Geus reports on experimental results from many countries that indicate increased use of N above current levels as a desirable practice. This is especially so in countries of the Indian peninsula and southeast Asia.

(U) Umali⁴⁶ compared the amounts of N used in the Philippines and Japan, 3 versus 85 kilograms per hectare, in an article designed to illustrate the need for his countrymen to use more fertilizers.

(U) Singh and Singh⁴⁷ report results of a four-year experiment on the influence of N and phosphorus fertilizers, date of transplanting, and spacing on rice yields in the Punjab: "Though nitrogen application, even up to 80 pounds per acre, has remarkable effects on grain yield of rice, 40 pounds of nitrogen per acre is the most profitable and economic dose."

* Personnal communication.

CONFIDENTIAL

CONFIDENTIAL

85

(U) Widespread use of commercial fertilizers on rice in the United States was not practiced until about ten years ago, when acreages were put under government control. Current recommendations⁴⁸ for N application are 30 to 60 pounds per acre in Louisiana, 40 to 50 (rarely 80 to 100) in Arkansas, and 40 to 80 in Texas. Recommendations for the use of 40 to 80 pounds of N per acre are being made by the Experiment Station at Crowley, Louisiana, but lodging is sometimes a problem even at the 40-pound rate. Blast was not commonly seen in Louisiana prior to 1955, after which time the acreage controls forced widespread use of fertilizer. Incidentally, the presence of blast can be used as an aid in judging the effectiveness of a farmer's fertilizer program in Louisiana. If blast is present then either N or new land has been used. If brown spot, caused by Helminthosporium oryzae, is present then either too little or no N has been applied.

(U) The International Rice Research Institute⁸ reports that ". . .the very practices which farmers in the tropics are being urged to adopt -- higher fertility levels and larger numbers of plants per unit area -- may present a new and serious problem unless resistant varieties (to blast) are made available." Some indication of how high these fertility levels in the tropics may go is also given. Their experiments on both Japonicas and Indicas at the Institute indicate that, for a given spacing between hills, fertilizer rates above certain levels may actually decrease yields (perhaps some sterility factor is involved). Yields of Japonicas either did not increase significantly or actually decreased with N applications above 60 kilograms per hectare, yields of Indicas similarly decreased above 40 kilograms per hectare (not to mention how badly they lodged).

(U) As a summary of this section several comments are appropriate. Irrespective of the rice blast problem, scientists are not going to recommend nor farmers use rates of N fertilizer that are too high to give economical yield responses and that contribute to lodging. The Japonica varieties give good yields and do not lodge severely at rates of N currently used in Japan and Taiwan. Blast is a problem at these rates whenever weather conditions favor the disease, and is controlled by one of the methods discussed in Section IX. The rates of N currently used or recommended in areas that grow chiefly Indicas do not seem to approach levels that would encourage epiphytic development of blast and it is doubtful that higher levels will be recommended until varieties are developed that give good yields without lodging at these higher levels. Rates of 120, 160, or 240 pounds of N per acre on Japonicas, 80 or 131 on Indicas, or 120 and 240 on the U.S. mixtures have been cited above as contributing to serious blast damage. These rates are not used now and are not likely to be widely used commercially in any country at any time in the foreseeable future.

CONFIDENTIAL

VII. (U) SPREAD

(U) The measurement of spread is complicated by the need to know the source(s) of the spores acting as inoculum. Otherwise, measurements of disease may indicate "occurrence" rather than "spread." Spread occurs within fields and, apparently, from field to field, although the latter is not well-documented.

A. (U) WITHIN A FIELD

(U) In 1954, the pathogen spread over most of a 12-acre field of Zenith rice growing in Florida. The original source of inoculum for this spread was an adjacent one-half acre containing a number of small plots of Zenith and other rice varieties that had been experimentally inoculated. Spread from this area of small plots was 450 feet in 13 days, 600 feet in 20 days, and 700 in 26 days. A decrease in disease severity occurred after about 20 days, and workers at the site associated this decrease with decreased soil fertility, especially decreased nitrogen.⁴⁹

(U) The following year another study on spread was made in Florida.¹ Eighteen acres were planted in an Archimedean spiral. Plots of Zenith and C.I. 8970 were alternated in 5-foot-wide strips out to the 300-foot perimenter beyond which Zenith alone was planted. Disease observations were made at sampling stations located at regular intervals along polar coordinates. An inoculation was made on 26 May in the center of the field and covered an area 48 feet in diameter. Little spread was found on 9 June. From 23 to 28 June, however, substantial disease increase was observed and was associated with weather conditions thought favorable for spore incubation from 10 to 12 June. Spread was definitely associated with the prevailing wind direction from 10 to 12 June. We cannot locate explicit information on the distances of spread with time.

(U) In 1964, the centers of 3 one-acre fields of Cultrose rice near Avon Park, Florida, were experimentally inoculated with Rice 1 (isolate 429). The center plots were of different sizes in each field and acted as foci for disease spread. A brief summary follows:^{*}

* Barksdale, T.H. Unpublished data, 1964, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

	<u>Paddy 4</u>	<u>Paddy 3</u>	<u>Paddy 5</u>
Size of focus, square feet	4	400	4356
Initial lesions per foot of row	4.25	17	15.9
Number of days from inoculation to first observation of:			
Buildup in focus	14	14	10
Spread from focus	17	17	11
Spread to 100 feet or more	23	22	14
Disease everywhere in field	48	35	14
Per cent panicle blast	94.5	64.3	68.3
Rough rice weight reduction, %	84.6	66.6	80.7

Buildup and spread were noted first in Paddy 5, which contained the largest focus. Data from Paddy 4 show that an initial infection of 17 lesions placed in the center of one acre can result in a serious epiphytotic. Perhaps fewer (one?) lesions could, also. Spread to 100 feet in some direction in all fields between 14 and 23 days does not mean that spread would not have been observed farther or sooner if larger fields had been used. Meteorological conditions and agronomic practices were considered ideal for disease development and were essentially the same in all fields.

B. (U) OVER LONG DISTANCES

(U) A serious, natural epiphytotic of rice blast occurred in Florida in 1953. Most of the fields affected were located more or less in a line near a canal running southeast to northwest. Workers⁵⁰ who observed this epiphytotic suggested that spread occurred from southeast to northwest, the direction of the prevailing winds at that time of year. We gained the impression from their report that first observations in most fields had been made after rice finished its tillering stage, and in some fields around heading time or thereafter. So, although their suggestion that spread occurred downwind for several miles may be correct, their observations may have been made too long after the fact to establish it with as much certainty as is desirable.

(U) One of the observations made in the 1953 survey⁵⁰ is especially interesting: The Turner-Hinman field had no observable leaf blast but did have a substantial amount of panicle blast. Similar observations were made by Willis and by Marchetti* for specific fields or varieties in areas where they worked in 1963. The supposition in all three instances is that the fields or varieties in question were inoculated from an outside source. If there really were no local sources of inoculum, these instances are excellent examples of spread. They would also lessen the reliability of any mathematical scheme designed to predict panicle blast or yield reduction on the basis of a disease parameter of the leaf stage.

(U) The analysis of Allison's data from Florida in 1961 revealed an association between yield in fields where blast occurred and the location of these fields (Figure 5). Fields were located at distances of about three miles from each other along a general line running southeast to northwest. Yield reductions of about 20 bushels per acre occurred for each 3-mile interval from the field on the upwind side of the site, although the design of the experiment did not permit a precise measurement of yield reduction for each increment of distance downwind. Allison's tentative conclusion was that the distance between fields was not sufficient to prevent significant spread of inoculum from upwind to downwind fields.**

(U) An experiment to test this hypothesis was performed in the same area the following year, and the same or adjacent field sites were used. The upwind field was not inoculated and was intended to serve as a check. The next field in line was uniformly inoculated. All other fields were left uninoculated. Disease eventually developed in all fields, but data on time of infection and amount of yield reduction do not substantiate the hypothesis.**

(U) There is no question that blast can spread; however, there are almost no quantitative data describing spread or the conditions influencing its direction, speed, or distance.

* Personal communication.

** Allison, W.H. Unpublished data and the associated Analyses 5219 (28 Mar 1962), 5312 (17 May 1962), and 5744 (16 Apr 1963), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

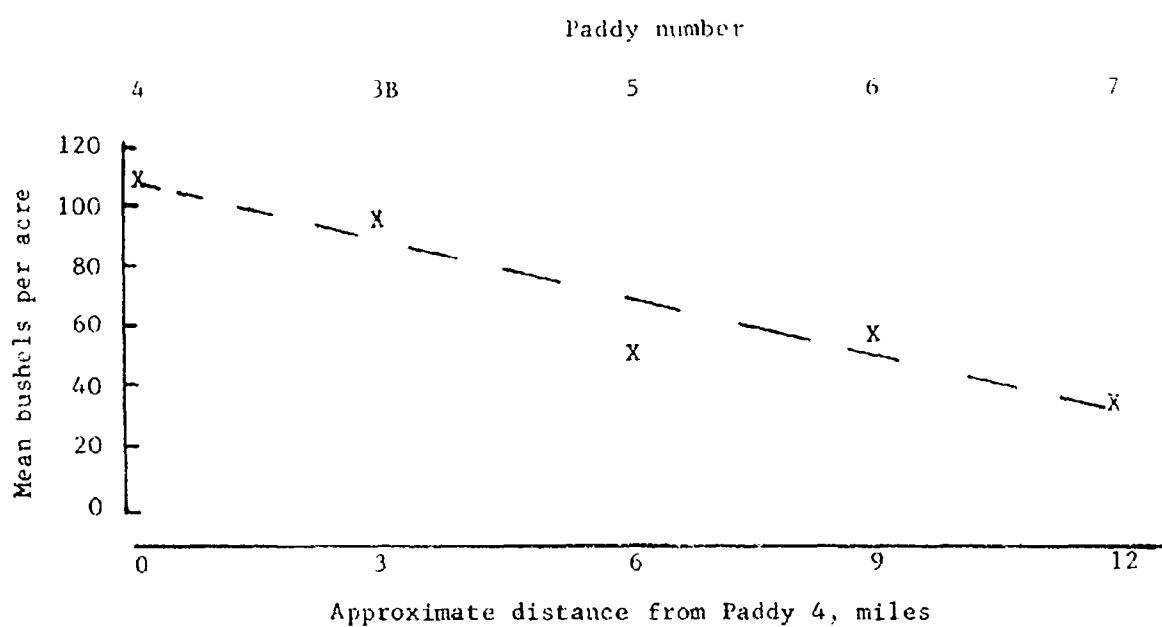


Figure 5. (U) Reduction in Yield with Distance Downwind. (U)
(Unpublished data, Allison)

CONFIDENTIALVIII. (C) YIELD REDUCTIONS

(U) Many statements about yield loss caused by rice blast can be found in the literature; some of the most recent are in the symposium held at the International Rice Research Institute.⁵ Padmanabhan^{5*} states that losses from blast in ten states in India in 1960 and 1961 ranged from 0.01 to 9.73% with an average loss of 0.8%. Goto^{5**} gives the losses from blast in Japan for the years 1953 through 1960 in order as 7.3, 1.4, 1.4, 4.0, 2.9, 2.5, 1.9, and 2.4%. When a percentage loss figure for a certain area is given, it is usually not clear how the estimate was made (and to what extent political considerations may have entered the estimating procedure). There have been few scientists reporting about the techniques of estimating loss.

(U) Yields can be reduced by disease on the leaves (Table 13) and by infections on the panicles. Infections on leaf sheaths and on nodes can also cause loss, but these infections are not usually considered. This may be either because they can be grouped under one of the other headings or because they are not common occurrences in the field. Infections on the leaves do not cause losses that are obvious to the casual observer unless the field is seriously affected or nearly destroyed. Infections on the panicle are usually so obvious as to be readily associated with yield loss.

(U) Since the correlation between rice yield and the number of productive tillers for a given rice variety is very high, we consider the percentage of panicle blast as a conservative estimate of yield loss. Dahlke*** substantiated this idea in 1963 during a study of epiphytotes occurring on several varieties of U.S. and foreign origin. Negative correlation coefficients were found between yield (measured in bushels per acre) and the percentage of panicle blast on those varieties that were severely affected on the leaves:

* Pages 203 through 222.

** Pages 195 through 202.

*** Dahlke, G.R. Unpublished data and the associated Analysis 6433 (5 Aug 1964), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

CONFIDENTIAL

91

<u>Variety</u>	<u>Geometric Mean Yield, pounds per acre</u>	<u>Mean Panicle Blast, %</u>	<u>Correlation Coefficient</u>
Toro	434.4	71.3	-0.912**
Arkrose	429.3	67.3	-0.891**
Colusa	665.9	59.9	-0.772**
Nato	380.9	80.6	-0.651*
Rexoro	1068.8	32.3	-0.972**
C.I. 8970	485.5	70.9	-0.888**
Fujisaka 5	163.0	88.3	-0.879**

* Significant at the 95% level.

** Significant at the 99% level.

(U) Goto^{s*} summarizes the work done on the correlation between yield loss (Y) and the severity of panicle blast (X) by two statistical offices in Japan. Here X was derived by examining each panicle in a sample and assigning it a disease severity rating using intervals of 10%:

$$X = \frac{\text{Sum of severity ratings for each panicle}}{\text{Total number of panicles in sample}}$$

An alternative method was just as satisfactory and required less work:

$$X = \frac{80(\text{Number of severely affected panicles}) + 50(\text{Number of moderately affected panicles}) + 20(\text{Number of lightly affected panicles})}{\text{Total number of panicles in sample}}$$

Samples were examined 25 days after heading. The method for determining yield loss was not stated. The formulas derived by the two offices are:

$$Y = 1.23 X - 6.4; \quad r = 0.955 \text{ for 45 samples}$$

$$Y = 1.32 X - 13.5; \quad r = 0.983 \text{ for 10 samples}$$

These two formulas are probably very useful in the local areas for which they were developed. They certainly indicate a high correlation between panicle blast and yield reduction.

* Pages 195 through 202.

CONFIDENTIAL

CONFIDENTIAL

(C) Another way of estimating loss is to compare actual grain yields in a diseased plot with yield expected if no disease had been present. The disease-free yield could be obtained from some suitable control plot in the same field. Fungicides were used by Dahlke and the senior writer to obtain checks in Florida and Okinawa.* An alternative method would be to assume that the conditions of the experiment would have produced a yield near that of the long-time average yield for the same variety. Because rice yields in the United States vary as much as 1,000 to 2,000 pounds per acre from year to year,³¹ this method is not very good.

(U) Examples of per cent loss recorded in Table 7 (Section IV, C) are based largely on percentage of panicle blast. Other examples of losses observed in the field can be found in published^{38,49,50} and unpublished data from Crops Division. These losses range from zero to nearly 100% for a given field. If losses were low, the reasons usually were thought to be one or more of the following: (i) an unsuitable race-variety combination, (ii) unfavorable weather for incubation during some part of the growing season, and (iii) predisposition of the plants toward resistance by drought, plant aging, high night temperatures, or low nitrogen. On the other hand, favorable weather, an abundance of inoculum, and high nitrogen levels are usually cited as the principal factors contributing to severe epiphytotics and to high yield reductions.

* Unpublished data, Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland.

CONFIDENTIAL

CONFIDENTIAL

93

IX. (C) PREDICTIONS AND CONTROL

(C) In van der Plank's analyses of epiphytotics,^{37,61} he emphasizes the rate of disease increase. He shows that for some diseases it is possible to evaluate the effectiveness of a particular control measure in terms of the number of days that the onset of the epiphytotic can be delayed. It may some day be possible to apply his concepts to rice blast and to answer questions like the following. (i) How resistant or tolerant a variety should the rice breeder develop so that the rate of disease increase in his area will not become too rapid? (ii) When should a farmer apply fungicide sprays so that onset of the epiphytotic will be delayed during those periods when plant growth stages or weather conditions are most favorable for epiphytotic development? (iii) When can the farmer apply topdressings of nitrogen fertilizer that will increase his yield without increasing the rate of disease increase?

(C) The ability to predict epiphytotics, i.e., to describe in some mathematical way disease occurrence, increase, and the resulting yield loss, can be a two-edged sword. Just as this knowledge can be used to control the disease, it can also be used to increase the likelihood or severity of disease, if that should be the interest of the experimenter. For example, van der Plank cites instances where some cultural practice or field control measure involving the removal of diseased plants or other source of inoculum (i.e., sanitation) is used to decrease the amount of inoculum present in the early growth stages of plants, and this decrease delays onset of the epiphytotic. But suppose, in the instance of rice blast, that the rate of increase were given a boost early in the rice-growing season by disseminating inoculum artificially? Prediction equations should indicate the quantity of such artificial inoculum and the timing of its application. For prediction purposes, it may eventually be necessary to develop several groups of equations, perhaps one group for Japonicas and one for Indicas. Within each group it may prove useful to consider short-, medium-, and late-maturing varieties separately. Some progress in this direction has been made and is discussed after the principal control measures now used commercially are described.

(L) The most widely used control method is growing resistant varieties. The most recent summary of the work being done to develop resistant varieties is found in several papers presented at the Symposium held at the International Rice Research Institute.⁶ For a number of years, breeding for resistance has been carried out in the United States, Taiwan, India and, especially, Japan. The breeder's problem is intensified by the existence of races of the pathogen, some of which appear to predominate in localized or regional areas of the world.⁷ Because the scientific community in most countries seems hesitant to import races or cultures of Piticularia found in other parts of the world for testing,

CONFIDENTIAL

rice varieties are being exchanged. On the basis of his experience in Taiwan, Thailand, and the Philippines, Dr. Ou⁸ of the International Rice Research Institute has standardized a nursery bed design to test the reaction of seedlings to blast in the field, and the institute hopes to establish similar test nurseries in other rice-growing countries. Such a nursery was constructed at the Institute in 1962 and it can be used to test 2,000 varieties or breeding lines at a time. Once the Institute establishes nurseries in other areas and furnishes local scientists with breeding material to be tested, international progress in developing resistant varieties will advance rapidly. In addition, the world pattern of distribution of races of Piricularia will become better understood.

(U) Fungicides can be used to control the disease in either the leaf or panicle stage. The Japanese have been leaders in the development of fungicides for blast control, and their progress has been reviewed.^{5*} Organic mercurial compounds, especially phenyl mercury acetate, have been on the market in Japan for years and are widely used there. Serious objections to the use of mercurials in the United States undoubtedly would arise for public health reasons. Recently, an antibiotic compound, Blasticidin S, has been developed and commercial formulations usually include a mixture of phenyl mercury acetate and the antibiotic. A recent summary of the research and development of this fungicide indicates that Japanese farmers used it on about 160,000 hectares in 1962, and its use was expected to double or triple in 1963.⁶² The senior author's experience with Blasticidin S on a limited experimental scale indicates that the material gives excellent control.

(U) Quite naturally, the Japanese have developed chemicals effective for the Japonica rice varieties. The mercurials and to some extent the commercial formulations of Blasticidin that contain mercury are phytotoxic to Indica varieties, however, and are not recommended for use on them. Thus, areas where Indicas are grown are left without as good a fungicide as would be desirable.

(U) The use of chemical control is determined by economics, whether the farmers can afford to buy the fungicides and application equipment. Japan is the only country in Asia that is economically developed to the point where farmers can afford to use fungicides on a large scale. Taiwanese farmers use them to a lesser extent, but their use elsewhere in Asia is practically nil. In 1963, Okamoto stated:^{6*}

"In Japan, the average yield of rice is about 4000 kilograms per hectare for brown rice and its official price is about \$200 per 1000 kilograms (\$800 per hectare on an average).

"Chemical control of blast with the most expensive dust costs about \$11 to \$33 per hectare."

* Pages 399 through 408.

CONFIDENTIAL

CONFIDENTIAL

95

(U) Equipment for the application of fungicides and insecticides is modern and in commercial use in Japan. Just as American rice farmers have turned to the airplane for disease and insect control, so have the Japanese, although the latter use mostly helicopters instead of fixed-wing aircraft because of the small fields, numerous telephone and electrical lines, and numerous buildings. Aerial applications of fungicides to control blast was made on 1,045 hectares in 1958 and 5 years later, in 1962, this aerially treated acreage had increased to 140,150 hectares.⁵⁴

(U) The use of various agronomic practices to control or moderate the effect of blast had already been discussed in Section VI, C.

(U) None of the prediction equations now available are complete in the sense that they can predict yield (or yield reduction) by combining terms relating to inoculum levels, agronomic practices, weather conditions, rates of disease buildup, levels of disease severity, and other variables into one equation. However, there are some beginnings.

(U) Rorie¹⁹ made a series of nightly inoculations in order to find out how several variables affected the establishment of primary infection following an artificial inoculation. From the analysis of his data, the following equations were obtained for the amount of inoculum indicated:

for 0.0 gm/acre, $X_1' = -2.3614 + 0.01304 X_2 + 0.04765 X_3 + 0.01520 X_{10}$;

0.1 gm/acre, $X_1' = -2.1974 + 0.003436 X_2 + 0.06753 X_3 + 0.01419 X_{10}$;

1.0 gm/acre, $X_1' = -2.3460 + 0.07117 X_2 + 0.1087 X_3 + 0.01284 X_{10}$;

10.0 gm/acre, $X_1' = -1.7344 + 0.1150 X_2 + 0.1321 X_3 + 0.008159 X_{10}$;

where X_1' is the log (average number of lesions plus 0.01),

X_2 is the amount of dew based on an arbitrary scale, 1 through 8,

X_3 is the length of dew period in hours, and

X_{10} is the leaf area in square centimeters at the time of inoculation.

(U) The first equation indicates the possibility of lesion occurrence without artificial inoculations, a fact due to the presence of a low level of natural inoculum. Temperature, a variable expected to enter into these estimates, was not limiting during his field study, or was not limiting on enough nights, and its effect on lesion production was not significant. The 95% confidence limits for the equation coefficients are given in his report.

* Pages 415 through 420.

CONFIDENTIAL

CONFIDENTIAL

(U) Kulik* did similar work. He used two varieties and only one inoculum level, ten grams per acre. Results of lesion counts were made on the basis of both a sample of 50 plants and a leaf area of 100 square centimeters. Estimating equations for a given variety and basis of lesion count indicated follow:

for Colusa per 50 plants, $Y' = -2.28 + 0.094 X_3 + 0.024 X_8$

for Colusa per 100 sq cm leaf area, $Y' = -2.29 + 0.125 X_3 + 0.028 X_8$

for 8970 per 50 plants, $Y' = -2.96 + 0.126 X_1 + 2.52 X_6 + 0.029 X_8$

for 8970 per 100 sq cm leaf area, $Y' = -3.00 + 0.152 X_1 + 3.19 X_6 + 0.029 X_8$

where Y' is the log (average number of lesions plus 0.01),

X_1 is the length of dew period in hours,

X_3 is the period of 100 per cent relative humidity in hours,

X_6 is the amount of daily rainfall in inches, and

X_8 is the average leaf area at the time of inoculation in square centimeters.

Again, in this test the effect of temperature could not be determined. The length of dew period contributed similarly to the estimate of lesions in both Kulik's and Rorie's data; however, in Kulik's work the contribution of leaf area at the time of inoculation was greater.

(C) Natural outbreaks of blast can be predicted in some areas on the basis of weather. The senior author thought that the first occurrence of blast on Okinawa for the first crop could be predicted for the week following the rise of minimum temperatures about 68 to 70 F, provided that there were dew periods of suitable length. A prediction made on this basis would have proved especially useful in 1962 when there was a rapid warming trend at the end of April.

(U) Ono^{6**} gives an introduction to the Japanese literature on blast disease forecasting, and examples of formulas derived at several Japanese Prefectural Experiment Stations follow. In Yamashiki Prefecture the area of disease outbreak (y) is given by:

* Kulik, M.M. Unpublished data and the associated Analyses 4451 (5 Dec 1960) and 4447 (20 Sept 1960), Crops and Biomathematics Divisions, U.S. Army Biological Laboratories, Frederick, Maryland.

** Pages 173 through 194.

CONFIDENTIAL

97

$$y = 12703.73 + 257.34 x \quad r = 0.761$$

where x is the average percentage of sunshine in June and July. In other words, the less sunshine (the more cloudy and rainy the weather) the more blast. Units of sunshine and area are not given. In Yamaguchi Prefecture a series of formulas has been developed:

$$y_1 = 144.8 + 5.35 a \quad r = -0.69$$

$$y_1 = 31.3 + 0.366 b \quad r = -0.79$$

$$y_1 = 0.18 c + 5.6 \quad r = +0.88$$

$$y_2 = 75590.7 - 2886.96 d \quad r = -0.65$$

where y_1 is the area of leaf blast outbreak,

y_2 is the area of damage,

a is the average temperature from 20 to 25 June,

b is the maximum temperature from 26 to 30 June,

c is the precipitation from 26 to 30 June, and

d is the average temperature from 1 to 5 July.

The units for these formulas are not given and it is difficult to describe what they mean in physical terms without knowledge of the plant growth stages for the dates given. It is interesting, however, that formulas of this type, which ignore inoculum potential and disease severity, can be derived and are presumably useful for specific rice-growing areas. A similar type of equation for estimating yield on the basis of rainfall and a degree-hour term was given above in Section VI, B, 2 from unpublished data of Allison.

(U) A formula taking fertilizer levels into account has been obtained also. In Iwata Prefecture the area of disease outbreak (z) is estimated:

$$z = 0.40 x + 6.67 y + 29.06 \quad r = 0.96$$

where x is the sum of the sunshine times in July and y is a mature index using the 1937 through 1939 level as an index value of 10. Units are not clear in this formula, either. Kobayashi and co-workers predict

CONFIDENTIAL

CONFIDENTIAL

outbreaks of neck blast by measuring the soluble nitrogen, total nitrogen, silica dioxide, or any combination of them in the top leaf of the rice plant. Ichikawa's group used the ash-to-nitrogen ratio to predict outbreaks. Hori is reported to have developed forecasting formulas using the number of silicated cells in sections of the top leaf collected at the time of ear formation.*

(U) Correlations between yield reduction and particle blast like those described in Section VIII should be useful for predictions.

(U) In Section IV, C, we attempted to use a curve (Figure 1; based on minimum requirements of dew period and temperature to predict whether infections were likely to occur on specific nights. An attempt was also made in the analysis of data from several epiphytotics (Appendix and Table 7) to use the percentage of nights with conditions described by points above the limits of this curve to gain information about the rate of disease buildup and yield reduction. Equations for the variables having a highly significant correlation coefficient with yield reduction were computed:

$$(1) y = -4.116 + 1.6278 x_1 \quad r = 0.705$$

$$(2) y = 18.389 + 0.12375 x_2 \quad r = 0.769$$

$$(3) y = -76.854 + 1.9296 x_3 \quad r = 0.749$$

where y is the percentage yield loss,

x_1 is the exponential rate of disease increase times 100,

x_2 is the highest lesion count observed, and

x_3 is the percentage of days with weather conditions favorable for incubation. The above equations, together with the observed values and lines indicating limits, are found in the Appendix. When all three variables were used in a regression equation for per cent yield loss, the coefficient for the rate term was not significant. Hence, a regression equation combining the other two variables was computed:

$$y = -50.94 + 0.08426 x_2 + 1.2343 x_3$$

and the multiple correlation coefficient was highly significant ($R = 0.88$). A table with y solved for several intervals of x_2 and x_3 is found in the Appendix.

* Pages 173 through 194.

CONFIDENTIAL

99

(U) In making predictions about some aspect of rice blast epiphytology, one naturally chooses those variables that seem most suitable for the purpose in mind. In some instances this will be a rate of disease increase calculated after van der Plank's formulas, in other instances certain weather data, and so on. At present, we do not know which information will permit development of the most accurate and most useful prediction equations. To use the equations immediately above, one must wait until the epiphytotic is nearly over to obtain lesion or rate data; or, one must be able to predict dew periods. We are still in an empirical stage where each time workers study epiphytotics they should gather as much data as time, facilities, and personnel will allow, and where statisticians should examine the data in a variety of ways.

(U) One of the best summaries of the present status of rice blast epiphytology has been made by Hashioka,^{6*} and his summary provides a suitable closing statement for this report, also.

"The effects of the individual environmental factors on the different phases of growth of the fungus, disease development, and host predisposition in the rice blast disease have been studied extensively. . . . On the other hand, experimental research on the complex simultaneous effects of more than two environmental factors has been limited to the present despite their importance for the population ecology of the disease, i.e., epidemiology."

(U) In this report, all factors known to affect the epiphytology of the rice blast disease are described individually, and, where known, in combination. Where possible, these factors have been quantified.

* Pages 153 through 162.

CONFIDENTIAL

(U) LITERATURE CITED

1. Minarik, C.E., and F.M. Latterell. August 1958. Anticrop agents (U), (Technical Study 11). Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland. SECRET (58-FDS-666).
2. Cavara, F. 1891. *Fungi Longobardiae exsiccati sive Mycetum specimina in Longobardia collecta, exsiccata et speciebus novis vel criticis, iconibus illustrata.* Pugillus I, No. 49.
3. Padwick, G. Watts. 1950. Manual of rice diseases. Commonwealth Mycological Institute, Kew, Surrey.
4. Latterell, F.M., and E.C. Tullis. June 1955. Screening Piricularia isolates for pathogenicity to rice, (Special Report 222). Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland. UNCLASSIFIED.
5. International Rice Research Institute. 1965. The Rice Blast Disease. Johns Hopkins Press, Baltimore, Maryland. From Symposium on the rice blast disease, Los Banos, Philippines, July 7-12, 1963.
6. Cherry, E., and R.E. Williamson. February 1958. A single-unit system for producing spores of Piricularia oryzae (U), (Interim Report 168). Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland. CONFIDENTIAL.
7. Latterell, F.M., M.A. Marchetti, and B.R. Grove. April 1964. Evaluation of rice varieties as differential hosts for races of P. oryzae, (Technical Manuscript 92). Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland. UNCLASSIFIED.
8. International Rice Research Institute, Los Banos, Philippines. Annual Report, 1961-1962. 1962. 55pp.
9. Grist, D.H. 1959. Rice. 3rd ed. Longmans, Green and Co., Ltd., London.
10. Matsuo, T. 1955. Rice culture in Japan. Yokendo, Tokyo.
11. Efferson, J.N. 1952. The production and marketing of rice. The Rice Journal, New Orleans.
12. Wilson, Emmett. 1962. The growth and development of the rice plant, p. 225-250. In Fifty-fourth annual progress report. Rice Experiment Station, Crowley, Louisiana.

13. Hemmi, T., and K. Yokogi. 1928. Experimental studies on the pathogenicity of certain fungi on rice seedlings. Mem. Coll. Agr., Kyoto Imperial Univ. 7:1-22.
14. Hemmi, T., and S. Endo. 1929. On the possibility of soil infection of Piricularia oryzae and its relation to soil moisture. Agr. Hart, Tokyo (Japan) 4:773-784.
15. Hashioka, Y. 1950. Studies on the mechanism of prevalence of the rice blast disease in the tropics. Taiwan Agr. Res. Inst. Tech. Bull. 8:1-237.
16. Andersen, A.L., B.W. Henry, and T.L. Morgan. 1948. The effect of temperature and relative humidity upon the viability of the conidia of Piricularia oryzae. Phytopathology 38:574.
17. Panzer, J.D., and R.D. Beier. 1958. A simple field inoculation technique. Plant Dis. Rep. 42:172-173.
18. Panzer, J.D., E.C. Tullis, and R.D. Beier. 1957. An explosion inoculation technique. Plant Dis. Rep. 41:225-226.
19. Rorie, F.G., and G.N. Asai. February 1960. The effects of certain environmental factors on initial infection by rice blast, (Technical Memorandum 9-13). Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland. UNCLASSIFIED.
20. Barksdale, T.H. June 1963. Rice blast epiphytology studies at Avon Park, Florida, 1960, (Technical Memorandum 27). Crops Division, U.S. Army Biological Laboratories, Frederick, Maryland. UNCLASSIFIED.
21. Kahn, R.P. May 1958. The blast disease of rice: I. Additional investigation of factors affecting infectivity and sporulation, (Interim Report 132). Crops Division, Fort Detrick, Frederick, Maryland. UNCLASSIFIED.
22. Barksdale, T.H., and G.N. Asai. 1961. Diurnal spore release of Piricularia oryzae from rice leaves. Phytopathology 51:331-317.
23. Hemmi, T., and J. Imura. 1939. On the relation of air humidity to conidial formation in the rice blast fungus, P. oryzae, and the characteristics in the germination of conidia produced by strains showing different pathogenicity. Ann. Phytopathol. Soc. Japan 9:147-156.
24. Johnson, T.W., and J.E. Halpin. 1954. Environmental effects on conidial variation in some fungi imperfecti. Elisha Mitchell Sci. Soc. 70:314-326.

25. Hemmi, T., T. Abe, Y. Inoue, and J. Ikeda. 1936. Studies on the rice blast disease: IV. Relation of the environments to the development of the blast disease and the physiologic specialization in the rice blast fungus. Mater. Rural Improvement Dept. Agr. Forestry, Japan 105:145 pp.
26. Andersen, A.L., B.W. Henry, and E.C. Tullis. 1947. Factors affecting infectivity, spread, and persistence of Piricularia oryzae Cav. Phytopathology 37:94-110.
27. Panzer, J.D., E.C. Tullis, and E.P. Van Arsdel. 1957. A simple 24-hour slide spore collector. Phytopathology 47:512-514.
28. Aerosol Laboratory, Department of Chemistry and Chemical Engineering, Stanford University, California. The rotorod sampler, (Second Semiannual Report, March to August 1957). (Contract DA-18-064-404-CML-186). UNCLASSIFIED.
29. Van Arsdel, E.P., E.C. Tullis, and J.D. Panzer. 1958. Movement of air in a rice paddy as indicated by colored smoke. Plant Disease Rep. 42:721-725.
30. Jones, J.W., C.R. Adair, H.M. Beachell, N.E. Jodon, and A.H. Williams. 1953. Rice varieties and their yields in the United States, 1939-1950. U.S. Dept. Agr. Cir. No. 915.
31. Rice Experiment Station, Crowley, Louisiana. 1962. Fifty-fourth annual progress report.
32. Kawai, I. 1952. Ecology and therapeutical studies on the rice blast. Shizuoka Agr. Exp. Sta. Tech. Bull. No. 4.
33. Kahn, R.P. October 1956. The blast disease of rice: I. Factors affecting infectivity (U), (Interim Report 132). Crops Division, Fort Detrick, Frederick, Maryland. CONFIDENTIAL.
34. Sueda, H. 1928. Studies on the rice blast disease, (Report 35). Dep. Agr. Res. Inst., Formosa.
35. Weintraub, R.L., W.E. Miller, and E.H. Schantz. 1958. Chemical stimulation of germination of spores of Piricularia oryzae. Phytopathology 48:7-10.
36. Sharp, E.L., C.G. Schmitt, J.M. Staley, and C.H. Kingsolver. 1958. Some critical factors involved in establishment of Puccinia graminis var. tritici. Phytopathology 48:469-474.

37. van der Plank, J.E. 1960. Analysis of epidemics, pp. 229-289. In J.G. Horsfall and A.E. Dimond. (ed.) Plant pathology, an advanced treatise. Vol. III. Academic Press, New York.
38. Beier, R.D., J.D. Panzer, and E.C. Tullis. 1959. The interrelationship of nitrogen and other factors affecting the blast disease of rice caused by Piricularia oryzae Cav. Plant Dis. Rep. 43:477-482.
39. Ono, K., and H. Suzuki. 1960. Studies on the mechanism of infection and ecology of blast and stem rot of rice plants. Special Report. Forecast Disease. Insect Pests. 156 p.
40. Atkins, J.G. 1956. An outlook of Piricularia on rice in 1955. Plant Dis. Rep. 40:372-373.
41. Volk, R.J., R.P. Kahn, and R.L. Weintraub. 1958. Silicon content of the rice plant as a factor influencing its resistance to infection by the blast fungus, Piricularia oryzae. Phytopathology 48:179-184.
42. Green, V.E. 1958. Observations on fungus diseases of rice in Florida 1951-1957. Plant Dis. Rep. 42:624-628.
43. Otani, Y. 1959. Studies on the relation between the principal components of rice plant and its susceptibility to the blast disease and physiological characters of the blast fungus. J. Fac. Agr. Hokkaido Univ. 51(1). 179 p.
44. Williams, M.S., and J.W. Couston. 1962. Crop production levels and fertilizer use. UN Food & Agr. Organization, Rome. 48 pages.
45. De Geus, J.G. 1954. Means of increasing rice production. Centre D'Etude de L'Azote, Geneva. 143 pages.
46. Umali, D.L. 1962. The rice dilemma, its causes, effects, and suggested remedies. Philippine Agriculturalist 45:395-420.
47. Singh, S.G., and S.J. Singh. 1962. Effect of some agronomic factors on yield of rice in Punjab. Indian J. Agr. Sci. 32:62-70.
48. Adair, C.R., M.D. Miller, and H.M. Beachell. 1962. Rice improvement and culture in the United States. Adv. Agron. 14:61-108.
49. Schein, R.D., E.C. Tullis, and J.E. Mitchell. August 1956. Epiphytology of the blast disease of rice (U), (Interim Report 130). Crops Division, Fort Detrick, Frederick, Maryland. CONFIDENTIAL.

50. Schein, R.D., and E.C. Tullis. July 1954. Analysis of the rice blast epiphytotic caused by Piricularia oryzae in Florida, 1953 U.S. (Special Report 208). Crops Division, Camp Detrick, Frederick, Maryland. CONFIDENTIAL.
51. van der Plank, J.E. 1964. Plant diseases; epidemics and control. Academic Press, New York. 349 pages.
52. Misato, T. 1963. Blasticidin S, a new antibiotic for rice blast control. Nat. Inst. Agr. Sci. Tokyo, Japan.

(U) APPENDIX

(U) BASES FOR PREDICTION OF RICE BLAST EPIDEMIOLOGY

I. (U) MODEL FOR MINIMUM LENGTH OF DEW PERIOD
REQUIRED FOR INFECTION, AS A
FUNCTION OF TEMPERATURE

(U) Various laboratory experiments on rice blast that involved temperature and dew period have been performed by various workers. The data were reviewed and observations selected that indicated, for a given temperature, the minimum length of dew period at which infection occurred. In order to find a mathematical expression to define a linear relationship between the two variables and simultaneously fulfill a limiting requirement, various functions were investigated.

(U) The search for a suitable function was initiated by stating the requirements for the limiting feature as follows:

1. $\lim_{T \rightarrow \infty} f(T, D) = D_{\text{asymptote}} : D \geq D_{\text{asymptote}}$
2. $\lim_{D \rightarrow \infty} f(T, D) = T_{\text{asymptote}} : T \geq T_{\text{asymptote}}$
3. T varies inversely with D
4. The number of parameters preferably should not exceed two.
5. The function should linearize the observed relationship between T and D .

(U) The mathematical properties of several candidate functions were investigated as shown below.

I.

$$DT = a$$

$$\log D = \log a - \log T$$

$$\lim_{\log D \rightarrow \infty} (\log a - \log T) = -\infty \quad T \rightarrow 0$$

II.

$$DT^b = a$$

$$\log D = \log a - b \log T$$

$$\begin{aligned} \lim_{\log D \rightarrow \infty} (\log a - b \log T) &= -\infty & T \rightarrow 0 : (b > 0) \\ \log D &\rightarrow \infty \end{aligned}$$

III.

$$\frac{1}{D} = a + \frac{1}{T}$$

$$\begin{aligned} \lim_{D \rightarrow \infty} (a + 1/T) &= 0 & T_{asymptote} = -1/a \\ a & & D_{asymptote} = 1/a \end{aligned}$$

$$\begin{aligned} \lim_{T \rightarrow \infty} (a + 1/T) &= a & \text{But } D_{asymptote} \neq -T_{asymptote} \\ a & & \end{aligned}$$

IV.

$$\frac{1}{D} = a + b/T$$

$$\begin{aligned} \lim_{D \rightarrow \infty} (a + b/T) &= 0 & T_{asymptote} = -b/a : b < 0 \\ a & & D_{asymptote} = 1/a : a > 0 \end{aligned}$$

$$\begin{aligned} \lim_{T \rightarrow \infty} (a + b/T) &= a & D_{asymptote} = 1/a : a > 0 \\ a & & \end{aligned}$$

V.

$$D^a + T^b = k$$

$$\begin{aligned} \lim_{D \rightarrow \infty} (k - T^b) &= 0 & T_{asymptote} = k^{1/b} : a > 0 \\ k & & D_{asymptote} = k : b < 0 \end{aligned}$$

$$\begin{aligned} \lim_{T \rightarrow \infty} (k - T^b) &= k & D_{asymptote} = k : b < 0 \\ k & & \end{aligned}$$

Difficult to estimate a, b, k.
A three-parameter function.

(U) Functions I, II, and III were not acceptable because of the asymptotic properties; function V would be difficult to employ. Function IV was deemed satisfactory for the first four requirements as listed above.

(U) It was instructive but not initially obvious to examine the plot of

$$\frac{\Delta D}{\Delta T} \text{ vs } \left(\frac{D}{T}\right)^2$$

as a measure of assessing linearity.

Approximating $\Delta D/\Delta T$ by dD/dT and writing

$$\frac{dD}{dT} = b \left(\frac{D}{T}\right)^2$$

we proceed to solve this differential equation as follows:

$$dD/D^2 = b/T^2 dT$$

$$-1/D = -b/T - a \quad (\text{where } -a \text{ is the constant of integration})$$

$$\text{or } 1/D = a + b/T$$

which corresponds to candidate function IV. From both approaches to the evaluation of function IV it was concluded that it was currently satisfactory and that efforts to deduce a more accurate model must await refined data.

(U) As a result of this investigation, it was found that by taking the reciprocal of both length of dew period and temperature, the desired linearity resulted, as can be seen from Figure 1. A model that adequately describes "minimum length of dew period required for infection" as a function of "temperature" is then:

$$\frac{1}{D} = a + \frac{b}{T} \quad (1)$$

where: D is the minimum length of dew period required for infection, and

T is temperature.

This model is also consistent with the knowledge of the effect of these two variables on infection, since both are known to be limiting factors. That is, some minimum dew period and some minimum temperature are both known to be required for germination of the rice blast spore. Equation (1) is characterized by asymptotes other than zero for both D and T.

(U) Upon estimating the parameters in Equation (1) from the available data, the following equation resulted:

$$\frac{1}{D} = 0.2650 - \frac{12.26}{T} \quad (2)$$

where the unit of D is hours, and the unit of T is degrees Fahrenheit. Standard errors for the two parameters a and b were 0.0058 and 3.44 respectively. Ninety-five per cent confidence limits for the mean function and 80% prediction limits for individual values are shown on Figure 1; the numbers in parentheses indicate number of observations.

(U) The curve describing the lower 95% confidence limit can be estimated directly, without reference to Figure 1 or to the mean function, by use of the equation:

$$\frac{1}{D} = 0.2650 - \frac{12.26}{T} + 2.145 \sqrt{0.000033513 + 11.86598(\frac{1}{T} - 0.014290)^2}$$

where D is hours of dew and T is temperature in degrees F.

(U) Figure 2 shows the observed points, computed line of regression, and limits in terms of the original scales for both variables. Computed asymptotes were 46.25 F for temperature and 3.78 hours for minimum length of dew period required for infection. That is, the minimum temperature at which infection can occur approaches 46.25 F as the length of dew period becomes infinitely long. Similarly, the minimum length of dew period required for infection approaches 3.78 hours as temperature becomes infinitely high.

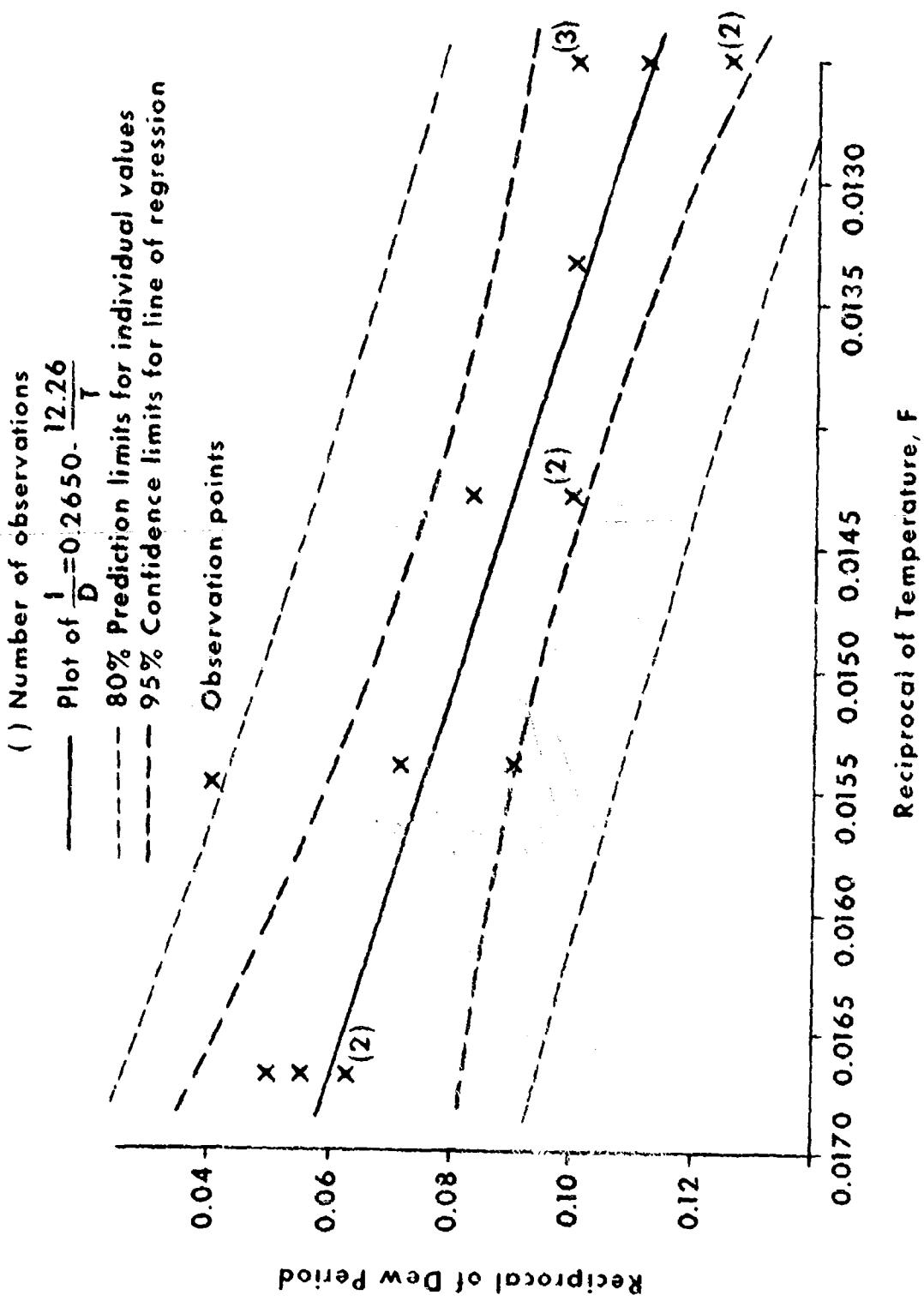


Figure 1. (Y) Minimum Length of Dew Period Required for Infection as a Function of Temperature. (U)

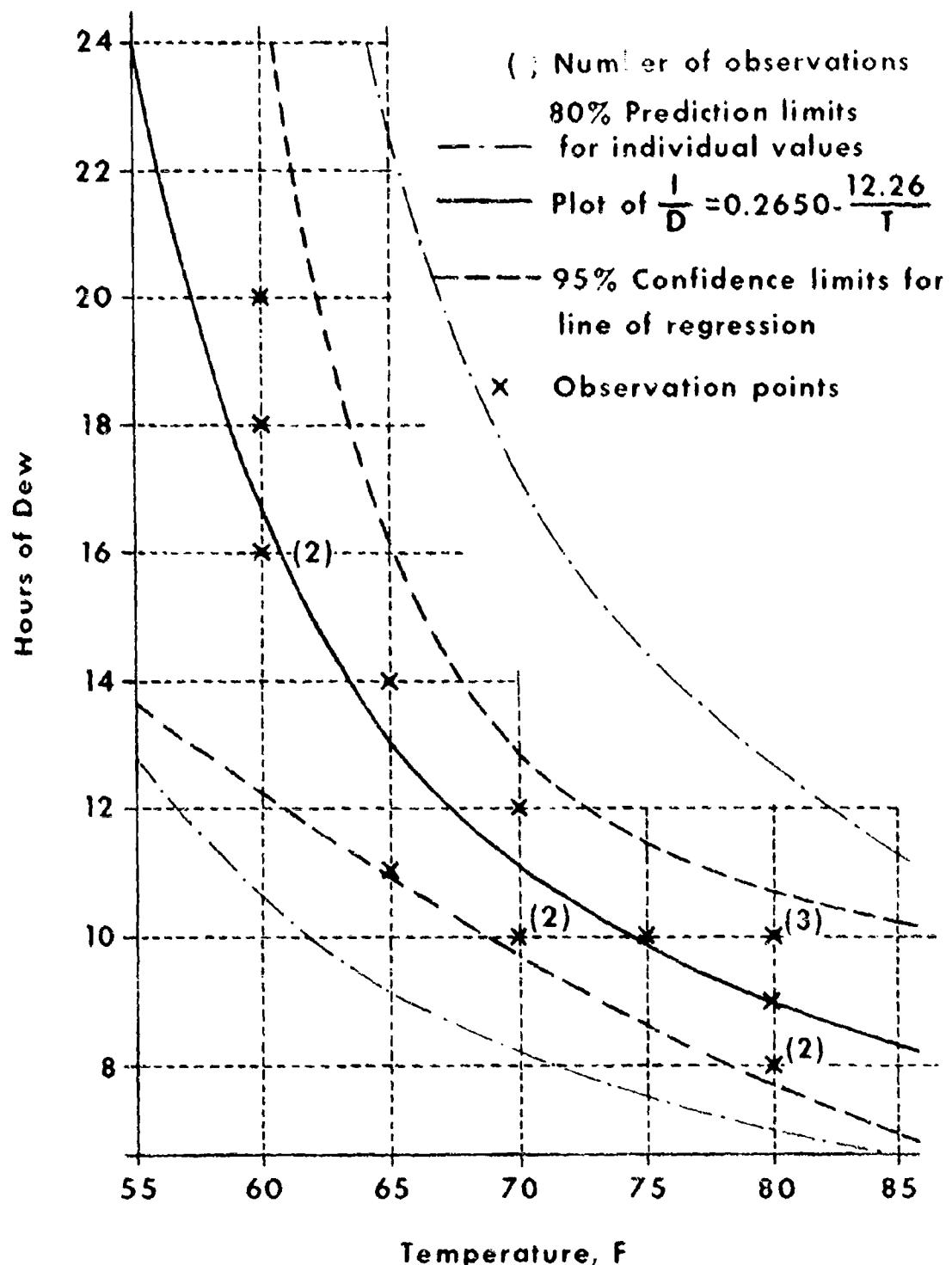


Figure 2. (U) Number of Lesions per Plant Obtained from Daily Field Inoculations at a Rate of Ten Grams per Acre Made by Rorie, Placed on Points Indicating the Average Night Temperature (2000 to 0800 hours) and Dew Period and Against a Background Indicating the Minimal Conditions for Establishment of Infection as Found in the Laboratory. (B)

II. AN EXAMINATION OF CANDIDATE VARIABLES
FOR USE AS PREDICTORS OF PER CENT YIELD
REDUCTION

(c) Available data from 24 rice blast epiphytotics were widely separated in time, location, rice variety, and race of the pathogen, and were obtained by various experimenters. Considerable variation was therefore to be expected. Should a basis that provides reasonably accurate estimates of epiphytotics be determined from these data, much more accuracy could be expected from application to a given location, rice variety, race of pathogen, time, and experimenter.

(d) Per cent yield reduction is considered the ultimate measure of an epiphytotic, and has therefore been used here to evaluate the efficiency as predictors of other measurements taken during the course of the epiphytotic. In most instances, the per cent yield reduction was taken as the per cent of panicle blast, which should give a conservative estimate. Candidate measures are described below.

1. (i) Exponential increase rate (100k), or per cent per day increase in number of lesions*

(i) In obtaining this measurement we used epiphytotics for which three or more periodic observations of number of leaf lesions were available. Using periods during which the buildup appeared to have reached the logarithmic rate, the equation

$$C_t = C_0 e^{kt} \quad (3)$$

where: C = Concentration in terms of number of lesions
 t = time

was fitted to the data for each epiphytotic. In this equation 100k is the exponential increase rate, or per cent per day increase in number of lesions.

2. (i) Log number of lesions 18 days after estimated time for one lesion

* Usually, leaf lesion counts were made per foot or row or per hill, depending on the cultural practices used.

(U) In addition to the rate computed above, a measure of the actual number of lesions during the early stage of the epiphytotic was desired. In order to place the epiphytetics on a comparable basis, and to estimate number of lesions as early as possible after the exponential rate of increase would be expected to begin, the time 18 days after the time estimated for one lesion to appear was chosen somewhat arbitrarily. The logarithm of the number of lesions expected at this time was computed by solving Equation (3) first for t , setting $C_t = 1$. This time was then incremented by 18 days, and the equation solved for C_t .

3. (U) Average daily increment of disease multiplication

(U) The investigator desired to use this measurement. It was computed by obtaining the difference between the greatest and smallest number of lesions observed for a given epiphytotic, and dividing this difference by the number of days between the two observations.

4. (U) Highest leaf lesion count observed

(U) The actual value of the highest lesion count observed during the period used for fitting Equation (3) was used as another measurement.

5. (U) Per cent of very favorable days for disease multiplication on the leaves

(U) For each epiphytotic six days (the period of incipient infection under temperature ranges usually found in the field) were subtracted from the end points of the period used for fitting Equation (3), to give the probable dates of incubation. The probable incubation periods were then examined with respect to average night temperatures and length of period. Those days that were characterized by points above the upper 95 per cent confidence limit for the line of regression shown in Figure 1 were considered very favorable for disease multiplication.

6. (U) Per cent of favorable days for disease multiplication on the leaves

(U) Those days characterized by points above the lower 95 per cent confidence limit for the line of regression shown in Figure 1 were considered favorable for disease multiplication.

(U) Table 1 gives observed values of the above variables for 24 epiphytotics. Simple correlation coefficients between each of the candidate variables and per cent yield loss are shown in Table 2. This table also shows simple correlation coefficients between "per cent days very favorable for disease multiplication" and each of the other measurements, and between "per cent days favorable for disease multiplication" and each of the other

TABLE I. (U) OBSERVED AND COMPUTED DATA FOR CANDIDATE VARIABLES IN 24 EPIPHYTOICS (U)

Epihytotic Number	Per Cent Yield Loss	1. Per Cent Per Day Increase in Lesions, 10 ⁻⁴	2. Log No. Lesions 18 Days After Est. Time for 1 Lesion	3. Avg. Daily Increment of Disease Multi- plication	4. Highest Lesion Count avg. per Field	5. Per Cent Days Very Favorable For Disease Multiplication	6. Per Cent Days Favorable for Disease Multi- plication
						Days	Count
1	95	39.9	3.1161	217.50	478.5	7.7	69.2
2	67	36.5	2.8769	131.20	288.6	15.4	69.2
3	91	37.7	2.9450	13.00	234.3	20.0	70.0
4	96	52.5	4.2642	138.20	829.3	20.0	70.0
5	88	30.0	2.3472	26.67	56.0	0	73.3
6	92	Unknown	Unknown	Unknown	738.6	0	70.0
7	66	50.1	3.9192	52.79	45.4	72.7	72.7
8	68	16.7	1.4631	0.86	30.6	57.1	85.7
9	95	35.2	2.7537	7.71	462.6	57.1	85.7
10	95	65.4	5.1094	396.86	555.6	57.1	85.7
11	18	10.9	0.8509	0.67	70.0	52.0	68.0
12	47	34.2	2.6703	222.22	60.0	52.0	68.0
13	20	36.3	2.8404	8.21	57.5	33.3	46.7
14	0	29.3	2.2921	1.40	28.0	18.2	27.2
15	20	27.7	2.1615	3.20	48.0	43.8	56.3
16	0	31.5	2.4630	21.50	43.0	10.0	35.0
17	11	36.1	2.8246	19.56	31.3	68.7	75.0
18	18	26.5	2.0721	10.00	21.0	54.5	63.6
19	1	24.6	1.9201	2.59	188.7	50.0	61.1
20	0	0	Unknown	0	0	0	42.9
21	0	0	Unknown	0	0	27.8	55.6
22	0	0	Unknown	0	0	13.3	40.0
23	0	0	Unknown	0	0	21.4	50.0
24	47	Unknown	Unknown	Unknown	Unknown	52.0	68.0

TABLE 2. (U) SIMPLE CORRELATION COEFFICIENTS (U)

Variables	Number of Observations	Correlation Coefficient
Per cent per day increase in lesions (100k) vs. per cent yield loss	22	0.705**
Log no. lesions 18 days after est. time for 1 lesion vs. per cent yield loss	18	0.585*
Avg. daily increment of disease multiplication vs. per cent yield loss	22	0.532*
Highest observed lesion count vs. per cent yield loss	22	0.769**
Per cent days <u>very</u> favorable for disease multiplication vs. per cent yield loss	24	-0.039
Per cent days favorable for disease multiplication vs. per cent yield loss	24	0.749**
Per cent days <u>very</u> favorable for disease multiplication vs. per cent per day increase in lesions (100k)	22	0.248
Per cent days favorable for disease multiplication vs. per cent per day increase in lesions (100k)	22	0.484*
Per cent days <u>very</u> favorable for disease multiplication vs. log no. lesions 18 days after est. time for 1 lesion	18	-0.096
Per cent days favorable for disease multiplication vs. log no. lesions 18 days after est. time for 1 lesion	18	0.250
Per cent days <u>very</u> favorable for disease multiplication vs. avg. daily increment of disease multiplication	22	0.099
Per cent days favorable for disease multiplication vs. avg. daily increment of disease multiplication	22	0.414
Per cent days <u>very</u> favorable for disease multiplication vs. highest lesion count observed	22	0.072
Per cent days favorable for disease multiplication vs. highest lesion count observed	22	0.499*

* Significant at the .05 level.
** Significant at the .01 level.

measurements. In addition to correlations shown in Table 2, correlation coefficients based on the arc sine transformation for all variables involving percentages, and on the logarithmic transformation of highest lesion count, were also computed. These coefficients differed so minutely from the coefficients based on original values that they are not considered here.

(U) Note that all candidate variables except "per cent days very favorable for disease multiplication" showed a significant correlation with "per cent yield loss." Variables for which coefficients were significant at the 0.01 level were:

1. Per cent per day increase in lesions (100k),
2. Highest observed lesion count, and
3. Per cent days favorable for disease multiplication.

(U) Computed equations for estimating "per cent yield loss" from each of the above variables were:

$$\hat{Y} = -4.116 + 1.6278 X_1 \quad \text{standard error of slope} = 0.3662 \quad (4)$$

$$\hat{Y} = 18.39 + 0.1238 X_2 \quad \text{standard error of slope} = 0.0230 \quad (5)$$

$$\hat{Y} = -76.85 + 1.930 X_3 \quad \text{standard error of slope} = 0.3642 \quad (6)$$

where: Y = per cent yield loss

X_1 = per cent per day increase in lesions (100k)

X_2 = highest observed lesion count, and

X_3 = per cent days favorable for disease multiplication.

The above equations, together with observed values, 95% confidence limits for the lines of regression, and 80% prediction limits for individual values are plotted in Figures 3, 4, and 5. Equations (4), (5), and (6) provide approximately the same precision of estimates of per cent yield loss, standard errors of estimate being 29.899% for Equation (4), 26.96% for Equation (5), and 27.556% for Equation (6).

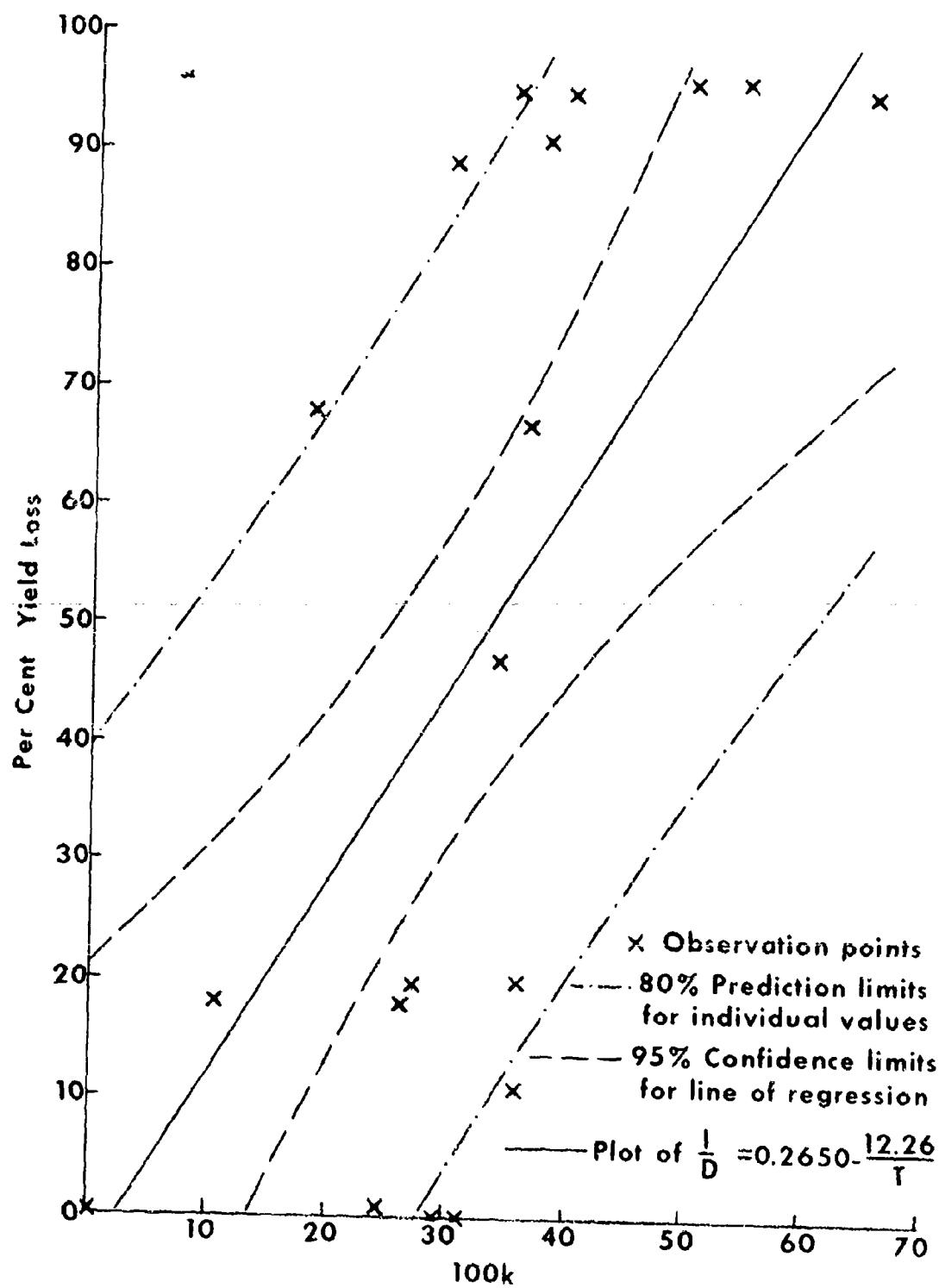


Figure 3. (U) Per Cent Yield Loss as a Function of 100k; Equation (4). (U)

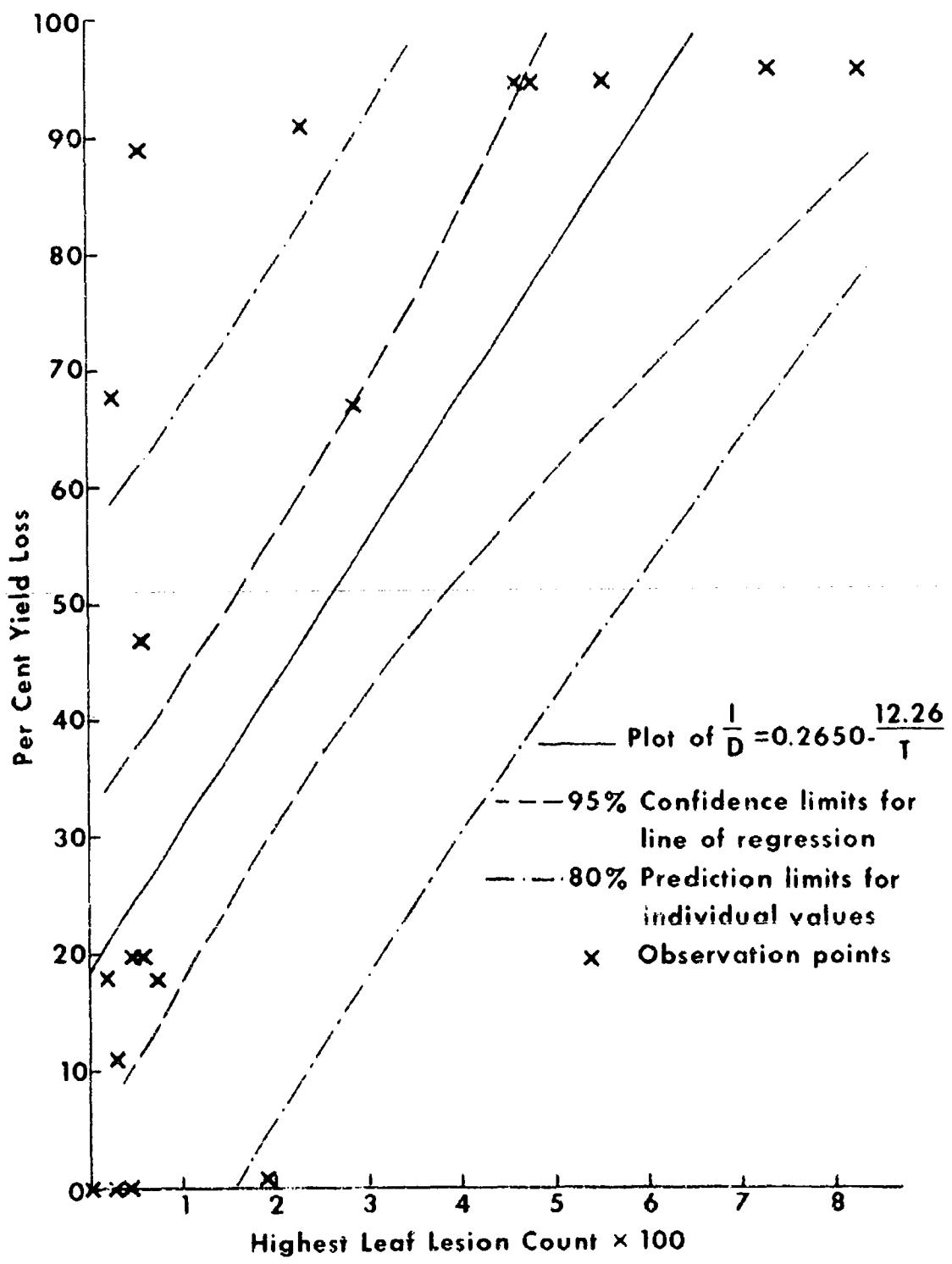


Figure 4. (U) Per Cent Yield Loss as a Function of Highest Leaf Lesion Count; Equation (5). (U)

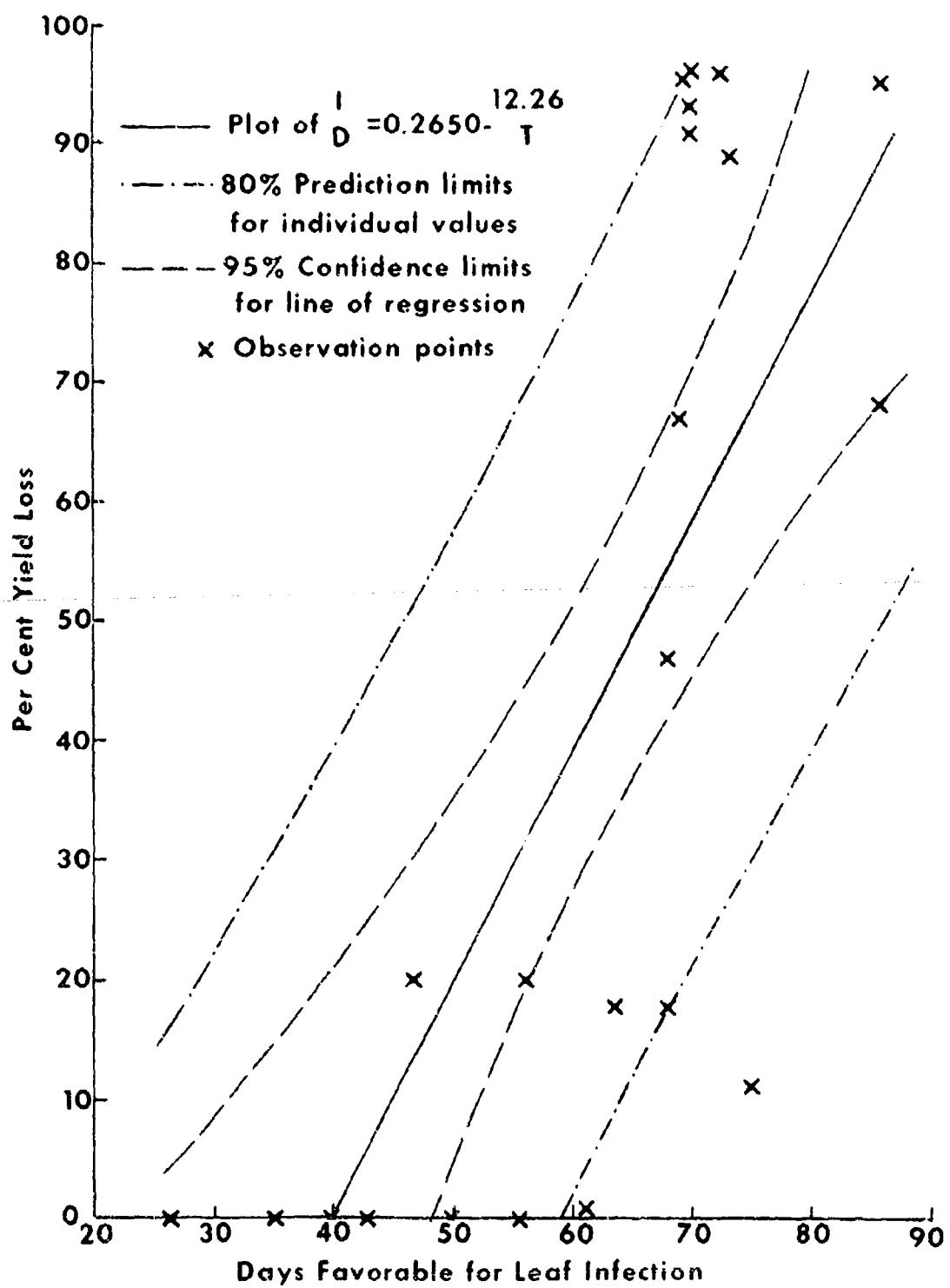


Figure 5. (U) Per Cent Yield Loss as a Function of Per Cent Days Favorable for Leaf Infection; Equation (6). (U)

(U) To determine whether the precision of estimate of per cent yield loss could be improved by simultaneous consideration of the other three variables, the multiple regression technique was used. Partial correlation coefficients (or correlation of per cent yield loss with a given independent variable holding the other two independent variables constant) and tests of significance were:

	<u>Partial Correlation Coefficient</u>	<u>Computed t</u>	<u>Approx. Probability</u>
r _{Y1.23}	0.2580	1.133	NS
r _{Y2.13}	0.4946	2.414	<0.05
r _{Y3.12}	0.6488	3.62	<0.01

where subscripts of r correspond with identification of variables given above.

(U) Thus X₁, or "per cent per day increase in lesions (100k)" in the presence of the other two independent variables did not contribute significantly to the estimate of per cent yield loss.

(U) Eliminating X₁ from the computations resulted in the following:

	<u>Partial Correlation Coefficient</u>	<u>Computed t</u>	<u>Approx. Probability</u>
r _{Y2.3}	0.6890	4.14	<0.01
r _{Y3.2}	0.6663	3.89	<0.01

with a multiple correlation coefficient, significant at the 0.01 level, of 0.879.

(U) The estimating equation for predicting per cent yield loss from known values of highest observed lesion count and per cent days favorable for disease multiplication was then:

$$\hat{Y} = -50.94 + 0.08426 X_2 + 1.2343 X_3 \quad (7)$$

Standard errors of partial regression coefficients were:

0.0281 for the coefficient of X₂, and

0.4250 for the coefficient of X₃.

The standard error of estimate of per cent yield loss from Equation (7) was 20.63%, a reduction of about 7% over estimates from Equations (4), (5), and (6).

(U) Equation (7) describes a plane, and is based on the assumption that a linear relationship exists among each pair of the three variables. In Table 3 values of per cent yield loss, as estimated from Equation (7), are cross-tabulated by highest observed lesion count in increments of 100 lesions and by per cent days favorable for disease multiplication in increments of 10%.

TABLE 3. (U) ESTIMATED PER CENT YIELD LOSS

Computed from the equation

$$\text{Y} = 50.94 + 0.08426 X_2 + 1.2343 X_3$$

Where: Y = estimated per cent yield loss

X_2 = highest observed lesion count

X_3 = per cent days favorable for disease multiplication

^o Days Favorable for Disease Multiplication	Highest Observed Lesion Count					
	100	200	300	400	500	600
10				3.53	11.96	20.38
20				7.45	15.88	24.30
30	2.94	11.37	19.79	28.22	36.64	45.07
40	6.86	15.28	23.71	32.14	40.56	48.99
50	19.20	27.63	36.05	44.48	52.90	61.33
60	31.54	39.97	48.40	56.82	65.25	73.67
70	43.89	52.31	60.74	69.16	77.59	86.02
80	56.23	64.66	73.08	81.51	89.93	98.36
90	68.57	77.00	85.42	93.85		

Unclassified

Security Classification

DOCUMENT CONTROL DATA - R&D

(Security classification of title, body of abstract and releasing instructions is reflected above the classification of this page)

1. ORIGINATING ACTIVITY (Corporate author) 2. ACQUISITION ACTIVITY (Corporate author) 3. DRAFTING ACTIVITY (Corporate author)

U.S. Army Biological Laboratories
Fort Detrick, Frederick, Maryland, 21701

Confidential

21-562-1 3

4. REPORT TITLE

RICE BLAST EPIPHYTOLOGY (U)

5. DESCRIPTIVE NOTES (Type of report and inclusive dates)

6. AUTHOR(S) (Last name, first name initial)

Barksdale, Thomas H.
Jones, Marian W.

7. REPORT DATE

June 1965

7a. TOTAL NO OF PAGES

125 pages

7b. NO OF REFS

52

8a. CONTRACT OR GRANT NO

8a. ORIGINATOR'S REPORT NUMBER(S)

8b. PROJECT NO

Technical Report 60

c. EC522301A06102

9b. OTHER REPORT NO(S). (Any other numbers that may be assigned)
Data report

d.

10. AVAILABILITY LIMITATION NOTICES

Qualified requestors may obtain copies of this publication from DDC. Foreign announcement and dissemination of this publication by DDC is not authorized. Release or announcement to the public is not authorized.

11. SUPPLEMENTARY NOTES

12. SPONSORING MILITARY ACTIVITY

U.S. Army Biological Laboratories
Fort Detrick, Frederick, Maryland, 21701

13. ABSTRACT

Data on rice blast disease from the field and laboratory experiments of many workers are reviewed and related to epiphytotes of the disease. Principal topics discussed are (i) sources of inoculum, (ii) spore dispersal, (iii) meteorological and other conditions required for establishment of infection and disease buildup, (iv) spread, (v) yield reduction, (vi) control measures, and (vii) the present ability to predict disease outbreak, buildup, and yield losses. A theoretical curve describing minimum requirements of dew period and temperature for infection was developed from laboratory data, and appears to fit two sets of field data. This curve was used to find the percentage of days favorable for disease development during 2+ epiphytotes, and regression equations were derived relating this parameter, along with other parameters derived from disease increase data, to yield loss caused by epiphytotes.

DD FORM 1 JAN 64 1473

Unclassified

Security Classification