# Environmental Conditions Conducive to Infection of Ryegrass by *Pyricularia grisea*

M. A. Moss and L. E. Trevathan

Former graduate research assistant and associate professor, Department of Plant Pathology and Weed Science, Mississippi State University, Mississippi State 39762.

Mississippi Agricultural and Forestry Experiment Station Publication 6161.

Portion of an M.S. thesis submitted by the first author to the Graduate School, Mississippi State University. Present address: Tropical Research and Education Center, 18905 S.W. 280th Street, Homestead, FL 33031.

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We thank Katy Porterfield and Herbert McClelland for technical assistance.

Accepted for publication 12 November 1986.

## ABSTRACT

Moss, M. A., and Trevathan, L. E. 1987. Environmental conditions conducive to infection of ryegrass by *Pyricularia grisea*. Phytopathology 77:863-866.

Environmental conditions conducive to infection of ryegrass (*Lolium multiflorum*) by *Pyricularia grisea* were characterized and the relative susceptibility of ryegrass cultivars was determined. Infection of 3-wk-old plants of the susceptible cultivar Gulf increased exponentially with increasing inoculum densities up to $8 \times 10^5$ conidia per milliliter. The youngest leaf of individual plants developed very few lesions in comparison with the more mature second, third, and fourth leaves. Maximum infection was predicted to occur on plants 4.7 wk old. The optimum temperature for infection was predicted to be 26 C. Few lesions occurred at 35 C, and none were observed at 5 C. A continual leaf-wetness period of at least 24 hr was required for maximum infection and may be the critical factor in epidemic disease development. Marshall, Sunbelt, and Tetrablend 444 cultivars developed fewer lesions than did the more susceptible cultivar Gulf.

*Additional key words*: epidemiology, ryegrass blast.

Annual ryegrass (*Lolium multiflorum* Lam.) is one of the most important winter annual forage crops planted in the southeastern United States (6). Ryegrass seeded alone, or in combination with other grasses and legumes, produces a high-quality forage for livestock. Ryegrass blast, caused by *Pyricularia grisea* (Cke.) Sacc. (perfect stage, *Magnaporthe grisea* (Hebert) Barr.), was first observed in ryegrass production areas in 1971 (1). During that year, widespread infection resulted in economic losses in Mississippi and Louisiana (2–5). Since 1971, the disease has become endemic in these areas, and localized outbreaks occur each year. The yearly threat of epidemic development underscores the need for understanding factors that govern development of the disease.

Characterization of conditions conducive to ryegrass infection by *P. grisea* could suggest cultural practices that would limit disease development. The objectives of this study were to determine the effects of inoculum density, temperature, and leaf wetness duration on infection and to determine plant age and cultivar susceptibility. A preliminary report has been given (9).

## MATERIALS AND METHODS

**Plant culture.** Cultural practices for ryegrass seedling production were similar for all experiments. Ryegrass seeds were surface-disinfested for 45 sec in 1% NaOCl (20% Clorox) and rinsed in distilled water. Seeds were planted in peat strips containing a growth medium of 2:2:1 (v:v:v) soil:sand:peat moss. The growth medium was pasteurized at 1.4 kg/cm$^2$ and 137 C for 6 hr, air-cooled, and amended with an 18-3-10 (N-P-K) formulation of Osmoscote fertilizer (Sierra Chemical Co., Milpitas, CA) at 216 g/m$^3$. Two weeks after emergence, plant populations were thinned to 36 plants per peat strip. Plants in a peat strip were fertilized weekly with 150 ml of a nitrogen fertilizer (Peters Fertilizer Products, Fogelsville, PA) at 473 ppm. Plants were watered three to four times a day. Insect pests were controlled using methomyl insecticide (Lannate; E. I. du Pont de Nemours & Co., Inc., Wilmington, DE).



**Inoculum production.** The isolate of *P. grisea* used in this study was obtained from a natural infection of annual ryegrass at Starkville, MS, in 1980 (12). Stock cultures were maintained on V-8 juice agar with periodic transfer to ryegrass agar (10 g of finely ground ryegrass leaves, 3 g of $CaCO_3$, and 15 g of agar per liter of distilled water) to maintain pathogen virulence.

Mycelial plugs from stock cultures were transferred to fresh V-8 juice agar in plastic petri dishes for inoculum production. Cultures were incubated 14 days on a 12-hr diurnal light period at 25 C. Seven milliliters of distilled water was added to each petri dish after the incubation period, and conidia were dislodged with a sterile loop. Conidial suspensions were combined in a beaker and filtered through cheesecloth. Conidial concentration was adjusted to the desired density using a hemacytometer. Gelatin (0.25%; w:v) was added to the inoculum suspension as an adhesive agent.

**Controlled environment studies.** Experiments were conducted in controlled environment chambers (Sherer Model CEL 37-14; Rheem Manufacturing Co., Asheville, NC) containing cool-white fluorescent lamps that yielded a photosynthetic photon flux density (400–700 nm) of 1.45 $\mu$mol/m$^2$ per second. Day length was based on a light period of 12 hr/day. Air temperatures were maintained at ±0.5 C, except during misting periods, when temperatures were maintained within ±2 C. Chambers were maintained at 75% relative humidity with a humidifier controlled with a humidistat and humidity-sensing elements (Model L15-3205 Humidistat; Hygrodynamics, Division of American Instrument Co., Silver Spring, MD), except during periods when plants were frequently misted to provide free moisture on the leaf surface for infection (9).

Each experiment was conducted according to randomization procedures in a randomized complete block design. In all experiments, except those testing effects of temperature, each chamber represented a block. In the temperature study, chambers represented experimental units and time represented a block.

Unless stated otherwise, all experiments were conducted using the following general experimental conditions. Plants were acclimated at 25 C in each chamber for 2 hr before inoculation. In each experiment, 3-wk-old Gulf ryegrass was inoculated with $2.0 \times 10^5$ spores per milliliter. Inoculations were made using an aerosol-propelled atomizer to apply 5 ml of spore suspension per

peat strip, each containing 36 plants. Plants were then incubated for 72 hr under periodic misting to maintain free water on the leaf surfaces. Following incubation in the growth chamber, plants were moved to an air-conditioned greenhouse (≈27 C), and total lesion number on leaves of individual plants was recorded after 72 hr.

Twenty-seven peat strips of plants were acclimated in each of three growth chambers. Three peat strips of plants from each chamber were randomly selected and inoculated with a spore suspension adjusted to one of nine inoculum concentrations. Treatments included 0, $6.25 \times 10^3$, $1.25 \times 10^4$, $2.5 \times 10^4$, $5.0 \times 10^4$, $1.0 \times 10^5$, $2.0 \times 10^5$, $4.0 \times 10^5$, and $8.0 \times 10^5$ spores per milliliter.

The effect of constant postinoculation temperature on infection was determined. For each treatment replication, three peat strips of plants were acclimated to one of the experimental temperatures, then inoculated and incubated as previously described at 5, 15, 25, or 35 C.

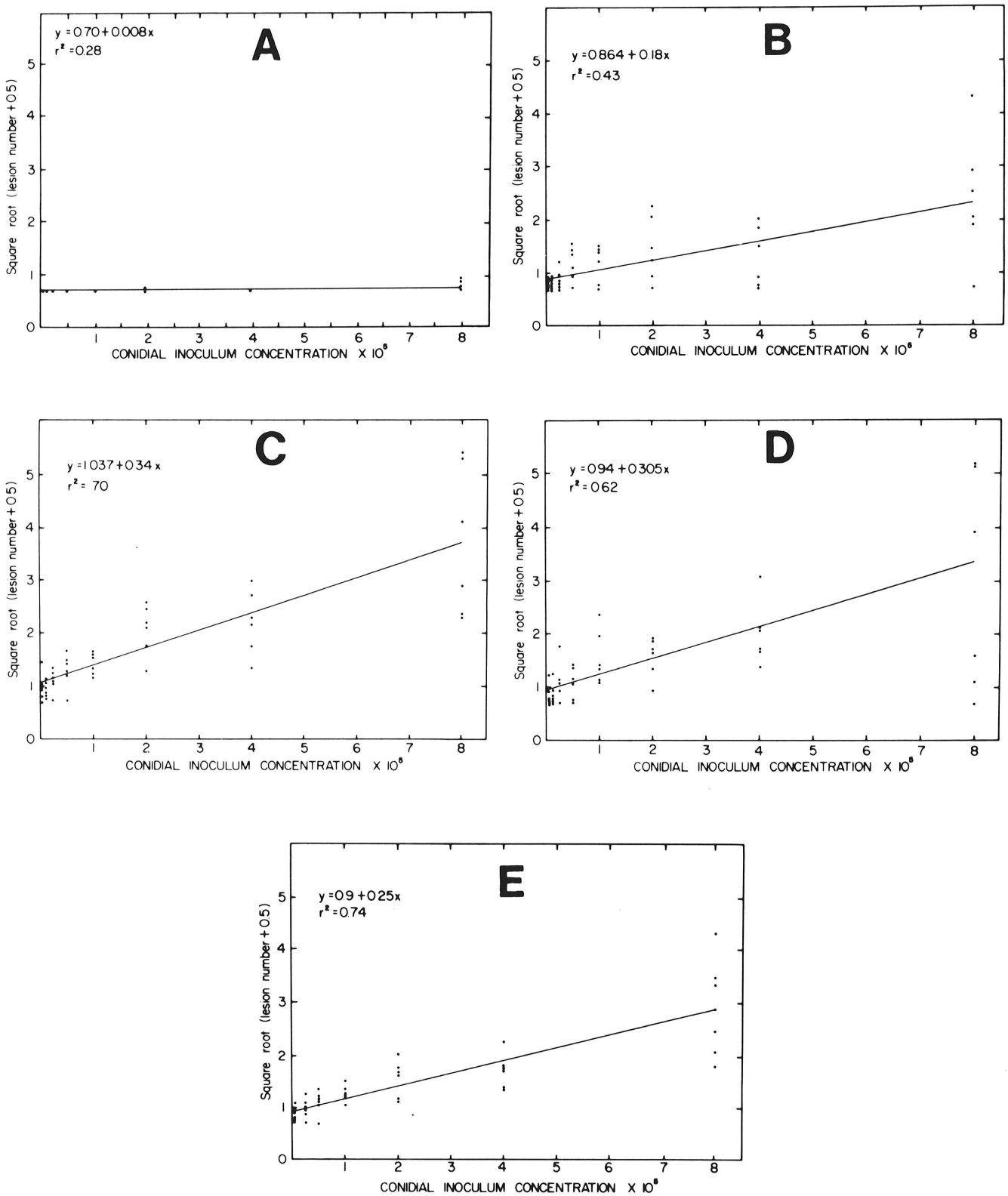


**Fig. 1.** Relationship of lesion number on **(A)** leaf one, **(B)** leaf two, **(C)** leaf three, **(D)** leaf four, and **(E)** whole plants of Gulf ryegrass to increasing inoculum concentrations of *Pyricularia grisea*.

The effect of continual leaf-wetness duration on infection of ryegrass was also tested. Twenty-one peat strips of plants were acclimated in each of three chambers. Following inoculation, plants were incubated under periodic misting, which provided a continuous film of free moisture on leaf surfaces. Following incubation periods of 0, 12, 24, 36, 48, 60, or 72 hr, three peat strips of plants were removed from each chamber and placed in the greenhouse. Leaf lesions were counted 6 days after inoculation.

The susceptibility of Gulf ryegrass to *P. grisea* was determined for different stages of plant maturity. Each week for 7 wk, nine peat strips of plants were cultured as described above. When plants of the final seeding emerged, the arbitrary age designation of 0 wk was assigned; the first planting was 6 wk old at this time. When the youngest plants had emerged, three peat strips of plants of each age were acclimated in each of three growth chambers. Plants were inoculated and incubated as described above.

Gulf, Marshall, Sunbelt, and Tetrablend 444 ryegrass cultivars were tested for relative susceptibility to *P. grisea*. Three peat strips of each cultivar were acclimated in each of three chambers. These plants were inoculated, incubated, and rated as previously described.

**Data analysis.** Each experiment was repeated and the data were combined for analysis. Data were analyzed using the General Linear Models procedure of SAS (10). Data for the study of effects of inoculum density on number of lesions produced on the four youngest ryegrass leaves and on the entire plant were linearized by square root transformation. The best-fit linear regression equation was then calculated for each leaf, and regression parameters were compared between leaves using $t$ tests according to Steel and Torrie (11). Data from plant age and temperature experiments were log transformed to improve normality and to stabilize variance. The best-fit polynomial regression equations were then determined for the effects of age and temperature on plant infection.

Regression analysis was also performed on continual wetness data. Because of a plateau in the data, all models attempted had a significant lack of fit. Therefore, continual wetness data were analyzed using analysis of variance and presented as means and standard errors.

## RESULTS

**Effect of inoculum density.** Total lesion number increased exponentially with increasing inoculum densities up to $8.0 \times 10^5$ conidia per milliliter on the second, third, and fourth youngest leaves of 3-wk-old Gulf ryegrass (Fig. 1A–D). Disease development on the youngest leaf was negligible. The linear model $Y = b_0 + b_1 X_1 + E$ was fit to the data for each leaf where $Y$ = SQRT (number of lesions + 0.5); $b_0$ and $b_1$ are parameter coefficients of intercept and slope, respectively; $X$ = inoculum density; and $E$ = random error.

When slope parameter estimates for each leaf were compared, the increased infection with increased inoculum densities was similar for all leaves except the youngest, which remained free of lesions (Table 1). Since regressions for leaves two through four were estimating the same population slope parameter, intercepts were compared to determine the relative susceptibility of each leaf. Mean lesion number was consistently higher on the third leaf, followed by the fourth and second leaves. Since these differences were not statistically significant, leaf data were combined and regression analyses performed on number of lesions produced on a per-plant basis. The regression of lesion number in response to increasing inoculum density accounted for 74% of the variation in the data as determined by the coefficient of determination, $r^2$ (Fig. 1E).

An inoculum density of $2.0 \times 10^5$ conidia per milliliter resulted in sufficient lesion numbers to develop response curves for subsequent experiments. Visual discernment of individual lesions was most efficient at this concentration.

**Effect of temperature.** Lesion numbers increased with increasing temperatures, up to 25 C. Lesions did not develop when plants were maintained at 5 C, and only a few were found at the highest temperature treatment of 35 C. A cubic polynomial model was the best-fit regression equation for the temperature data (Fig. 2). The

TABLE 1. Comparison of parameter estimates of linear regression equations for effect of inoculum density of *Pyricularia grisea* on number of lesions on four youngest leaves of 3-wk-old Gulf ryegrass

| Leaf comparsion[a] | $t$ tests for parameter comparison | |
|---|---|---|
| | Slopes ($b_1$) | Intercepts ($b_0$) |
| Leaf 1 vs. leaf 2 | 2.3*[b] | ...[c] |
| Leaf 1 vs. leaf 3 | 4.1*** | ... |
| Leaf 1 vs. leaf 4 | 3.5** | ... |
| Leaf 2 vs. leaf 3 | $1.5^{ns}$ | $0.7^{ns}$ |
| Leaf 2 vs. leaf 4 | $1.1^{ns}$ | $0.3^{ns}$ |
| Leaf 3 vs. leaf 4 | $0.3^{ns}$ | $0.4^{ns}$ |

[a] Leaves 1 and 4 the youngest and the most mature, respectively.
[b] Critical $t_{.05,94}$ value = 1.99; ns = not significant; * = $P \leq .05$, ** = $P \leq .01$, and *** = $P < .001$.
[c] No comparison was made because slopes were significantly different.

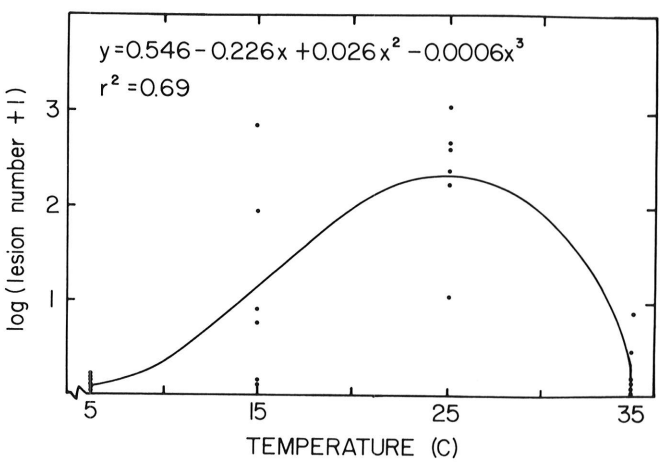


**Fig. 2.** Relationship of lesion number on 3-wk-old Gulf ryegrass plants to incubation at different temperatures for 72 hr following inoculation with *Pyricularia grisea* at $2.0 \times 10^5$ conidia per milliliter.

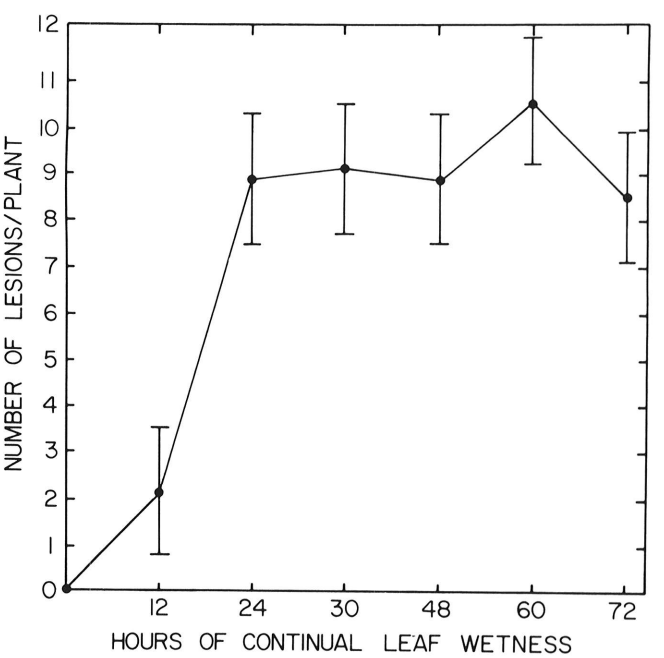


**Fig. 3.** Number of lesions produced on 3-wk-old Gulf ryegrass plants exposed to increasing periods of continual leaf wetness after inoculation with *Pyricularia grisea* at $2.0 \times 10^5$ conidia per milliliter.

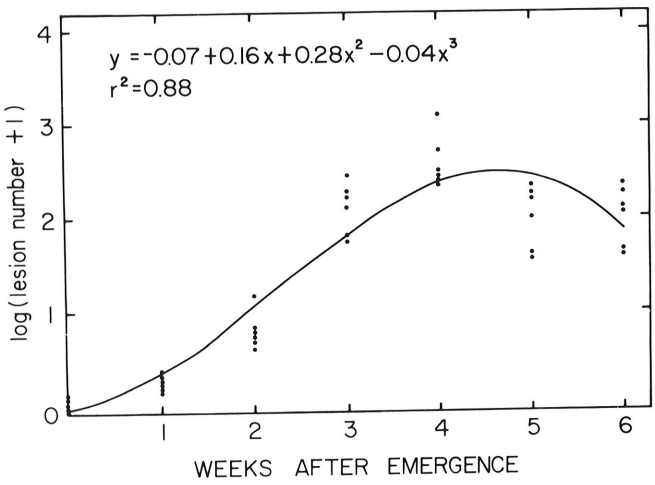


**Fig. 4.** Relationship of lesion number to increasing age of Gulf ryegrass plants inoculated with *Pyricularia grisea* at $2.0 \times 10^5$ conidia per milliliter.

model resulted in a coefficient of determination of 0.69 and predicted that 26 C was the optimum temperature for infection of 3-wk-old Gulf ryegrass by *P. grisea*.

**Effect of continual wetness period.** Lesion numbers increased exponentially with increasing periods of free moisture on leaf surfaces up to 24 hr (Fig. 3). Continual wetness periods beyond 24 hr did not increase the number of lesions produced.

**Plant age and cultivar susceptibility.** Gulf ryegrass was susceptible to *P. grisea* in all stages of development, although only a few lesions were observed on 1-wk-old plants. Lesion numbers increased with plant age until about 4–5 wk of age, then began to decrease (Fig. 4). A cubic polynomial regression equation, which accounted for 88% of the variation within the data, predicted that 4.7-wk-old plants were most susceptible.

When four ryegrass cultivars were inoculated with *P. grisea*, Gulf was most susceptible, with an average of 3.2 lesions per plant. Marshall, Sunbelt, and Tetrablend 444 cultivars developed 0.12, 0.13, and 0.05 lesions per plant, respectively.

## DISCUSSION

Ryegrass blast has occurred annually in southern Mississippi and Louisiana since it was first reported in 1971. However, the disease has not been as widespread or caused the economic losses that occurred during the 1971 epidemic. The endemic nature of the disease in these areas results in an annual inoculum source. Thus, the potential for epidemic disease development poses a yearly threat to ryegrass production in these areas.

Ryegrass cultivar Gulf was the primary cultivar planted during 1971. Since that time, the availability of additional cultivars more tolerant to ryegrass blast may partially explain the decrease in disease incidence and severity. The inoculum densities required for lesion development on Gulf ryegrass resulted in a negligible number of lesions on other cultivars in this study.

The susceptibility of leaves two, three, and four on 3-wk-old plants did not differ, and the maturity of these leaves at this stage of development had little or no effect on susceptibility. As plants reached 5–6 wk of age, lesion numbers began to decline. This may reflect the onset of adult plant resistance; however, ryegrass was susceptible to the fungus in all stages of development over the period of the study.

The range of temperatures at which lesions developed was broad, falling within the temperatures generally experienced in Mississippi and Louisiana during initial stages of ryegrass culture. For this reason, temperature is probably not a limiting factor in epidemic development. However, the longer planting can be delayed to take advantage of cooler fall temperatures, the lower will be the risk of infection.

Maintenance of a film of moisture on leaf surfaces was essential for infection. Meredith (8) reported maximum germination of conidia of *P. grisea* when a film of water was available. Infection of St. Augustine grass (*Stenotaphrum secundatum* (Walt.) Kuntze) occurred after wetness periods of 24 hr or more (7). An abnormal period of wet weather that coincided with the landfall of hurricane Edith during the last 2 wk of September may have provided moisture conditions critical for epidemic development in 1971.

Infection of ryegrass by *P. grisea* may occur in susceptible cultivars planted in late summer to early fall and exposed to 24 hr of continual leaf wetness. For polycyclic disease development leading to ryegrass blast epidemic, the availability of free moisture on the leaf surface is critical. Delayed planting of tolerant cultivars would reduce the risk of epidemic disease development.

## LITERATURE CITED

1. Bain, D. C. 1972. Blast, an undescribed disease of ryegrass. Miss. Agric. For. Exp. Stn. Info. Sheet 1188.
2. Bain, D. C. 1972. Blast fungus caused ryegrass kills. Miss. Farm Res. 35(4):2.
3. Bain, D. C. 1972. Special report: Ryegrass disease agent isolated. Miss. Farm Res. 35(12):7.
4. Bain, D. C., Patel, B. S., and Patel, M. V. 1972. Blast of ryegrass in Mississippi. Plant Dis. Rep. 56:210.
5. Carver, R. B., Rush, M. C., and Lindberg, G. D. 1972. An epiphytotic of ryegrass blast in Louisiana. Plant Dis. Rep. 56:157-159.
6. Frakes, R. V. 1976. The ryegrasses. Pages 307-313 in: Forages: The Science of Grassland Agriculture. 3rd ed. M. E. Heath, D. S. Metcalfe, and R. F. Barnes, eds. Iowa State University Press, Ames.
7. Isaac, M. M., and Owen, J. H. 1957. The gray-leaf-spot disease of St. Augustinegrass. Plant Dis. Rep. 41:871-875.
8. Meredith, D. S. 1963. *Pyricularia grisea* (Cooke) Sacc. causing pitting disease of bananas in Central America. Ann. Appl. Biol. 52:453-463.
9. Moss, M. A., and Trevathan, L. E. 1982. Environmental conditions conducive to development of ryegrass blast. (Abstr.) Phytopathology 72:994.
10. SAS Institute. 1982. SAS User's Guide: Statistics. SAS Institute, Inc., Cary, NC.
11. Steel, R. G. D., and Torrie, J. H. 1980. Principles and Procedures of Statistics: A Biometrical Approach. 2nd ed. McGraw-Hill, New York.
12. Trevathan, L. E. 1982. Pathogenicity on ryegrass and cultural variability of Mississippi isolates of *Pyricularia grisea*. Plant Dis. Rep. 65:592-594.