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Interaction of Temperature and Moisture on Infection of Wild Rice by *Bipolaris oryzae* in the Growth Chamber

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ABSTRACT

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Infection of wild rice (*Zizania palustris*) flag leaves by *Bipolaris oryzae* was studied at temperatures of 5 to 35°C and wet periods of 2 to 36 h after inoculation. Lesion densities (lesions/cm^2) increased with increasing wet periods depending on optimum temperature. High rates of infection occurred at 25 and 30°C and generally increased with continuous wet periods of 16, 18, 24, and 28 h. There were no lesions at 5°C and few at 10 and 35°C. Lesion densities declined when wet periods of 2, 4, or 6 h were interrupted by dry periods of 4, 6, 8, 10, or 12 h followed by a final 14 h of wetness. Lesion densities decreased at all temperatures with increased dry periods regardless of the initial wet period. The interaction of dry period length × wet period length × temperature was significant at the 0.5% level. With continuous wet periods, lesion numbers were highest at 25 to 30°C.

Fungal brown spot of wild rice, caused by *Bipolaris oryzae* (Breda de Haan) Shoemaker, is a severe disease that occurs on leaves, stems, and flowers of cultivated wild rice (*Zizania palustris* L.) in Minnesota (7). Under favorable environmental conditions for disease, fungal brown spot can cause up to 67% yield loss (9,10). Based on symptomatology and etiology, the disease commonly referred to as fungal brown spot has been separated into two diseases; fungal brown spot, caused by *B. oryzae*, and spot blotch caused by *B. sorokiniana* (Sacc.) Shoemaker (14). Previously reported yield losses to this disease likely resulted from both fungal brown spot and spot blotch.

The effects of moisture and temperature on infection by *B. oryzae* have been investigated under both controlled and field conditions for brown spot disease of rice (*Oryzae sativa*). Results from these studies, however, are often contradictory (1,2,5,6,8). This may be partially due to physiological differences between isolates from the Philippines and the southern United States (15).

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Germination of *B. oryzae* conidia differ between isolates from rice and wild rice. Generally, conidia from isolates obtained from rice germinate on a selective medium at 16 to 40°C, with optimal germination and growth occurring at 28°C (15). Infection of rice occurs at temperatures of 20 to 30°C, with optimal infection at 20 to 25°C with 4 or more h at 100% relative humidity (RH; 6,17). Conidia from isolates obtained from wild rice, however, germinate at 5 to 45°C on water agar, with optimal infection at 28 to 30°C with 8 or more h of 96 to 100% RH. The survival, primary inoculum dispersal, early infection, and subsequent spread of *B. oryzae* in commercial wild rice fields in Minnesota have been studied (3).

Currently, the fungicide propiconazole (Tilt) is used to control fungal brown spot on cultivated wild rice in Minnesota. The fungicide is normally applied to wild rice on a calendar schedule beginning in early July or when environmental conditions are thought to be favorable for disease to occur (9,10,16). Therefore, it is necessary to understand the precise environmental conditions necessary for infection and disease development in order to more effectively time the application of the fungicide and perhaps implement alternative disease control measures (7,12).

There have been no studies on the effects of environment on infection of cultivated wild rice by *B. oryzae* under controlled conditions. Therefore, the objective of this study was to determine the effects of temperature and continuous or intermittent moisture on infection of wild rice by *B. oryzae* in the growth chamber.

MATERIALS AND METHODS

Dew chambers were constructed of polyvinyl chloride (PVC) pipe covered with clear polyethylene plastic (0.01 mm) by the method of Krupinsky and Scharen (11). These chambers were assembled inside environmental growth chambers (1.2 by 2.3 by 1.7 m; Integrated Development & MFG., Environmental Growth Chambers). Pipe (PVC, 5 cm diameter) had 1.3-mm-diameter holes placed at 10-cm intervals to evenly distribute mist above the plants.

Wild rice seed (cultivar K-2) was stored for 4 months at 2°C, then germinated in tap water at 24°C. Seedlings were placed individually in plastic pots (15 cm diameter) previously filled within 2 cm of the top with a soil mix (sandy loam/sand/peat: composted manure, 7:3:2:1), pH 6.9, amended with 3.5 g of a 10-10-10 fertilizer. The remaining 2 cm of each pot was filled with washed silica sand to control growth of algae. Pots then were placed in wooden frames (91 by 71 cm) lined with 4 layers of 0.01 mm black polypropylene plastic. The frames were filled with tap water to a depth of 13 cm, and maintained throughout the experiment. Supplemental lighting was a mixture of 60 W incandescent, 160 W cool white, and Gro-lux fluorescent bulbs (ratio 5:5:3; Sylvania Lighting Products Group, Danvers, MA) for 16.5 h at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$, measured at mid-foliage with a Quantum Radiometer Photometer (Model L1-185, LI-COR Inc. Lincoln, NE). An additional granular 2.5 g of urea (46-0-0) fertilizer was placed into the water of each flat during the early boot stage of plant development.

B. oryzae isolate (B08055) was isolated originally from a wild rice plant in a commercial Minnesota field and was inoculated to wild rice every 6 months to maintain pathogenicity. BO8055 did not differ significantly in percent conidial germination or germ tube numbers when compared to nine other *B. oryzae* isolates on either water or potato dextrose agar (PDA; 13). Also, germ tube length, appressorium development, and infection efficiency on wild rice (cultivar K-2) did not differ from other *B. oryzae* isolates (13). BO8055 was cultured on PDA (Difco Laboratories, Detroit) in petri dishes for 4 weeks at 24°C in the dark to produce conidia. Conidia were removed from cultures by suspension in Soltrol 120 oil (Phillips Chemical Co.,

equation (Excel, Microsoft Corp., Redmond, WA).

RESULTS AND DISCUSSION

A highly significant ($P < 0.005$) interaction existed between the continuous wet periods and temperature. These two factors greatly influenced lesion density on the flag leaf (Table 1). Disease symptoms occurred at temperatures of 10 to 35°C and continuous wetness periods of 10 to 36 h, with maximum numbers of lesions/cm² occurring at 25°C and 20 to 24 h of wetness (Table 1). No disease symptoms occurred at 5°C (data not shown) and few at 10 or 35°C. Continuous moisture of 2 to 8 h (data not shown), regardless of temperature, did not result in disease expression. In general, lesion density increased as continuous wet periods increased from 10 to 24 h, and decreased at continuous wet periods of 28 to 36 h. The R^2 values (Table 1) reveal relationships between incubation temperature, continuous wet periods, and lesion numbers. R^2 values for continuous wet periods versus lesion numbers were

ie at various temperatures and periods of continuous

ure (°C)		
30	35	R^{2a}
2.0	0.1	0.44
4.5	0.1	0.55
6.8	0.6	0.68
9.4	0.4	0.64
11.4	0.2	0.60
15.8	0.3	0.50
18.6	0.4	0.45
14.0	0.1	0.50
11.0	0.2	0.56
0.92	0.14	

ryzae at various temperatures and intermittent wet

perature (°C)	
30	35
7.3	0.1
5.7	2.6
1.9	0.8
0.6	0.5
0.1	0.5
–0.96	–0.27
5.5	2.2
0.2	1.9
1.1	0.7
0.0	0.0
0.0	0.0
–0.80	–0.94
1.3	0.2
2.7	0.4
2.0	0.1
0.7	0.0
1.2	0.1
–0.37	–0.38

ures.

inconsistent across various incubation periods; however, the R^2 (0.95) was the highest at 25°C (Table 1).

Significant disease development by *B. oryzae*, indicated by lesion numbers in the continuous wet period treatments, occurred in a narrower temperature range than spore germination. In previous studies *B. oryzae* germinated 80 to 90% in vitro after 2 h at 5 to 35°C, but longer wet periods are needed for infection to be successful (13). The majority (95%) of *B. oryzae* conidia produced two bipolar germ tubes on wild rice at 5 or 10°C. However, germination was very slow with little infection at these temperatures even after continuous wet periods of 24 h or more (13). The importance of intercalary cell germination to infection during intermittent wet periods has not been investigated. An extended dry period following a wet one may damage bipolar germ tubes, but would probably have little or no effect on the ability of the intercalary cells at a later time to produce germ tubes during favorable environmental conditions. Also, *B. oryzae* isolates differ in the speed at which conidial germination and subsequent infection takes place (13). Consequently, these two factors may be important, because the paddy environment in Minnesota during July and August is often characterized by long periods of wet nights with intermittent dry days (7,9).

Temperature in the intermittent wet period study (Table 2) significantly ($P < 0.005$) influenced lesion density. Lengths of the initial wet and dry periods at a given temperature were negatively related to lesion density in a linear manner. The linear relationship (R^2) was strongest at 25 to 30°C ($R^2 = 0.95$–0.97). Lesion density generally increased with decreasing dry periods and increasing temperature of 15 to 25°C (Table 2). Lesion density declined at 30 and 35°C. No lesions developed at 5 or 10°C (data not shown), regardless of the length of wet and dry periods. Increasing the length of the wet periods beyond 4 h and the dry period beyond 12 h caused a decrease in the number of lesions per cm² at incubation temperatures of 15 to 35°C.

The wet period × temperature interaction was not significant ($P < 0.5$). This indicates the incubation temperature during the short initial wet period did not significantly influence the effect of wet period on lesion density. The interaction of dry period length and incubation temperature, however, was significant ($P < 0.005$).

Lesion density from the intermittent wet period study was lower than equivalent continuous wet period lengths. The fungus had fewer successful infections with increasing lengths of dryness after initial wet periods. Lesion formation by *B. oryzae* on wild rice cultivar K-2 occurred by 36 h after inoculation. Hyphae emerged through the cuticle and stomata by 48 h (96 to 100% RH, 28 to 30°C) and conidiophore initials and mats of hyphae on the cuticle surface occurred at 48 to 72 h (96 to 100% RH) (13). Isolates of *B. oryzae* from rice, *O. sativa*, produced germ tubes 10 to 14 h after inoculation at 100% RH (17). Because varying periods of high relative humidity occur frequently in wild rice stands, it is likely the conidial germination of *B. oryzae* during July and August in Minnesota may occur after only 6 to 8 h (9). Dew periods in Minnesota may occur nightly, and are often 12 or more h in duration in the middle of the plant canopy (10). Cultivated wild rice tillers profusely, and at maturity may be greater than 2 m in height with leaves 1 m long. This growth produces an understory of densely packed leaves and stems that is characterized by long dew periods and poor penetration by wind or aerially applied fungicides. The upper canopy is less dense and has shorter dew periods, good air circulation, and excellent fungicide penetration (9). Because of differences in wet periods and fungicide deposition, the flag leaf may have only a few small lesions, while older leaves in the understory may have a very high disease severity rating (9,10,16). The future use of dwarf wild rice cultivars and the more precise control of plant density will reduce canopy development and long periods of leaf wetness. Thus, these factors should result in reduced infection and better fungicide coverage resulting in improved FBS management.

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Pasadena, TX), and the concentration was adjusted to approximately 1.0×10^3 conidia/ml.

The middle portion (8 to 10 cm in length) of the uppermost fully expanded leaf from each plant in the boot stage of development was delineated as the inoculation area with a permanent felt tip marker. Each inoculation area was uniformly inoculated using a DeVilbiss atomizer with a conidial suspension until lightly wet as described by Browder (4).

Continuous and intermittent wet period treatments were conducted at 5, 10, 15, 20, 25, 30, or 35°C. Prior to placement in the dew chambers, plants were misted with a mixture of 0.026% Tween 40 (Polyoxyethylene titan monopalmitate, Sigma Chemical Co., St. Louis) in deionized water. In the continuous wet period, plants were wet for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 28, or 36 h after inoculation. In the intermittent wet period, plants were initially kept wet for 2, 4, or 6 h, followed by dry periods (85% RH) when plants were removed from the dew chamber for 4, 6, 8, 10, 12, or 14 h. After the dry periods, plants were placed back in the dew chamber for a final 14 h wet period. After each wet period, plants were returned to the greenhouse for lesion development. Each treatment consisted of 11 plants and was replicated three times. The entire experiment was repeated twice.

Seven days after initiation of the wet periods, inoculated areas were excised from the plants, placed in head bags, pressed, and dried at 24°C to later determine lesions/cm^2. Pressed samples were rehydrated for 30 min in 250 ml water mixed with 0.3 ml Tween 40 and examined with a Quebec colony counter (American Optical). Leaf area was determined with a portable area meter and transparent belt conveyer accessory (models LI-3000 and LI-3050A, LI-COR).

Data were analyzed with analysis of variance (ANOVA) using the IVAN interactive statistical computer program (18). Coefficients of determination (R^2) were calculated using simple nonlinear regression, based on a secondary polynomial equation (Excel, Microsoft Corp., Redmond, WA).

RESULTS AND DISCUSSION

A highly significant ($P < 0.005$) interaction existed between the continuous wet periods and temperature. These two factors greatly influenced lesion density on the flag leaf (Table 1). Disease symptoms occurred at temperatures of 10 to 35°C and continuous wetness periods of 10 to 36 h, with maximum numbers of lesions/cm^2 occurring at 25°C and 20 to 24 h of wetness (Table 1). No disease symptoms occurred at 5°C (data not shown) and few at 10 or 35°C. Continuous moisture of 2 to 8 h (data not shown), regardless of temperature, did not result in disease expression. In general, lesion density increased as continuous wet periods increased from 10 to 24 h, and decreased at continuous wet periods of 28 to 36 h. The R^2 values (Table 1) reveal relationships between incubation temperature, continuous wet periods, and lesion numbers. R^2 values for continuous wet periods versus lesion numbers were

Table 1. Mean number of lesions/cm^2 on the flag leaf of *Zizania palustris* infected with *Bipolaris oryzae* at various temperatures and periods of continuous wetness

Continuous wet periods (h)	Incubation temperature (°C)						
	10	15	20	25	30	35	R^{2a}
10	0.0	0.0	0.5[b]	1.0	2.0	0.1	0.44
12	0.0	0.0	2.1	8.0	4.5	0.1	0.55
14	0.2	2.0	3.6	9.6	6.8	0.6	0.68
16	0.2	2.4	4.3	12.6	9.4	0.4	0.64
18	0.3	2.5	4.8	16.2	11.4	0.2	0.60
20	0.5	2.6	3.8	22.6	15.8	0.3	0.50
24	1.2	2.5	3.0	22.7	18.6	0.4	0.45
28	0.4	3.2	3.1	20.0	14.0	0.1	0.50
36	0.3	2.5	3.5	14.6	11.0	0.2	0.56
R^{2c}	0.58	0.84	0.44	0.95	0.92	0.14	

[a] Coefficient of determination (R^2) for incubation temperature vs. lesion number.
[b] Mean value of 11 plants in each of three replications.
[c] Coefficient of determination (R^2) for continuous wet period vs. lesion number.

Table 2. Mean number of lesions/cm^2 on the flag leaves of *Zizania palustris* infected with *Bipolaris oryzae* at various temperatures and intermittent wet and dry periods. The dry periods were followed by 14 h of continuous wetness

Wet h	Dry h		Incubation temperature (°C)				
			15	20	25	30	35
2	4		0.8[a]	0.5	11.5	7.3	0.1
	6		5.1	0.7	4.2	5.7	2.6
	8		1.4	0.2	11.2	1.9	0.8
	10		1.6	1.1	3.2	0.6	0.5
	12		0.2	0.4	1.3	0.1	0.5
		R^{2b}	–0.42	0	–0.52	–0.96	–0.27
4	4		0.3	0.1	10.0	5.5	2.2
	6		1.0	1.7	8.8	0.2	1.9
	8		0.2	1.1	4.5	1.1	0.7
	10		0.3	0.6	1.0	0.0	0.0
	12		0.4	0.1	0.3	0.0	0.0
		R^{2b}	0	–0.70	–0.95	–0.80	–0.94
6	4		1.0	0.4	7.8	1.3	0.2
	6		1.0	0.4	9.4	2.7	0.4
	8		0.5	0.3	7.2	2.0	0.1
	10		1.5	1.3	3.4	0.7	0.0
	12		0.0	0.6	0.2	1.2	0.1
		R^{2b}	–0.30	–0.39	–0.97	–0.37	–0.38

[a] Mean of 11 flag leaves in each of three replications.
[b] Coefficient of determination (R^2) for incubation dry h vs. lesion numbers at various incubation temperatures.