Comparison of Aerial Concentration, Deposition, and Infectiousness of Conidia of *Pyricularia grisea* by Spore-Sampling Techniques

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ABSTRACT

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In three field trials on the epidemiology of blast disease in upland rice, different methods of sampling spores of *Pyricularia grisea* were compared to study aerial concentration, deposition, survival, and infectiousness of conidia of the pathogen. The methods included a Burkard spore trap, glass slides and rods, several trap-plant treatments, and leaf prints from trap plants. The results were mostly highly intercorrelated and revealed a unimodal pattern of disease progression, with peaks before or at the middle of the cropping seasons. The number of conidia per square centimeter deposited on leaf surfaces as measured by leaf prints made up one-fourth of the number caught with glass slides. The amount of deposited and potentially infective spores on leaves of trap plants of upland rice cultivar C22 incubated in a dew chamber after exposure in the field was 0.05–0.11, 0.42, and 0.44 of the spore density as measured by glass slides, leaf prints, and trap plants of the highly susceptible upland rice cultivar Co 39, respectively. The number of actually infectious spores on trap plants incubated in the greenhouse after exposure was 0.31–0.39 of total lesions observed on dew chamber-incubated trap plants. Few spores were observed on glass rods, indicating that sedimentation rather than impaction was the major factor for spore deposition. Nonlinear regression analysis revealed that the spore-catch results of the Burkard spore trap, glass slides, and leaf prints explained 0.58–0.65, 0.72–0.77, and 0.66 of the variability of the number of lesions on trap plants incubated in dew chambers, respectively. Similarly, the spore-catch results of the Burkard spore trap and the total lesions on dew chamber-incubated trap plants explained 0.42–0.76 and 0.65–0.79 of the variability of susceptible-type lesions on trap plants incubated in greenhouse conditions, respectively. The number of potentially infective spores tended to increase toward an asymptote at high inoculum concentrations. Depending on the method, the spore-catch results were significantly affected by several weather variables. Estimation of deposited and infectious conidia and of inoculum potential by means of comparison of spore-sampling methods can be improved considerably by adjusting for the effects of these variables.

Additional keywords: rice blast, spore catch, weather effects.

Forecasting techniques for rice blast disease progress usually emphasize the importance of aerial spore density as an essential predictor because the number of infected sites on plants in the field is closely related to aerial spore density (3,9,16,19,31,32). Therefore, accurately measuring the amount of airborne inoculum is crucial for developing and applying blast forecasting systems.

Methods that have been used to measure the amount of airborne spores of the blast pathogen *Pyricularia grisea* Sacc. include glass slides (20,31), trap plants (29), and volumetric samplers such as

rotorods (6,16,18,19,42,44) and suction samplers (33,36). However, there has been no detailed quantitative comparison of these methods.

Jenkyn (14) and Dutzmann (8) compared methods for sampling conidia of *Erysiphe graminis* f. sp. *hordei*. Such comparisons are useful if spore-catch data obtained with one method are to be used with forecasting techniques based on spore-catch data obtained with another method. Also, by comparing the results of different spore-trapping techniques, one can examine the relative contribution of impaction and sedimentation in spore deposition (4).

Besides knowing total aerial inoculum concentrations, it is equally important to obtain estimates of the inoculum potential (5), which includes the spores deposited on leaves, spore viability, and the infection efficiency of viable spores under given field conditions. Rotem (37) discussed the benefits of using trap plants for studying such aspects.

This study makes a quantitative comparison of various methods for measuring the airborne inoculum density of *P. grisea*. We emphasize the use of techniques involving trap plants because they reflect factors that determine inoculum potential and its partial aspects with more biological relevance than do other methods (37). The influence of weather on spore deposition and infection also is studied.

MATERIALS AND METHODS

Field layout. Data were obtained during a field study (21,22) on the epidemiology of rice blast in upland rice at the International Rice Research Institute (IRRI) in Los Baños, Laguna, Philippines (34). Three experiments were conducted at the IRRI upland field site from September 1984 to August 1986. Upland rice cultivar C22 was sown in 400-m^2 plots on 13 September 1984, 18 April 1985, and 19 February 1986. Plots were surrounded with a 1-m border of sorghum planted at the same time. Standard crop-management practices for upland rice were used (13). Sprinkler irrigation was applied when signs of severe drought stress appeared; otherwise, the crops were rainfed. To initiate the epidemic, 25 pots with infected seedlings from the IRRI blast nursery were distributed within the plot.

Weather data. Two hygrothermographs with wetness sensors (Lufft, Stuttgart, Germany) and a wind-cup anemometer (Makino Applied Instruments Inc., Tokyo, Japan) were placed in the center of the plot to form a field lab (23). One hygrothermograph was placed on the ground with the wetness sensor attached to a wooden stick in the upper third of the canopy. The second hygrothermograph was installed 1.5 m above the ground with the wetness sensor in the lower third of the canopy. The anemometer was placed just above the canopy. Data were recorded hourly. Additional data on sunshine duration (hourly), rainfall (hourly), and irradiation intensity (daily) were obtained from the nearby IRRI upland weather station.

Spore traps and data collection. A Burkard volumetric suction sampler (Burkard Manufacturing Co. Ltd., Rickmansworth, Hertfordshire, England) was installed in the middle of the field (method BST, Table 1). The suction rate of the Burkard trap was adjusted to 10 L of air per minute and was checked regularly. Five wooden stands for holding glass slides and rods and trap plants were distributed within the plot. The Burkard spore trap and wooden stands were adjusted regularly to place the orifice of the volumetric spore trap and other trapping surfaces in the upper third of the canopy. All spore traps were unprotected from rain except for the Burkard spore trap, which is equipped with a rain shield.

Melinex tape (ICI Americas Inc., Wilmington, DE) with a thin layer of petroleum jelly plus 10% paraffin was wrapped around the revolving drum of the Burkard spore trap. Glass slides (72 × 25 mm) were coated with a thin layer of the petroleum jelly-paraffin mixture, and glass rods (14), 5 mm in diameter, were wrapped with cellophane dipped in a heated mixture of the same adhesive material. One slide and one glass rod were placed horizontally and vertically, respectively, on each wooden stand in the field. After exposure for 24 h, slides and cellophane strips were placed in petri dishes and taken to the laboratory. To obtain spore counts, microscopic observation was done with polyvinal acetate (Gelvatol, Monsanto Chemical Co., St. Louis, MO) plus aniline blue as staining medium. The number of conidia on the Melinex tape and slides was estimated by counting the number in a microscope field from three positions along the middle of the tape or slide. All conidia on the cellophane strip were counted.

Seeds of cv. C22 were sown in 300-ml plastic pots containing field soil supplemented with 6 g of complete fertilizer per kilogram of soil and grown in the greenhouse. Plants were thinned for uniformity at the three-leaf stage. Ten pots were taken to the field, and two pots were placed on each wooden stand. After exposure, the plants were separated into two groups of five pots each. The first group was incubated for 24 h in dew chambers (Percival MFG, Boone, IA) at 26 C and 100% relative humidity (RH) and then was transferred to the greenhouse. The second group was transferred directly to the greenhouse. For each exposure, a new set of trap plants was used.

Six days after exposure, lesions on the third leaf were counted. All lesions, susceptible (S)-type lesions (dark green, water-soaked spots that later develop grey centers and sporulate) and hypersensitive (H)-type lesions (necrotic spots about 1 mm in diameter or smaller) were counted. The mean leaf area of the third leaf was determined from a sample of 30 leaves per cultivar with an automatic area meter (AAM-7, Hayashi Denkoh, Tokyo, Japan), and these values were used to standardize lesion counts.

Leaf prints (38) were obtained from another set of C22 trap plants. After exposure in the field, 10 plants were taken to the

TABLE 1. Survey of spore-sampling methods used

Acronym	Item measured[a]	Sampling result used to determine
BST	No. of spores/m^3 of air as measured by the Burkard spore trap	Aerial spore load
GR	No. of spores/cm^2 on vertically exposed glass rods	No. of impacted spores
LP-spo	No. of spores/cm^2 on leaf prints	No. of spores deposited on leaves
LP-tot	No. of spores/cm^2 on leaf prints plus no. of S-type plus H-type lesions/cm^2 on incubated leaves of upland rice cv. C22 trap plants	No. of spores deposited on leaves
GS	No. of spores/cm^2 on horizontally exposed glass slides	No. of sedimented spores
TG-C22	Total no. of lesions/cm^2 on leaves of greenhouse-incubated cv. C22 trap plants	No. of spores deposited on leaves and potentially infective on cv. C22 under field conditions
SG-C22	No. of S-type lesions/cm^2 on leaves of greenhouse-incubated cv. C22 trap plants	No. of spores deposited on leaves and able to infect cv. C22 under field conditions on the day of dispersal
SF-C22	No. of S-type lesions/cm^2 on leaves of field-incubated cv. C22 trap plants	Like SG-C22 plus no. of spores able to infect cv. C22 after survival for 1 day under field conditions.
SD-C22	No. of S-type lesions/cm^2 on leaves of dew chamber-incubated cv. C22 trap plants	No. of spores deposited on leaves and potentially able to cause S-type lesions on cv. C22
TD-C22	Total no. of lesions/cm^2 on leaves of dew chamber-incubated cv. C22 trap plants	No. of spores deposited on leaves and potentially infective on cv. C22
TD-Co 39	Total no. of lesions/cm^2 on leaves of dew chamber-incubated cv. Co 39 trap plants (highly susceptible)	No. of spores deposited on leaves and potentially infective

[a] Susceptible (S)-type and hypersensitive (H)-type lesions are described in text.

laboratory. To obtain a leaf print, a thin layer of transparent glue (Technicoll, Beiersdorf AG, Hamburg, Germany) was applied to a glass slide. After drying, the upper side of a leaf blade was gently pressed against the glue and then removed gently. Mounting medium was applied, a coverslip was placed over the leaf print, and the conidia were counted along a 50- × 0.92-mm strip in the middle of the print. Immediately after the leaf prints were made, leaves were placed in petri dishes containing 0.5% water agar plus 30 ppm of benzimidazole and incubated for 5 days at 26 C with a 12-h photoperiod per day. The total number of lesions on each leaf blade was counted. The total number of spores on the leaf prints was calculated by adding lesion numbers counted on the blades and estimating the number of conidia on the leaf print after conversion to standard units.

The Burkard spore trap and trap plants (cv. C22) were used throughout the study. Glass slides were used in 1985 and 1986. Glass rods and leaf prints obtained from leaves of C22 were used in 1985. In 1985, a set of trap plants of Co 39, a highly susceptible cultivar, was grown and placed in the dew chamber for 24 h after exposure (method TD-Co 39, Table 1). Slides, glass rods, and plants were exposed for a period of 24 h beginning at 0900, and conidia released during the corresponding period were sampled with the Burkard spore trap. Traps were usually placed in the field two to three times a week during the course of the epidemic. In 1986, trap plants of C22 were exposed on a daily basis.

Data analyses. Data for the Burkard spore trap were expressed as the number of conidia per cubic meter per day. All other data were expressed as conidia (and/or lesions) per square centimeter, and daily means were used. Data for comparable sampling times were used in the subsequent analyses. All methods were subjected to linear correlation. In addition, the efficiency of the different methods, compared with that of C22 trap plants placed in the dew chamber, was determined by linear regression through the origin. The efficiency of the different methods was based on the regression coefficient; the efficiency of a method was greater than that of trap plants placed in the dew chamber if the regression coefficient was greater than one and less efficient if it was less than one.

For a more detailed comparison of selected methods, the data were transformed and subjected to further linear and nonlinear regression analyses. In most cases, residual plots of linear regression equations showed curvilinear trends. Homoscedasticity was obtained only when both dependent and independent variables were transformed to the cube root.

Finally, correlation analysis was performed on the residuals of some selected regression equations with the weather variables as covariates.

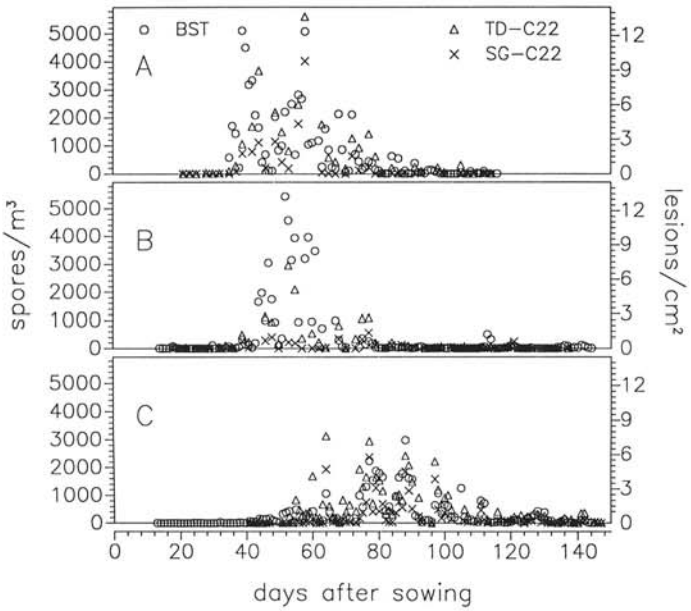

Fig. 1. Daily spore catch of the Burkard spore trap (BST) and of trap plants of upland rice cv. C22 incubated either in the dew chamber (TD-C22) or in the greenhouse (SG-C22) in three field experiments. **A,** 1984, **B,** 1985, and **C,** 1986. (Table 1 provides a detailed description of spore-trapping methods.)

RESULTS

The number of spores sampled by the Burkard spore trap followed the disease progress in all years the experiment was conducted. Overall, as disease severity increased, the number of spores increased. Later in the season, as disease severity decreased because of leaf senescence and increasing plant resistance, the number of spores decreased. This pattern was observed for all methods (Fig. 1) and accounted for the positive correlations between all methods (Table 2).

Most correlations between methods were highly significant ($P < 0.01$; Table 2). Most nonsignificant correlations involved C22 trap plants transferred to the greenhouse after exposure in 1985. Lesion counts on these plants were poorly correlated with spore counts on glass rods and leaf prints.

Because the units of the Burkard spore trap were in spores per volume and the other methods were in spores per area, it was not appropriate to compare any methods with the Burkard spore trap. Nevertheless, the relative number of spores trapped by the Burkard spore trap was greater than the relative number of lesions on the standard trap plants on most occasions (Fig. 1). Linear regression was used to compare sampling efficiency among the other methods. Total lesion counts on dew chamber-incubated trap plants of cv. C22 served as the reference method, and all other methods were regressed on these. Thus, the resulting regression coefficients served as a measure of the relative sampling efficiency of the various methods, as compared to dew chamber-incubated C22 trap plants. The sampling efficiency of the glass

TABLE 2. Correlation matrix for various methods of sampling airborne conidia of *Pyricularia grisea*[a]

Sampling method[b]	Year	BST	TD-C22	SG-C22	TD-Co 39	SF-C22	GS	GR
TD-C22	1984	0.69**						
	1985	0.92**						
	1986	0.77**						
SG-C22	1984	0.77**	0.91**					
	1985	0.42**	0.69**					
	1986	0.80**	0.87**					
TD-Co 39	1985	0.70**	0.92**	0.83**				
SF-C22	1985	0.49*	0.68**	0.89**	0.84**			
	1986	0.85**	0.82**	0.97**				
GS	1985	0.85**	0.88**	0.43*	0.83**	0.60*		
	1986	0.65**	0.85**			0.80**		
GR	1985	0.62**	0.52**	0.25		0.53*	0.80**	
LP-tot	1985	0.95**	0.89**	0.33		0.62	0.97**	0.94**

[a] Based on sampling result per day; * = significant at $P = 0.01$; ** = significant at $P = 0.001$; one-tailed.
[b] Table 1 describes spore-trapping methods.

slides was greater than that of the trap plants in both 1985 and 1986, whereas efficiency was lower on the glass rods in 1985 (Table 3). However, on rainy days, the petroleum jelly-paraffin film was removed from glass slides, and no spores were observed.

Comparing deposition on trap-plant leaves, only regression coefficients derived for total conidia on leaf prints of C22 and lesions on Co 39 indicated greater sampling efficiencies than did trap plants of C22 incubated in the dew chamber. Although leaf prints gave adequate counts for the number of conidia on leaves, it was not always possible to count lesions on leaves incubated on water agar, because of saprophytic activity due to other leaf colonists. Nevertheless, the total number of conidia determined by leaf prints, based on nondeteriorated leaves, was approximately equal to the number on Co 39 trap plants but contained only 0.26 of the number of conidia found on the glass slides. Conidial counts on the Technicoll film accounted for roughly half the total number of conidia determined by the technique.

In 1984, the total number of lesions on trap plants incubated in the greenhouse was 0.66 of the number of total lesions on trap plants incubated in the dew chamber. The number of susceptible lesions on these plants represented 0.02–0.04, 0.14, 0.15, and 0.31–0.39 of conidia or lesions on glass slides, leaf prints, and trap plants of Co 39 and C22 placed in dew chambers, respectively. Also, transferring trap plants directly to the greenhouse resulted in lower sampling efficiency as determined by the number of occasions when no lesions were recorded on days when lesions were found on trap plants transferred to the dew chamber (Fig. 1). The proportion of susceptible lesions was 0.5 on plants incubated in the greenhouse in 1984 and 0.77 and 0.55 in 1984 and 1985, respectively, on plants incubated in dew chambers. Lower proportions of susceptible lesions on plants transferred to the greenhouse were observed in all years of the study (results not shown). In 1985 and 1986, the number of susceptible lesions on trap plants left in gauze-covered cages in the field for a second day after 1 day of exposure was higher than on plants directly transferred to the greenhouse after 1 day of exposure.

After further regression analyses, nonlinear regression involving data transformed to the cube root gave the best fit in most cases, with the coefficient of determination ranging from 0.42 to 0.85 (Figs. 2–5; Table 4). Lesions on dew chamber-incubated trap plants of C22 and Co 39 gave results indicating a linear relationship (Fig. 4), so did conidia on leaf prints or glass slides and on the Burkard spore trap (Fig. 5).

The number of lesions on dew chamber-incubated trap plants increased in a logarithmic manner with increasing spore concentrations, and regression coefficients were similar for all years. The coefficients indicated an asymptotic value at very high inoculum densities (Fig. 2A; Table 4). Similar trends were observed with regressions involving conidial counts on glass slides in 1985 and 1986 (Fig. 3; Table 4). The relationship between leaf-print data and lesion number on dew chamber-incubated plants was nearly linear.

In contrast, lesion numbers on greenhouse-incubated trap plants increased exponentially in 1984 and 1986 and almost linearly in 1985 as the number of conidia sampled by the Burkard spore trap increased (Fig. 2B). Regressing the same variables (excluding plants with zero lesions) resulted in the same trends, but intercepts and coefficients differed (H. O. Pinnschmidt, *unpublished data*). In 2 out of 3 yr, trends were similar when lesion numbers on trap plants placed in the greenhouse were regressed on dew chamber-incubated trap plant data (Fig. 4; Table 4).

Regressions of conidial counts on leaf prints or glass slides with the volumetric spore-trap counts revealed linear relationships with R^2 values similar to those obtained by comparing other spore-sampling methods (Fig. 5; Table 4).

Correlations of residuals with weather variables. A significant proportion of total variability was not explained by regressions summarized in Table 4. Correlations of residuals of selected models and weather parameters varied with the regression model

TABLE 3. Regression coefficients and adjusted coefficients of determination to compare the relative efficiency of various methods of sampling airborne conidia of *Pyricularia grisea*[a]

Year	Sampling method[b]	Regression + SE coefficient	Adjusted R^2
1984	SD-C22	0.77 ± 0.03	0.96
1985		0.55 ± 0.02	0.94
1984	TG-C22	0.66 ± 0.05	0.94
1984	SG-C22	0.31 ± 0.04	0.69
1985		0.34 ± 0.02	0.85
1986		0.39 ± 0.03	0.71
1985	TD-Co 39	2.26 ± 0.12	0.92
1985	SF-C22	0.43 ± 0.05	0.80
1986		0.44 ± 0.04	0.66
1985	GS	9.18 ± 1.11	0.69
1986		18.48 ± 1.39	0.76
1985	GR	0.34 ± 0.06	0.50
1985	LP-spo	1.38 ± 0.24	0.48
1985	LP-tot	2.38 ± 0.37	0.68

[a] Simple linear regression through the origin, using TD-C22 as the independent variable. TD-C22 is the reference trapping method.
[b] Table 1 describes spore-trapping methods.

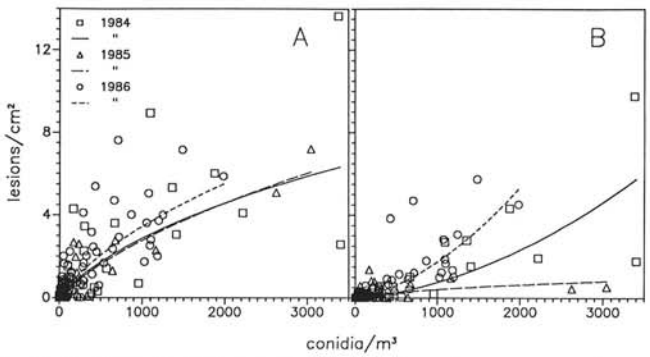

Fig. 2. Relationship between the airborne inoculum concentration as measured with the Burkard spore trap (BST) and the number of lesions per square centimeter on the third leaf of trap plants of upland rice cv. C22 (**A**, TD-C22 and **B**, SG-C22) (Tables 1 and 4 describe spore-trapping methods and regression equations, respectively.)

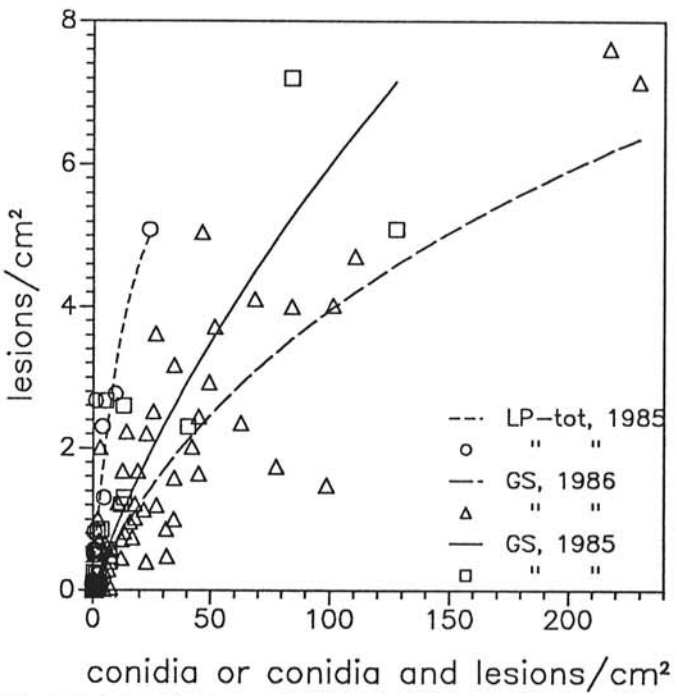

Fig. 3. Relationship between the spore catch of leaf prints (LP-tot) or glass slides (GS) and the total number of lesions per square centimeter of leaf area on dew chamber-incubated trap plants of upland rice cv. C22 (TD-C22). (Tables 1 and 4 describe spore-trapping methods and regression equations, respectively.)

(Table 5). Only wind gave consistent results over the four regression models and was negatively correlated with the residuals, although the correlation was not significant for residuals obtained from the regression including lesion counts on leaves of trap plants incubated in the dew chamber and spore counts on glass slides. All other correlations depended on what independent and dependent variables were used.

Using data from the Burkard spore trap to predict the number of lesions on trap plants placed in the dew chamber, rainfall and wind were negatively correlated and radiation and hours of sunshine positively correlated with the residuals. In contrast, using spore counts on glass slides as a predictor, the residuals were significantly positively correlated with rainfall and RH. All other correlations were not significant for deviations from predicted numbers of lesions on trap plants incubated in the dew chamber.

RH and leaf wetness were positively correlated, whereas wind and temperature were negatively correlated with the residuals of the regressions of the number of lesions on trap plants transferred to the greenhouse on either the number of conidia collected in the Burkard spore trap or the number of lesions on trap plants in the dew chamber. Rain was positively correlated with residuals when the second predictor was used.

DISCUSSION

The volumetric spore sampler measured the density of conidia in the volume of air sampled. Because there were no other plots

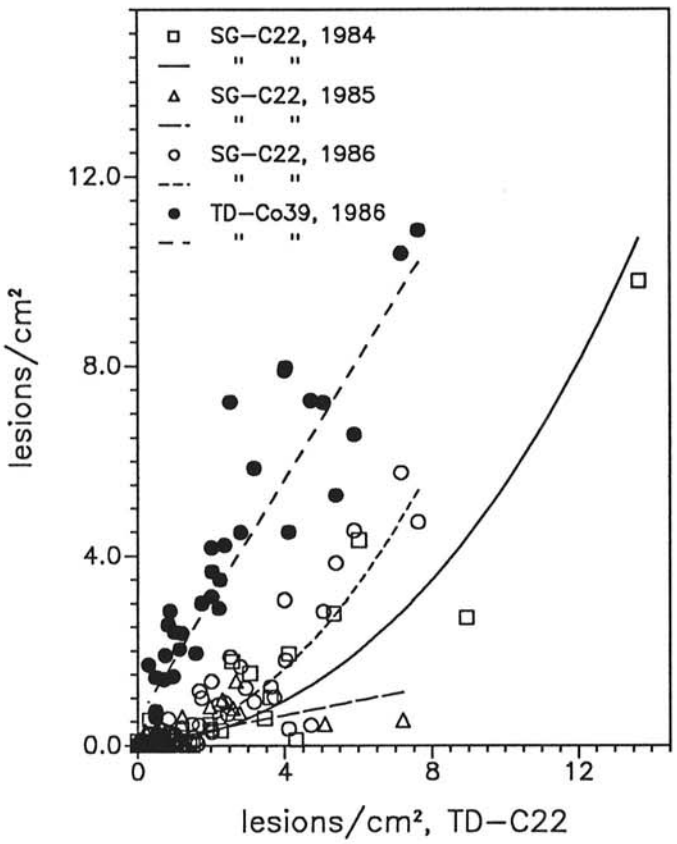

Fig. 4. Relationship between the total number of lesions on dew chamber-incubated trap plants of upland rice cv. C22 (TD-C22) and other trap-plant methods (SG-C22 and TD-Co 39). (Tables 1 and 4 describe spore-trapping methods and regression equations, respectively.)

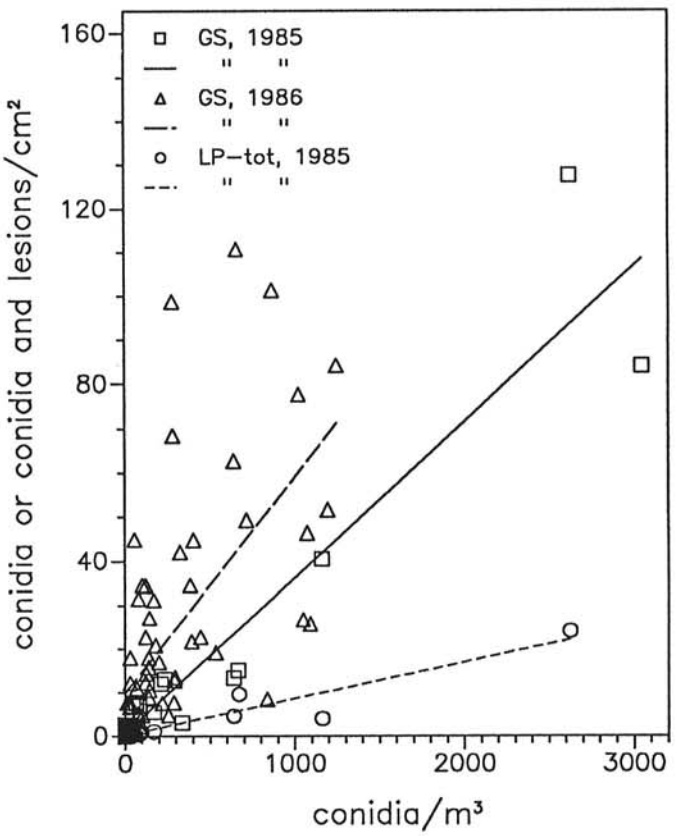

Fig. 5. Relationship between the spore catch of the Burkard spore trap (BST) and the spore catch of leaf prints (LP-tot) and glass slides (GS). (Tables 1 and 4 describe spore-trapping methods and regression equations, respectively.)

TABLE 4. Regression equations and test statistics for comparison of spore-trapping methods in Figures 2–5[a]

Figure	Year	Regression equation	R^2	n	F value
1A	1984	TD-C22 = $2.922\,(1 - e^{-0.039BST})$	0.58	31	19.49
1A	1985	TD-C22 = $3.341\,(1 - e^{-0.032BST})$	0.65	64	57.40
1A	1986	TD-C22 = $3.179\,(1 - e^{-0.037BST})$	0.58	81	53.20
1B	1984	SG-C22 = $-0.839\,(1 - e^{0.045BST}) - 0.85$	0.76	31	28.66
1B	1985	SG-C22 = $1.430\,(1 - e^{-0.039BST}) + 0.047$	0.42	65	14.48
1B	1986	SG-C22 = $-0.910\,(1 - e^{0.051BST}) - 0.046$	0.68	82	54.00
2	1985	TD-C22 = $-6.249\,(1 - e^{0.080LP}) + 0.194$	0.66	22	11.60
2	1985	TD-C22 = $4.053\,(1 - e^{-0.133GS}) - 0.052$	0.77	40	40.40
2	1986	TD-C22 = $2.711\,(1 - e^{-0.219GS}) - 0.150$	0.72	56	44.35
3	1984	SG-C22 = $-0.441\,(1 - e^{0.756\,TD\text{-}C22}) - 0.040$	0.77	32	31.60
3	1985	SG-C22 = $6.008\,(1 - e^{-0.104\,TD\text{-}C22}) - 0.048$	0.65	68	38.93
3	1986	SG-C22 = $-0.512\,(1 - e^{0.761\,TD\text{-}C22}) - 0.027$	0.79	83	99.66
3	1986	TD-Co 39 = $0.914\ TD\text{-}C22 + 0.368$	0.85	32	174.50
4	1985	GS = $0.272\ BST + 0.465$	0.84	38	182.20
4	1986	GS = $0.314\ BST + 0.710$	0.52	62	64.91
4	1985	LP = $0.140\ BST + 0.486$	0.71	19	42.54

[a] All variables are transformed to the cube root. All F values are significant at $P < 0.01$. Table 1 describes spore-trapping methods.

or rice fields in the immediate vicinity of the experimental site, the Burkard spore trap estimated the number of spores released into the air from sporulating lesions in the plot. The Burkard spore trap is more efficient than methods based on passive deposition mechanisms, especially at low aerial spore concentrations (8,30). This was confirmed by our results on occasions when few spores were sampled by the Burkard spore trap, and no lesions or spores were detected on slides, rods, or leaves. However, the efficiency of a method may depend on the spatial pattern of initial disease foci within the plot. Because of multiple placements in the plot, glass slides and trap plants may be more efficient than one Burkard spore trap placed in the center of the plot, particularly at the early stages of the epidemic.

Spore deposition is governed by two processes: impaction and sedimentation (4,10,25). Vertically exposed glass rods were used to determine the relative number of conidia deposited by impaction. Horizontally exposed glass slides sampled conidia deposited by sedimentation and impaction. Impaction on glass slides occurs because of turbulence in the canopy; it occurs at the edge of the glass slide under gusty, windy conditions. Under calm conditions, deposition is mainly by sedimentation, but as wind speed increases, impaction increases in importance. In this study, the number of conidia sampled by glass rods was relatively low, about one-thirtieth of the number on glass slides (Table 3). Also, spore dispersal occurred almost exclusively at night when there was little or no wind at the experimental site (34). Thus, sedimentation was deemed more important than impaction.

Leaf prints and trap plants were less efficient than glass slides for estimating total spores. Spore-deposition efficiency is affected by leaf-angle and -surface characteristics (27,28,39). The estimated number of conidia on leaf prints was only one-fourth of the number on glass slides. At the growth stage at which the trap plants were exposed in the field, leaf-print zones were oriented toward the horizontal. Thus, leaf angle could not account for the difference. It is possible that the leaf prints did not account for all spores on the leaf segments. The proportion of conidia removed by pressing the Technicoll film against the leaf is not known. Furthermore, multiple infections on incubated leaves from which leaf prints were obtained could be important; the technique may have underestimated the total number of spores.

TABLE 5. Correlation between residuals of the relationship between spore-sampling methods and weather variables[a]

Weather variable[b]	Residuals of relationship			
	TD-C22 vs. BST	TD-C22 vs. GS	SG-C22 vs. BST	SG-C22 vs. TD-C22
WETNESS	0.047	0.110	0.328**	0.354**
WETNESSN	0.077	0.047	0.321**	0.326**
RH	−0.060	0.274*	0.239**	0.403**
RHN	−0.002	0.329**	0.290**	0.416**
TEMP	−0.007	−0.050	−0.142	−0.273**
TEMPN	−0.064	−0.038	−0.179*	−0.257**
SUN	0.237**	0.040	0.096	−0.205*
RAIN	−0.253*	0.503**	−0.050	0.179
RAINN	−0.317*	0.730**	−0.032	0.204
RAINNoN	−0.404**	0.697**	−0.057	0.270
WIND	−0.338**	−0.035	−0.605**	−0.408**
WINDN	−0.405**	−0.117	−0.600**	−0.395**
IRRAD	0.191*	0.070	0.090	−0.149

[a] * = significant at $P = 0.01$; ** = significant at $P = 0.001$; two-tailed. Table 1 describes spore-trapping methods. For TD-C22 vs. BST, $n = 177$; for TD-C22 vs. GS, $n = 98$; for SG-C22 vs. BST, $n = 184$; for SG-C22 vs. TD-C22, $n = 192$.

[b] IRRAD = irradiation intensity per day in milliwatts per hour per square centimeter; RAIN = rainfall per entire day in millimeters; RAINN = rainfall per night in millimeters; RAINNoN = hours with rain during night; SUN = hours with sunshine per day; RH = mean reltive humidity per entire day within canopy in percents; RHN = mean relative humidity per night within canopy in percents; TEMP = mean temperature per entire day within canopy in degrees Celsius; TEMPN = mean temperature per night within canopy in degrees Celsius; WETNESS = hours with leaf wetness per entire day in upper third of canopy; WETNESSN = hours with leaf wetness per night in upper third of the canopy; WIND = mean wind velocity per day in meters per second; WINDN = mean wind velocity per night in meters per second.

The efficiency of using trap plants to estimate the total number of conidia deposited on the leaves depended on how plants were incubated after exposure in the field. Trap plants incubated in dew chambers after exposure had a greater number of lesions than did those placed in the greenhouse. Using susceptible cultivars as trap plants may be more suitable for estimating inoculum density; the lesion number on Co 39 was greater than that on C22.

Despite lower efficiency in estimating spore concentrations, trap plants could be more reliable than other methods for estimating parameters of epidemiological importance. The combined total number of lesions and spores from leaf prints estimated the total number of spores deposited on leaves; dew chamber-incubated trap plants provided estimates of the total number of viable spores deposited on leaves; and trap plants transferred from the field to the greenhouse provided estimates of the proportion of viable spores that infected the leaves and produced susceptible lesions under the prevailing microclimatic conditions during the exposure period. We assumed that the H-type lesions were not due to the presence of incompatible races, because spores from cv. C22 were the only inoculum source present at the site. The number of lesions on dew chamber-incubated plants accounted for less than half the number of conidia determined for leaf prints. If the efficiency of leaf prints for estimating total spores is assumed to be about 50% (as was determined by Merchan Vergas (26) for *Erysiphe graminis* on wheat), viable spores on leaves account for only about 20% of the total spores deposited. The susceptible lesions formed on trap plants under field conditions accounted for about 30–40% of the viable spores. Only these spores can potentially affect progress of the epidemic. Hence, they represent inoculum potential as determined by weather and pathogen factors on a given day.

At low spore densities, the number of susceptible lesions produced increased exponentially with increasing density, indicating possible synergistic effects among conidia. Alternatively, conidia produced in a given night could be divided into three categories depending on onset time and duration of leaf wetness in relation to deposition: conidia germinating, producing appressoria, and successfully infecting leaves; conidia germinating on leaves with no infection occurring; and ungerminated conidia. Initially, the number of conidia released and deposited during a given night increases exponentially. Only those conidia deposited during the initial period of spore release are likely to infect plants because penetration is likely to have occurred before the end of the wetness period. Although sporulation occurs at RH > 92%, sporulation, release, and infection occur simultaneously during periods of leaf wetness. Increasing the wetness period increases the number of conidia produced and released and the proportion of conidia able to complete the infection process. Unless there is a long wetness period, no conidia produced later in the night are able to infect the plant. Only conidia produced during periods of high humidity or leaf wetness, followed by a sufficient period of leaf wetness, complete the infection process and yield susceptible lesions.

On the other hand, a logarithmic relationship with spore density was observed for the total number of susceptible- and resistant-type lesions produced under optimum conditions in the dew chambers. This result confirms El Refaei's (9) and Kim's (17) findings and indicates decreasing infection efficiency at high inoculum densities due to phenomena, such as competition and autoinhibition among conidia. Taken together, the overall relationship between spore density and infection over a wide range of inoculum concentrations would be sigmoidal (21).

Infectivity of conidia, produced during the previous night and during the day, depends on survival, which is determined by RH, temperature, exposure to the sun, and other conditions (1,24, 35,42). In controlled experiments, viability of germinated conidia decreased after a period of drying (35). There was an increase in the number of susceptible lesions when another group of trap plants was incubated for another 24 h in a gauze-covered cage in the field before being transferred to the greenhouse. The increase was about 30% of the lesion numbers on trap plants transferred

to the greenhouse a day earlier. This indicates the possible importance of space survival for epidemic progress.

Although trap plants proved to be a reasonable alternative for estimating inoculum density, the results were extremely variable. The number of lesions and infection types is conditioned by cultural methods and environmental conditions prior to exposure (7) and by environmental conditions during exposure (Table 5). It is recognized that variability resulted in unreliable estimates for the regression coefficients. Nevertheless, trends accounted for a significant proportion of the total variation, and microclimatic variables were significantly correlated with deviations from these trends.

The trends observed in the regressions depended on variable combinations. This result could be attributed to conidial viability and multiple infections at higher densities affecting the shape of the curve depicting the relationship between total lesion number and spore dose. The curvilinear relationships for the number of lesions on trap plants incubated in the dew chamber and total aerial spore concentrations were similar in all years. In contrast, trends were not consistent over years when susceptible lesions on trap plants placed in the greenhouse were used as a dependent variable in the regressions. This could reflect relative differences in onset times for sporulation and infection because of microclimate differences in the canopy during the wet and dry seasons.

It is likely that not all correlations between deviations and weather data are the direct results of cause and effect relations. These included weather variables that were correlated with another variable that was the contributing factor in determining variability. For example, deviations from the mean trends of infection were negatively correlated with temperature and positively correlated with RH and leaf wetness (Table 5). Other microclimatic conditions may exert indirect effects on sporulation of lesions on crop plants and infection on trap plants by affecting the host. Sunlight would be expected to affect leaf wetness, especially in the morning. Furthermore, only simple correlations were calculated. When considering the effects of microclimate on the observed variability, however, one should note the importance of interactions between microclimatic variables such as effects of temperature and leaf wetness on infection.

Most correlations of residuals and microclimatic variables agreed with published results. Wind was negatively correlated with infection in fields from a number of locations in Japan as a result of effects on inoculum dispersion and shortening the leaf wetness period (43,45). Leaf wetness, RH, temperature, and rain were correlated with deviations from the predicted number of S-type lesions on trap plants, using the potential number of lesions as a predictor. These correlations agreed with results of Andersen et al (2), Asai et al (3), El Refaei (9), Hemmi and Abe (12), Kahn and Libby (15), and Yoshino (46).

Rainfall decreased the inoculum concentration in the atmosphere but increased infection ratios (41). Suzuki (40) assumed that rainfall effects on deposition were largely positive because of impaction of spores in rain drops. However, negative correlations were found with deviations from the observed regression of the potential number of lesions on leaves with the number of conidia sampled by the Burkard space trap and the wind and rain covariates, possibly through removal of spores from dry surfaces by rain or wind. The number of conidia removed by distilled water from a Teflon surface depended on time after deposition and the flow rate of water used to remove the spores (11). Spores adhered to the wet surface by means of a gelatinous matrix at the spore tip. Deviations from the predicted number of spores on trap plants based on results from glass slides were positively correlated with rain (Table 5). There was a relative increase in the total potential number of lesions on trap plants as rain intensity increased because spores were removed from glass slides during rain; removal rates on leaves, however, were lower because of adhesion and impaction of conidia by rain. Clearly, alternative techniques or other reliable statistical methods must be found to remove the adverse effects of rain on sampling efficiency.

It should be possible to improve estimation of inoculum dose through the use of trap plants by correcting for multiple infections. Also, by adjusting for effects of weather variables, predictions of infection by the various methods could be improved. For example, the use of wind and rain as covariates could improve the estimation of inoculum dose and/or infection based on the number of lesions on trap plants and/or the number of conidia on other spore traps. This would require more detailed information from controlled experiments to determine mathematical relationships on the direct or indirect effects of weather variables such as rain, rain intensity, or sunlight on deposition, removal, and infection. Several models of infection already developed incorporate equations for predicting infection ratios based on leaf wetness duration and temperature (9,46).

For estimating the number of blast lesions formed in a rice canopy during a given exposure period, one has to consider that infection decreases with leaf age and position at a given plant age and plant age (15). Because only trap plants at the three-leaf stage were used, any model must adjust the number of infections at given levels of inoculum doses and environmental conditions by accounting for leaf age and position and plant age. By using the same cultivar for trap plants and crop, these adjustments may be more accurate because of differences in deposition and infection efficiency between cultivars.

The results showed reasonably good correlations among spore-sampling methods. This is important for research in developing countries where more expensive equipment is not always available. The alternate methods can be used to obtain a data base for epidemiological modeling and, in some cases, forecasting epidemic outbreaks.

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