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Effect of Continuous and Intermittent Wet Periods at Various Temperatures on Infection of Wild Rice by *Bipolaris oryzae.*

J. A. PERCICH, Professor, Department of Plant Pathology, University of Minnesota, St. Paul 55108; R. F. NYVALL, Professor, Department of Plant Pathology, University of Minnesota, St. Paul 55108; C. L. KOHLS, Plant Pathologist, American Cyanamid Company, P. O. Box 700, Princeton, NJ 08540; and D. K. MALVICIK, Reserch Associate, Department of Plant Pathology, University of Minnesota, St. Paul 55108.

ABSTRACT

Percich, J. A., Nyvall, R. E., Kohls, C. L., and Malvick, M. K. 1994. Effect of continuous and intermittent wet periods a various temperatures on infection of wild rice by *Bipolaris oryzae.*

Infection of wild rice (*Zizania palustris*) flag leaves by *Bipolaris oryzae* was studied at temperatures of 5-35 C and wet periods of 2-36 hr after inoculation. Lesion densities (lesions/ cm^2) increased with increasing wet periods depending on optimum temperature. High rates of infection occurred at 25 and 35 C with continuous wet periods of 16, 18, 20 and 24 hr, respectively. There were no lesions at 5 and few at 10 C. Lesion densities declined when wet periods of 2, 4, or 8 hr were interrupted by dry periods of 4, 6, 8 or 12 hr followed by a final 14 hr of wetness. Lesion densities decreased at all temperatures with increased dry periods regardless of the initial wet period. The interaction of dry period length x wet period length x temperature was significant at the O.5% level.

LITERATURE REVIEW

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 (10). Under favorable environmental conditions for disease, fungal brown spot can cause up to 67% yield loss (11, 12). The effect of environment (sunlight, moisture, and temperature) and plant nutrition on infection by *B. oryzae* has been investigated for brown spot disease of rice (*Oryzae sativa* L.), but results are often contradictory (1, 2, 4, 5, 6, 7, 8, 9, 10. 20). *Bipolaris oryzae* isolates from the Southern U. S. were morphologically and physiologically distinct than those from the Philippines (17).

Conidial germination behavior of *B. oryzae* differs if it isolated from rice or wild rice. In general, conidia of *B. oryzae* from rice, regardless of isolate and/or

source, germinate on a selective medium at 16-40 C with optimal germination and growth occurring at 28 C (17). Successful infection of rice, however, occurs at temperatures of (20-30 C) with optimal infection at 20-25 C with 4 hr or more of continuous moisture (100% RH) (9, 21). Whereas conidial germination of *B. oryzae* isolates from wild rice can germinate at 5-45 C on water agar with optimal infection at 28-30 C (96-100% RH) after 8 hr (16).

Currently, fungicide spray scheduling to control fungal brown spot of cultivated wild rice is based on either a calendar schedule beginning in early July or when forecast weather is thought to be favorable for severe disease to occur (11, 12, 18). A better understanding of the environmental conditions necessary for infection and disease progress in commercial fields in Minnesota is needed or consistent and effective integrated management of fungal brown spot will remain illusive (9, 15).

There have been no studies of the effect of environment on infection of wild rice by *B. oryzae*. The objectives of this study were to determine the effects of a) temperature under conditions of continuous wetness and b), intermittent wet and dry periods at various temperatures on infection of wild rice by *B. oryzae*.

MATERIALS AND METHODS

Dew chambers were constructed of polyvinyl chloride (PVC) pipe covered with clear polyethylene plastic (4 mil) by the method of Krupinsky and Scharen (14). These chambers were assembled inside environmental growth chambers (1.2 x 2.3 x 1.7 m) (Integrated Development & MFG., Environmental Growth Chambers, P.0. Box 407, Chagrin Falls, OH). Pipe (PVC, 5 cm diam) had holes (1.3 mm diam) placed at 10 cm intervals above the plants to evenly distribute mist. A single mister (Herrimidifer Co, Inc., Model 707 SM, Lancaster, Penn) was connected to one end of the pipe. Plastic lined wooden frames, 13 cm in depth were placed in the dew chambers and filled with water.

Wild rice seed (cultivar K-2), was stored for 4 mo at 2 C then germinated in tap water at 24 C. Seedlings were placed individually in plastic pots (15 cm diam) previously filled within 2 cm of the top with a soil mix (7 parts field soil: 3 sand: 2 peat: 1 manure), 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 were then placed in wooden frames (91 x 71 cm) lined with four layers of 4 mil black polypropylene plastic. The frames were filled with tap water to a depth of 13 cm that was maintained throughout the experiment. Supplemental lighting was a mixture of 60 W incandescent and 160 W cool white and Gro-lux fluorescent bulbs (Sylvania , Danvers, AMA) (ratio 5:5:3) for 16.5 hr at 300 μM m^{-2} s^{-1}, measured at midfoliage with a LiCor Quantum Radiometer Photometer (Model L1-185, Lincoln, NE 68504). 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.

The middle portion of uppermost fully expanded leaf (8-10 cm in length) from plants in the boot stage of development was delineated as the inoculation areas with a permanent felt tip marker. Each inoculation area was uniformly inoculated by hand with a conidial suspension until lightly wet as described by Browder (3). The isolate of *B. oryzae* (BO 8305) used in this study was originally isolated from a plant in a commercial Minnesota field in 1983. Conidia were produced on PDA (Difco Corp., Detroit, MI) in 100 x 15 mm plastic petri dishes for 4 wk at 24 C in the dark. Conidia were removed from cultures by suspending them in Soltrol 120 oil (Phillips Chem. Co., Borger, TX 79007), and their concentration adjusted to approximately 1.0×10^5 conidia/ml.

Continuous and intermittent wet period treatments were incubated at 5, 10, 15, 20, 25, 30 or 35 C. Prior to placement in the dew chambers, plants were misted with a mixture of 1 ml Tween 40 (Polyoxyethylene titan monopalmitate, Sigma Chem. Co.) per 3.8 L of deionized water. In the Continuous wet period, plants were wet for 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 24, 28, and 36 hr after inoculation. In the intermittent wet period, plants were initially kept wet for 2, 4, or 6 hr followed by dry periods (85% RH) when plants were removed from the dew chamber for 4, 6, 8, 10, 12, or 14 hr. After the dry periods, plants were placed in the dew chamber for a final 14 hr 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.

Seven days after initiation of the wet periods, the inoculated areas were excised from the plants, placed in breeding head bags, pressed and dried at 24 C to determine lesions (lesions /cm^2) later. 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 (model 3327, American Optical, Scientific Instrument Division, Buffalo, NY 14215). Lesions averaged 0.5 to 1.5 mm dia after 20 and 36 hr incubation, respectively at temperatures above 15 C. Leaf area measurements were determined with a portable area meter and transparent belt conveyer accessory (Model L1-3000 and L1-3050A Li-cor Inc., Lincoln, NE).

Data was analyzed using the IVAN statistical package, an interactive computer program for factorial design analysis (21), and the University of Minnesota's Cyber 74 computer.

RESULTS

Disease occurred at temperatures of 10-35 C and continuous wetness periods of 10-36 hr with maximum numbers of lesions/cm^2 occurring at 25 C and 24 hr of wetness (Table 1). No disease occurred at 5 C and little at 35 C. Continuous moistures of 2-8 hr, regardless of temperature did not result in disease. However, lesion density increased at continuous wet periods up to 24 hr but decreased at wet

periods of 28-36 hr. The analysis of variance (ANOVA) for the continuous wet period study (Table 2) demonstrated factors, such as length of the wet period, temperature at incubation and the interaction of wet period and temperature of infection had highly significant (0.005 level) effects on lesion density in a linear fashion.

Lesion density in the Intermittent wet period study generally increased with decreasing dry periods and increasing temperature of 15-25 C (Table 3). Lesion density declined at 30 and 35 C. No lesions developed at 5 or 10 C, regardless of the length of the wet and dry periods. The ANOVA for the intermittent wet period study indicated temperature was highly significant (0.005 level) related to lesion density (Table 4). Lengths of the initial wet and dry periods, at a given temperature, were negatively related to the lesion density in a linear manner. Thus, increasing the length of the wet and dry period from 4 and 12 hr, respectively, caused a decrease in the number of lesions per cm^2 at incubation temperatures of 15-25 C.

The lack of significance of the wet period x dry period x temperature interaction in the ANOVA indicates the temperature of incubation 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 temperature of incubation was significant (P=0.005). If all three factors, wet period (W), dry period (D), and temperature of incubation (T), are taken into consideration together in a W x D x T their interaction is significant (P=0.005).

DISCUSSION

Significant disease development by *B. oryzae*, indicated by lesion numbers in the continuous wet period treatments occurs in a narrower temperature range than germination. *Bipolaris oryzae* germinated 80-90% in 2 hr at temperatures of 15-35 C, however, significant levels of infection require at least 12 hr of continuous wetness. Germination of *B. oryzae* on wild rice at 5 or 10 C is very slow with little infection at these temperatures even after continuous wet periods of 24 hr or more. At 10 C at least 18 hr of continuous wetness was required for even a very low level of successful infection. *Bipolaris oryzae* conidia are multicellular and initially germinate bipolarly (5) to create more than one point of infection (16). However, the importance of the middle conidial cells to infection during intermittent wet periods has not been investigated. Additionally, *B. oryzae* isolates differ in the speed at which conidial germination and subsequent infection takes place (17). Consequently, these two factors may be important in a paddy environment where long nightly wet periods are common (9, 11).

The intermittent wet period results for *B. oryzae* on wild rice are similar to those of *Coccomyces hiemalis* Higgins on sour cherry (6). Lesion density from the intermittent wet period study was lower that equivalent continuous wet period length in both cases. Both pathogens had fewer successful infections with increasing

lengths of dryness after initial wet periods. Lesion formation by *B. oryzae* on wild rice cultivar K-2 occurs by 24 hr after inoculation. Hyphae emerged through the cuticle and stomata by 48 hr (96 - 100% RH, 28 - 30 C) and conidiophore initials and mats of hyphae on the cuticle surface occurred at 48-72 hr (96 - 100% RH) (16). Isolates of *B. oryzae* from rice, *Oryzae sativa* L, produced conidiophores 5 - 14 hr after inoculation at 100% RH (20). Because periods of high relative humidity occur frequently in wild rice stands it is likely the latent period of *B. oryzae* during July and August in Minnesota may frequently be only 6 - 8 days (11). Also, because wild rice is an aquatic plant the microenvironment in the paddy is different from that of dryland cereal. Dew periods in Minnesota may occur nightly and are often 10 hr or more in the middle of the plant canopy (12). Cultivated wild rice tillers profusely and at maturity may be greater than 2 m in height with leaves 1 m long to produce an understory of densely packed leaves and stems, that is characterized by long dew periods and poor penetration by wind or aerially applied fungicide. The upper canopy is less dense and has shorter dew periods, good air circulation and excellent fungicide penetration (11). Thus, because of differences in wet periods and fungicide deposition, the flag leaf may have only a few small lesions, while leaves in the understory may have a very high disease severity rating (12, 18).

Experiments are underway to study the survival, primary inoculum dispersal, early infection, and subsequent spread of *B. oryzae* in commercial wild rice fields in Minnesota.

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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 (hr)	Incubation temperature (C)					
	10	15	20	25	30	35
10	0.0	0.0	0.5[z]	1.0	2.0	0.1
12	0.0	0.0	2.1	8.0	4.5	0.1
14	0.2	2.0	3.6	9.6	6.8	0.6
16	0.2	2.4	4.3	12.6	9.4	0.4
18	0.3	2.5	4.8	16.2	11.4	0.2
20	0.5	2.6	3.8	22.6	15.8	0.3
24	1.2	2.5	3.0	22.7	18.6	0.4
28	0.4	3.2	3.1	20.0	14.0	0.1
36	0.3	2.5	3.5	14.6	11.0	0.2

[z]Mean value of 11 plants in each of three replications.

Table 2. The analysis of variance for lesion density data from the continuous wet period infection study of *Bipolaris oryzae* on wild rice.

Source	df	Mean Square	F^z
Wet period	13	136.25	22.67***
linear	1	1,374.79	212.64
remainder	12	422.61	
Temperature	6	435.73	68.66***
Wet x temperature	78	42.18	5.23***

[z]Two asterisks indicate that the F value was significant at $P<0.005$.

Table 3. Mean number of lesions/cm^2 on the flag leaves of *Zizania palustris* infected with *Bipolaris oryzae* at various temperature and intermittent wet and dry periods. The dry periods were followed by 14 hr of continuous wetness.

Wet hr	Dry hr	Incubation temperature (C)				
		15	20	25	30	35
2	4	0.8[z]	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
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
6	4	1.0	0.0	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

[z]Mean of eleven flag leaves in each of three replications.

Table 4. Analysis of variance for lesion density data from the intermittent wet period infection study of *Bipolaris oryzae* on wild rice.

Source	df	Mean Square	F^z value
Wet period	2	8.06	4.38
linear	2	12.66	6.88**
remainder	1	3.45	
Dry period	4	33.19	18.03
linear	1	128.56	69.85***
remainder	3	4.20	
Temperature	6	115.08	62.53***
Wet x dry	8	2.14	1.16
linear x linear	1	8.26	4.49**
remainder	7	8.86	
Wet x temperature	12	2.51	1.37
Dry x temperature	24	14.37	7.80***
Wet x dry x temperature	48	3.37	1.83
Error	105	1.84	

[z]Two asterisks indicate that the F value was significant at $P<0.05$; Three asterisks at $P<0.005$.