Ecology and Epidemiology

# Interaction of Wet Period and Temperature on *Pyrenophora tritici-repentis* Infection and Development in Wheats of Differing Resistance

R. M. Hosford, Jr., C. R. Larez, and J. J. Hammond

Professor, Department of Plant Pathology, North Dakota State University (NDSU) Fargo 58105; associate professor, Departamento de Agronomia, Universidad de Oriente, Jusepin-Monagas, Venezuela; and professor, Department of Agronomy, NDSU, respectively.

Portion of a thesis submitted by the second author in partial fulfillment of the requirements for the Ph.D. degree at North Dakota State University, reanalyzed by the first and third authors.
Published with the approval of the director of the North Dakota Agricultural Experiment Station as Journal Series Article 1451.
We thank Dr. H. A. Lamey for critical manuscript review and Mrs. Phyllis Hellem for repeated manuscript typing.
Accepted for publication 30 December 1986 (submitted for electronic processing).

## ABSTRACT

Hosford, R. M., Jr., Larez, C. R., and Hammond, J. J. 1987. Interaction of wet period and temperature on *Pyrenophora tritici-repentis* infection and development in wheats of differing resistance. Phytopathology 77:1021-1027.

Increasing postinoculation wet period and/or temperature increased infection and disease development, but disease development in the resistant wheat was always less than in the susceptible. Infections (penetrations) were few, and lesions did not grow to visible size after 6 hr wetting at 10 C. Infections and visible lesions were few after 6 hr wetting at 20 and 30 C. Number and size of lesions increased and became abundant with lengthening wet period (12, 24, and 48 hr) at 10, 20, and 30 C. On tan spot resistant BH1146 and susceptible ND495 wheats in all wet periods and temperatures, conidia of *Pyrenophora tritici-repentis* produced similar numbers of germ tubes, appressoria, penetrations, and lesions. Germ tube growth and appressoria production increased with increasing wet period and temperature and stopped with termination of the wet period. As the wet period increased, papilla formation became twice as numerous in BH1146 as in ND495 but not enough to significantly affect the number of penetrations or lesions. Compared with ND495, resistance in BH1146 kept hyphal growth in the lesions, lesion growth, and growth in leaf area damage to approximately one-half and growth in lesion length to two thirds, with lengthening wet period and rising temperature. Resistance in BH1146 stopped lesion growth with rising temperature between 20 and 30 C. This resistance appeared to operate on a level smaller than organelle (molecular) in the leaf cells. Similar lesion spread beyond the mycelium in both wheats indicated a diffusible toxin from the mycelium that was not affected by lesion restricting resistance.

*Additional key words*: *Drechslera tritici-repentis, Helminthosporium tritici-repentis, Pyrenophora trichostoma*, yellow leaf spot.

Tan spot, caused by *Pyrenophora tritici-repentis* (Died.) Drechs. (syn. *P. trichostoma* (Fr.) Fckl.), anamorph *Drechslera tritici-repentis* (Died.) Shoem. (syn. *Helminthosporium tritici-repentis* Died.), has recently become recognized as one of the important and widespread diseases of wheat (17). Losses due to tan spot have been chronically 3–15% and as high as 50% of grain yield (17). The fungus has a wide host range, including 26 species in the Gramineae (22). It is the major leaf spot pathogen of important prairie grasses in Saskatchewan (19,27). Physiological races for virulence in *P. tritici-repentis* have been reported (7,10,11). Resistance to *P. tritici-repentis* in wheat has been associated with length of the postinoculation wet period. Highly susceptible wheat selections and cultivars have been severely spotted after only 6–12 hr of leaf wetness (12). In resistant wheats, disease severity has increased in differing degrees with increasing duration of the wet period, with the most resistant wheats requiring more than a 48-hr wet period for severe spotting to develop (17). Wheats identified as resistant in seedling and adult plants in the greenhouse are resistant in field evaluations (10–12,18,35). Field inoculation procedures have been developed (8,36). Tan spot severity has been greater on each lower leaf than on the leaf above it (35). Some wheat varieties have been resistant in one part of the world and susceptible in another (7,10,11). Resistance has been reported as polygenic (3,28) and monogenic (25) and of intermediate to high heritability (3,28). Leaf glaucousness is unrelated to resistance (36). Recently incubation period (inoculation to first symptoms) was shown to rapidly decrease from over 150 hr as temperature rises from 12 and 15 C to around 40 hr at 18, 20, 24, 28, and 32 C. Incubation period also increases in the presence of *Cochliobolus sativus* Ito & Kurib. (5). Wetting of foliage before inoculation has had no effect on tan spot (10,11). The fungus persists in wheat straw 2 cm above the soil but not on or below (33). Sexual sporulation has been common in standing and partly buried stubble. Reproduction (asexual) in leaf lesions has been absent to abundant (17). Asexual (10–12,17,35) and sexual (10–12,32) reproduction have been accomplished in the laboratory. The fungus has been reported to store well in liquid nitrogen for 16 wk (20). We have recovered it in good condition after 4 yr in liquid nitrogen.

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In mixed inoculations of wheat leaf spotting pathogens, *P. tritici-repentis* and *Leptosphaeria nodorum* Müller have not affected each other's disease scores. *P. tritici-repentis* and *C. sativus* have antagonistically reduced their combined leaf scores and their induced yield losses. *C. sativus* has inhibited conidial germination, germ tube growth, and appressorium formation of *P. tritici-repentis*, reduced its recovery (reisolation) from lesions, and inhibited its perithecial formation and growth in culture (5).

The objective of this study on tan spot was to determine the effect of postinoculation wet periods alone and combined with temperature on infection and pathogen and lesion development and host resistance.

## MATERIALS AND METHODS

**Inoculum and plants.** Hard red spring wheat breeding line ND495, highly susceptible to race 11 and almost all other races of *P. tritici-repentis*, and wheat cultivar BH1146 (PI185831), resistant to race 11 and most other races of this fungus, were used in this study. ND495 is severely spotted by the fungus in wet periods of only 6–12 hr. BH1146 is relatively resistant even after wet periods of 50 hr (5,7,12,28).

Conidial inoculum of *P. tritici-repentis* race 11 (isolate PyD7) (7) was produced by the technique of Raymond et al (35). The conidia were suspended in deionized water and filtered through four layers of cheesecloth. One drop of Tween 20 (12) was added to each 100 ml of conidial suspension. The concentration was adjusted to approximately 2,500 conidia per milliliter. Plants were

sprayed to runoff (4 ml per plant) with the conidial suspension using a DeVilbiss No. 26 sprayer, at 0.55 kg $cm^{-2}$ pressure.

**Wet period and disease development at constant temperature.** Plants were grown in autoclaved soil in 15-cm-diameter clay pots in greenhouse for 30 days to the four- to six-leaf stage of susceptibility (35) and inoculated as described above. After inoculation, plants were placed in a mist chamber at 20 C under continuous fluorescent light. Misting was provided with a DeVilbiss No. 841 nebulizer. After completion of the wet periods, plants were dried for 30 min with a fan and placed on a greenhouse bench (16–23 C).

The experimental design was a split-plot with three replicates. Whole plots were combinations of ND495 and BH1146 at each of five wet periods (6, 12, 24, 48, and 60 hr) in a randomized complete block arrangement, with five sampling times after inoculation (6, 12, 24, 48, and 72 hr) as subplots within each whole plot. For each replication there were five plants per pot, five pots per cultivar, one pot of each cultivar per wet period, and one plant per sample. At each sampling time, the second youngest leaf of each plant was cut into three pieces, each 3 cm long, and placed in alcoholic lactophenol (three parts 95% ethanol, one part of lactophenol) until examined. In each sample, number and length of germ tubes on the leaf surface, number of appressoria, number of hyphae penetrating the epidermal cells that had papillae form around the hyphae, and number of hyphae that grew through the papillae or grew without papillae forming to establish colonies were determined for 40 germinated conidia.

To study symptom expression, the experimental design was a randomized complete block with subsamplings and three replicates. Treatments consisted of combinations of ND495 and BH1146 and five postinoculation wet periods (6, 12, 24, 48, and 60 hr). For each replication there were eight plants per pot, five pots per cultivar, and one pot of each cultivar per each wet period. Subsamplings were the eight plants of each pot. Eight days after inoculation a 15-cm-long section of the second youngest leaf of each plant was excised, mounted on a cardboard, and placed in a plant press for 8 days. For each 15 cm of leaf, the number of lesions per square centimeter of tissue and the length and width of the lesions were measured microscopically at 100×. Length of lesions in each leaf was expressed as the average length of 15 lesions, whereas width was determined by averaging the width of 10 lesions. An approximation of the average size of lesions on each leaf was obtained by multiplying the average lesion length by the average width. The surface area of each leaf piece was determined by multiplying the average width on each leaf by the length (15 cm).

Hyphal growth within the host tissue was studied in the same leaf samples used for determination of symptom expression. Five 3-cm-long leaf pieces from each treatment in each of the three replicates were placed in standard formalin-aceto-alcohol for 24 hr, boiled in alcoholic lactophenol for 3 min, placed in lactophenol cotton blue for 2 hr, and then destained in a concentrated solution of chloral hydrate for 1–3 days. Samples were rinsed in deionized water and mounted in lactophenol. The longitudinal hyphal growth in 30 lesions per treatment was measured microscopically.

**Wet period and disease development at three temperatures.** Seedlings were grown in a mixture of Jiffy Mix (Ball Jiffy Co., Chicago, IL), Sunshine Peat Moss (Fisons Western Corp., Vancouver, B.C., Canada), and No. 3 vermiculite (Silbrico Corp., Chicago, IL) in a 1:2:2 (w/w) ratio in six-well plastic trays (Cel-Pak, Bedding Plant Containers Inc., Twin City Plastic, Minneapolis, MN). Two seedlings per well were used for a total of 12 plants per tray. Each 10 kg of the mixture was supplemented with 1 kg of dolomitic lime, 0.1 kg of 0-20-0 fertilizer, 0.45 kg of calcium nitrate, 0.035 kg of Peter's soluble trace elements mixture (Peters Fertilizer Products, W. R. Grace & Co., Allentown, PA) and 0.18 kg of 14-14-14 controlled release fertilizer (Sierra Chemical Company, Milpitas, CA). Wells in the trays were 4 cm long × 4 cm wide × 5.5 cm deep.

Twenty-day-old seedlings in the trays were inoculated and placed in a mist chamber. Each chamber consisted of two plastic transparent boxes, 37 cm long × 27 cm wide × 15 cm high, which were sealed at the junctures with masking tape. Misting in each chamber was provided with a DeVilbiss No. 272 electric humidifier through a 5-cm-diameter hole bored on one side of the mist chamber cover. A total of eight trays, six inoculated and two noninoculated controls, were accommodated in each chamber. Each mist chamber was placed in a Percival 135LLVL temperature-controlled chamber (Percival Mfg. Co., Boone, IA), set at the desired temperatures.

ND495 and BH1146, three temperatures (10, 20, and 30 C), and three postinoculation wet periods (6, 12, and 24 hr) were studied. After each wet period, seedlings were dried for 15 min with a fan and returned to the temperature-controlled chamber, outside of the mist chamber, to complete a postinoculation period of 24 hr at the same temperature, and then moved to a greenhouse bench (16–23 C) until the experiment was completed.

The experimental design was a split-split-plot. Main plots were the temperatures, as randomized complete blocks with three replicates. Wheats were the subplots, and the three wet periods were the sub subplots. Three replications were done in time. For each replicate there were six trays per temperature, three trays per wheat, and one tray per wet period.

Forty-eight hours after inoculation the top leaf of two seedlings in each treatment was prepared as outlined in the preceding constant temperature study. Forty conidia on the two leaves were recorded for spore germination, number of germ tubes, length of germ tubes, number of appressoria, number of infecting hyphae beneath which papillae formed, and number of infecting hyphae colonizing host cells.

To study symptom expression, the top leaf of eight seedlings in each treatment was cut in 10-cm-long pieces 8 days after inoculation and processed and measured as outlined in the constant temperature study. All microscopic observations and measurements were made with a Leitz Laborlux bright field microscope and an ocular micrometer.

Analyses of variance and differences between means were performed using the ANOVA or General Linear Model of the Statistical Analysis Systems (SAS Institute Inc., Cary, NC). The interactions involving wet period relate to Tables 1–4, those involving time of observation to Table 1, and temperature to Tables 3 and 4.

No spotting developed on water inoculated check plants used in all the studies.

## RESULTS

Conidial germination at 6 hr wetting and 10 C was significantly higher on ND495 (82%) than on BH1146 (71%). Increasing the wet period and/or raising the temperature resulted in a nonsignificantly different range of 92–100% germination on the two wheats (Table 3). The interactions wheat selection × temperature and wheat selection × wet period were nonsignificant. The interaction temperature × wet period was significant, reflecting the reduced germination at 10 C × 6 hr wetting.

Germ tubes per conidium at 20 C ranged from 2.0 to 2.7 with some significant differences among wheats, wet periods, and observation times that did not follow any discernible pattern. Germ tubes per conidium were not significantly different among wheats over the combined range of wet periods and observation times (Tables 1 and 3). Germ tubes per conidium increased significantly with rising temperature over the range 10, 20, and 30 C and with increase in wet period from 6 to 12 hr but not 12 to 24 hr. Temperature and length of wet period appeared to affect germ tube production independently of each other. The interactions wheat selection × temperature, wheat selection × wet period, temperature × wet period, and wet period × time of observation were nonsignificant. The interaction wheat selection × time of observation was significant, reflecting the significant differences that did not follow any discernible pattern.

Length of germ tubes on the leaf surface significantly increased with lenthening wet period (Tables 1 and 3) and with temperature rising from 10 to 20 C (Table 3). From 20 to 30 C increase occurred but was nonsignificant. The interactions wheat selection × temperature, wheat selection × observation time, and wet period × observation time were nonsignificant. The interaction temperature

× wet period was significant, reflecting the plateauing at 12 and 24 hr wet period at 30 C on both varieties and the drop to 215.8 $\mu$m on BH1146 after 24 hr wetting at 30 C (Table 3). The interaction wheat selection × wet period was nonsignificant in the study reported in Table 3 and significant in the study reported in Table 1.

Appressoria production was significantly higher on ND495 than on BH1146 at the longer wet periods of 48 and 60 hr combined with the highest observation time of 72 hr (Table 1). It was not significantly different on the two wheats over the entire range of wet periods and observation times (Table 1). It increased significantly on both wheats with increasing wet period and rising temperature (Tables 1 and 3). Production of appressoria stopped when the plants were returned to dry conditions (Table 1). The interactions of wheat selection × temperature, temperature × wet

TABLE 1. Effect of wet period on infection of ND495 (susceptible) and BH1146 (resistant) hard red spring wheats inoculated with conidia of *Pyrenophora tritici-repentis*[a]

| Wet period[a]/ Observation time[a]/ Conidia observed[a] | Conidia on leaves: Germ tubes/ conidium | | | Length of germ tubes ($\mu$m) | | | Appressoria: Per 100 conidia | | | Percent with papilla around infection hypha | | | Colonizing host cells (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 495 | 1146 | PDIFF | 495 | 1146 | PDIFF | 495 | 1146 | PDIFF | 495 | 1146 | PDIFF | 495 | 1146 | PDIFF |
| 6/6/600 | 2.3 | 2.1 | 0.05 | 82.3 | 77.1 | 0.70 | 54.8 | 53.0 | 0.88 | 7.0 | 19.00 | 0.0001 | 6.4 | 7.2 | 0.80 |
| 6/12/120 | 2.6 | 2.2 | 0.11 | 95.3 | 91.9 | 0.90 | 49.2 | 54.2 | 0.85 | 0.0 | 8.8 | 0.13 | 7.8 | 14.5 | 0.37 |
| 6/24/120 | 2.4 | 2.4 | 0.87 | 83.2 | 89.2 | 0.82 | 56.7 | 58.3 | 0.95 | 9.3 | 7.5 | 0.76 | 12.8 | 7.5 | 0.48 |
| 6/48/120 | 2.4 | 2.4 | 0.75 | 89.7 | 97.8 | 0.76 | 45.0 | 41.7 | 0.90 | 2.2 | 8.8 | 0.26 | 4.5 | 6.4 | 0.80 |
| 6/72/120 | 2.4 | 2.6 | 0.20 | ··· | ··· | | 55.8 | 70.0 | 0.59 | 7.2 | 9.4 | 0.71 | 16.6 | 6.4 | 0.17 |
| 12/12/480 | 2.4 | 2.4 | 0.87 | 180.7 | 179.7 | 0.95 | 121.7 | 127.7 | 0.65 | 8.3 | 16.2 | 0.01 | 34.3 | 31.3 | 0.41 |
| 12/24/120 | 2.7 | 2.4 | 0.15 | 214.3 | 207.0 | 0.79 | 98.3 | 111.7 | 0.61 | 3.2 | 14.6 | 0.05 | 47.7 | 40.8 | 0.35 |
| 12/48/120 | 2.4 | 2.5 | 0.87 | 185.0 | 201.8 | 0.54 | 121.7 | 118.3 | 0.90 | 11.0 | 12.0 | 0.86 | 47.5 | 45.1 | 0.75 |
| 12/72/120 | 2.3 | 2.7 | 0.04 | ··· | ··· | | 98.3 | 113.3 | 0.57 | 5.7 | 13.1 | 0.20 | 56.9 | 53.7 | 0.67 |
| 24/24/360 | 2.4 | 2.3 | 0.20 | 328.7 | 323.7 | 0.79 | 219.7 | 191.1 | 0.07 | 10.0 | 20.7 | 0.002 | 29.6 | 32.7 | 0.47 |
| 24/48/120 | 2.3 | 2.4 | 0.63 | 408.6 | 318.0 | 0.002 | 158.3 | 173.3 | 0.57 | 6.1 | 15.1 | 0.12 | 48.5 | 42.0 | 0.39 |
| 24/72/120 | 2.3 | 2.4 | 0.52 | ··· | ··· | | 179.2 | 184.2 | 0.85 | 2.8 | 14.2 | 0.05 | 47.2 | 33.9 | 0.08 |
| 48/48/240 | 2.3 | 2.0 | 0.03 | 428.9 | 497.6 | 0.02 | 290.4 | 260.8 | 0.12 | 15.1 | 24.9 | 0.02 | 27.7 | 31.8 | 0.44 |
| 48/72/120 | 2.5 | 2.3 | 0.34 | ··· | ··· | | 352.5 | 295.8 | 0.03 | 9.1 | 20.8 | 0.05 | 26.8 | 25.9 | 0.90 |
| 60/72/120 | 2.2 | 2.4 | 0.27 | ··· | ··· | | 375.0 | 310.8 | 0.02 | 20.1 | 30.3 | 0.08 | 26.0 | 30.3 | 0.57 |
| 6–60/6–72 3,000 | 2.4 A | 2.3 A | | 189.6 A[b] | 187.4 A[b] | | 143.6 A | 136.1 A | | 8.2 A | 17.0 B | | 26.2 A | 25.2 A | |
| 6/6–72/1,080 | 2.4 | 2.3 | | 85.3[c] | 83.9[c] | | 53.4 | 54.4 | | 5.9 | 14.4 | | 8.2 | 7.9 | |
| 12/12–72/840 | 2.4 | 2.5 | | 188.3[d] | 189.6[d] | | 115.0 | 122.0 | | 7.6 | 14.9 | | 41.31 | 37.8 | |
| 24/24–72/600 | 2.4 | 2.3 | | 355.4[e] | 321.8[e] | | 199.3 | 186.2 | | 7.8 | 18.3 | | 36.9 | 34.8 | |
| 48/48–72/360 | 2.4 | 2.1 | | 428.9[f] | 497.6[f] | | 311.1 | 272.5 | | 13.0 | 23.5 | | 27.4 | 29.8 | |
| 60/72/120 | 2.2 | 2.4 | | ··· | ··· | | 375.0 | 310.8 | | 20.1 | 30.3 | | 26.0 | 30.3 | |
| | 495 & 1146 | | | 495 & 1146 | | | 495 & 1146 | | | 495 & 1146 | | | 495 & 1146 | | |
| 6/6–72/2,160 | 2.3 AB | | | 84.6 A[g] | | | 53.9 A | | | 10.2 A | | | 8.0 A | | |
| 12/12–72/ 1,680 | 2.4 A | | | 188.9 B[h] | | | 118.5 B | | | 11.2 A | | | 39.6 C | | |
| 24/24–72/ 1,200 | 2.3 AB | | | 388.6 C[i] | | | 192.8 C | | | 13.0 A | | | 35.9 C | | |
| 48/48–72/720 | 2.2 B | | | 463.2 D[j] | | | 291.8 D | | | 18.3 B | | | 28.6 C | | |
| 60/72/240 | 2.3 AB | | | ··· | | | 342.9 E | | | 25.2 C | | | 28.1 B | | |
| 6/6/1,200 | 2.2 | | | 79.7[k] | | | 53.9 | | | 13.0 | | | 6.8 | | |
| 6–12/12/ 1,200 | 2.4 | | | 150.5[k] | | | 100.0 | | | 10.7 | | | 28.4 | | |
| 6–24/24/ 1,200 | 2.4 | | | 237.3[k] | | | 155.8 | | | 12.7 | | | 29.6 | | |
| 6–48/48/ 1,200 | 2.3 | | | 278.4[k] | | | 176.1 | | | 13.5 | | | 31.3 | | |
| 6–60/72/ 1,200 | 2.4 | | | ··· | | | 203.5 | | | 13.3 | | | 32.4 | | |
| LSD | 0.1 | | | 27.1 | | | 21.7 | | | 4.7 | | | 5.9 | | |

[a] After inoculation hosts were misted in a lighted chamber at 20 C for 6, 12, 24, 48, or 60 hr (wet periods), then placed on a glasshouse bench at 16–23 C. At observation times 6, 12, 24, 48, and 72 hr after inoculation the second youngest leaf from each of three replications of each wet period was placedin alcoholic lactophenol. On each leaf the infection activities of 40 conidia were recorded. The results were analyzed by SAS General Linear Models Procedure using differing tests for balanced and unbalanced parts of the study. PDIFF is probability of difference of LS means. Means followed by the same capital letter are not significantly different at the 0.05 level of the Duncan's multiple range test. LSD values are at the 0.05 level of significance. Unreal assumptions, such as a 12-hr wetting–6-hr observation in the original dissertation, prevented statistical comparisons of the cultivars for a given wet period over all observation times.

[b] When no observations on length of germ tubes were made at 72 hr, 1,920 conidia were observed (instead of 3,000).

[c] When no observations on length of germ tubes were made at 72 hr, 840 conidia were observed.

[d] When no observations on length of germ tubes were made at 72 hr, 600 conidia were observed.

[e] When no observations on length of germ tubes were made at 72 hr, 360 conidia were observed.

[f] When no observations on length of germ tubes were made at 72 hr, 120 conidia were observed.

[g] When no observations on length of germ tubes were made at 72 hr, 1,680 conidia were observed.

[h] When no observations on length of germ tubes were made at 72 hr, 1,200 conidia were observed.

[i] When no observations on length of germ tubes were made at 72 hr, 720 conidia were observed.

[j] When no observations on length of germ tubes were made at 72 hr, 240 conidia were observed.

[k] When no observations on length of germ tubes were made at 72 hr, 960 conidia were observed.

period, wheat selection × observation time, and wet period × observation time were nonsignificant. The interaction wheat selection × wet period was nonsignificant in the study reported in Table 3 and significant in the study reported in Table 1. There was a significant correlation ($r$ = 0.80) between the number of appressoria and the length of the germ tubes.

Papilla formation did not occur around the appressorial infecting hypha in the host epidermal cell in a 6-hr wet period at 10 C. The percentage of infecting hyphae with papilla formation around the hypha rose with lengthening wet period (Tables 1 and 3) and rising temperature (Table 3) but not with lengthening observation time (Table 1). The percentage was greater to significantly greater in BH1146 than in ND495 but not consistently so until the wet period exceeded 12 hr (Table 1 and 3). Over the combined range of wet periods and wet periods × temperatures this percentage was significantly twice as high in BH1146 as in ND495 (17 vs. 5.82% in Table 1 and 21.7 vs. 10.9% in Table 3). In one study, an unusually high 19% was recorded for 6-hr wetting at 20 C on BH1146 (Table 1). The interactions of wheat selection × wet periods, wheat selection × temperature, wheat selection ×

TABLE 2. Effect of wet period on disease development in ND495 (susceptible) and BH1146 (resistant) hard red spring wheat leaves inoculated with conidia of *Pyrenophora tritici-repentis*[a]

| Wet period (hr) | Lesions $cm^{-2}$ | | | Mycelial longitudinal growth in lesion (mm) | | | Lesion length (mm) | | | Lesion length minus mycelial growth (mm) | | | Lesion width (mm) | | | Lesion size ($mm^2$) | | | Leaf area affected (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 495 | 1146 | LSD | 495 | 1146 | LSD | 495 | 1146 | LSD | 495 | 1146 | LSD | 495 | 1146 | LSD | 495 | 1146 | LSD | 495 | 1146 | LSD |
| 6 | 1.0 | 1.1 | 1.9 | 1.3[b] | 0.7 | 0.6 | 2.3[d] | 1.4 | 0.5 | 1.0[b] | 0.7 | 0.8 | 0.7[f] | 0.5 | 0.1 | 1.5 | 0.7 | 0.4 | 1.6 | 0.8 | 6.7 |
| 12 | 8.3 | 9.1 | 1.9 | 1.9 | 0.9 | 0.6 | 2.7 | 1.7 | 0.5 | 0.8 | 0.8 | 0.8 | 0.8 | 0.6 | 0.1 | 2.3 | 1.0 | 0.4 | 18.8 | 9.3 | 6.7 |
| 24 | 10.7 | 11.8 | 1.9 | 2.2 | 1.1 | 0.6 | 3.3 | 2.4 | 0.5 | 1.1 | 1.3 | 0.8 | 0.9 | 0.7 | 0.1 | 2.9 | 1.6 | 0.4 | 30.9 | 18.9 | 6.7 |
| 48 | 11.8 | 13.4 | 1.9 | 3.0 | 1.5 | 0.6 | 3.9 | 2.6 | 0.5 | 0.9 | 1.1 | 0.8 | 0.9 | 0.7 | 0.1 | 3.6 | 1.7 | 0.4 | 42.4 | 22.4 | 6.7 |
| 60 | 13.0 | 13.9 | 1.9 | ··· | ··· | ··· | 4.2 | 2.7 | 0.5 | ··· | ··· | ··· | 0.9 | 0.7 | 0.1 | 3.8 | 1.9 | 0.4 | 49.9 | 27.1 | 6.7 |
| LSD | 1.9 | 1.9 | | 0.6 | 0.6 | | 0.5 | 0.5 | | 0.8 | 0.8 | | 0.1 | 0.1 | | 0.4 | 0.4 | | 6.7 | 6.7 | |
| 6–48 | ··· | ··· | ··· | 2.0 | 1.0 | 0.3 | 3.3 | 2.1 | 0.2 | 1.0 | 1.0 | 0.4 | ··· | ··· | ··· | ··· | ··· | ··· | | | |
| 6–60 | 9.0 | 9.9 | 0.8 | ··· | ··· | ··· | ··· | ··· | ··· | ··· | ··· | ··· | 0.8 | 0.6 | 0.04 | 2.8 | 1.4 | 0.2 | 28.7 | 15.7 | 3.0 |

| Wet period (hr) | Lesions $cm^{-2}$ 495 & 1146 | Mycelial longitudinal growth 495 & 1146 | Lesion length 495 & 1146 | Lesion length minus mycelial growth 495 & 1146 | Lesion width 495 & 1146 | Lesion size 495 & 1146 | Leaf area affected 495 & 1146 |
|---|---|---|---|---|---|---|---|
| 6 | 1.0 | 1.0[c] | 1.8[e] | 0.8[c] | 0.6[g] | 1.1 | 1.2 |
| 12 | 8.7 | 1.4 | 2.2 | 0.8 | 0.7 | 1.7 | 14.1 |
| 24 | 11.2 | 1.6 | 2.8 | 1.2 | 0.8 | 2.3 | 24.9 |
| 48 | 12.6 | 2.2 | 3.2 | 1.0 | 0.8 | 2.6 | 32.4 |
| 60 | 13.5 | ··· | 3.4 | ··· | 0.8 | 2.9 | 38.5 |
| LSD | 1.3 | 0.4 | 0.4 | 0.5 | 0.06 | 0.3 | 4.8 |

[a] After inoculation hosts were misted in a lighted chamber at 20 C for 6, 12, 24, 48, and 60 hr, then placed on a glasshouse bench at 16–23 C. Observations were made 8 days after inoculation on the second youngest leaf of four- to six-leaf stage seedlings. They were analyzed by SAS ANOVA procedure (LSD $P = 0.05$).

[b-g] Each number is the average of 90 observations (30 lesions × 3 replications) for b, 180 (30 × 3 × 2 selections) for c, 360 (15 × 3 × 8 leaves) for d, 720 (15 × 3 × 2 × 8) for e, 240 (10 × 3 × 8) for f, and 480 (10 × 3 × 2 × 8) for g.

TABLE 3. Effect of wet period × temperature on infection of ND495 (susceptible) and BH1146 (resistant) hard red spring wheats inoculated with conidia of *Pyrenophora tritici-repentis*[a]

| Wet period (hr)/ temp. (C) | Conidia on leaves: Percent germination | | | Germ tubes/ conidium | | | Length of germ tubes ($\mu$m) | | | Appressoria: Per 100 conidium | | | Percent with papilla formed around infecting hypha | | | Colonizing host cells (%) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 495 | 1146 | LSD | 495 | 1146 | LSD | 495 | 1146 | LSD | 495 | 1146 | LSD | 495 | 1146 | LSD | 495 | 1146 | LSD |
| 6/10 | 82[b] | 71 | 8 | 1.7 | 1.5 | 0.4 | 34.2 | 40.9 | 75.7 | 21.7 | 10.0 | 53.1 | 0.0 | 0.0 | 14.3 | 1.9 | 13.1 | 14.5 |
| 6/20 | 94 | 96 | 8 | 2.1 | 2.0 | 0.4 | 79.0 | 71.5 | 75.7 | 52.5 | 56.7 | 53.1 | 7.1 | 6.2 | 14.3 | 3.5 | 10.6 | 14.5 |
| 6/30 | 92 | 96 | 8 | 2.4 | 2.9 | 0.4 | 81.3 | 88.0 | 75.7 | 68.3 | 109.1 | 53.1 | 1.2 | 22.8 | 14.3 | 5.3 | 1.6 | 14.5 |
| 12/10 | 93 | 98 | 8 | 1.9 | 2.0 | 0.4 | 52.6 | 59.2 | 75.7 | 58.3 | 72.5 | 53.1 | 4.9 | 19.1 | 14.3 | 3.6 | 11.7 | 14.5 |
| 12/20 | 100 | 98 | 8 | 2.5 | 2.3 | 0.4 | 139.4 | 126.1 | 75.7 | 128.3 | 132.5 | 53.1 | 13.4 | 17.7 | 14.3 | 25.4 | 33.3 | 14.5 |
| 12/30 | 99 | 100 | 8 | 3.1 | 3.0 | 0.4 | 231.5 | 220.1 | 75.7 | 170.0 | 170.0 | 53.1 | 15.0 | 25.5 | 14.3 | 29.7 | 26.4 | 14.5 |
| 24/10 | 97 | 100 | 8 | 1.9 | 2.0 | 0.4 | 125.6 | 122.2 | 75.7 | 112.5 | 141.7 | 53.1 | 17.5 | 29.2 | 14.9 | 34.1 | 24.2 | 14.5 |
| 24/20 | 100 | 100 | 8 | 2.3 | 2.6 | 0.4 | 255.6 | 345.6 | 75.7 | 199.2 | 225.8 | 53.1 | 16.8 | 42.9 | 14.3 | 37.2 | 26.7 | 14.5 |
| 24/30 | 100 | 100 | 8 | 2.7 | 2.7 | 0.4 | 298.6 | 215.8 | 75.7 | 269.2 | 219.2 | 53.1 | 21.8 | 31.6 | 14.3 | 26.8 | 31.0 | 14.5 |
| LSD[d] | 8 | 8 | | 0.4 | 0.4 | | 75.7 | 75.7 | | 53.1 | 53.1 | | 14.3 | 14.3 | | 14.5 | 14.5 | |
| 6–24/10–30 | 95 | 95 | 3 | 2.3 | 2.3 | 0.1 | 144.2 | 143.3 | 25.2 | 120.0 | 126.4 | 17.7 | 10.9 | 21.7 | 4.8 | 18.6 | 19.9 | 4.8 |

| Wet period (hr)/ temp. (C) | Percent germination 495 & 1146 | Germ tubes/ conidium 495 & 1146 | Length of germ tubes 495 & 1146 | Per 100 conidium 495 & 1146 | Percent with papilla 495 & 1146 | Colonizing host cells 495 & 1146 |
|---|---|---|---|---|---|---|
| 6/10–30 | 88[c] | 2.1 | 65.8 | 53.1 | 6.3 | 10.0 |
| 12/10–30 | 98 | 2.5 | 138.2 | 121.9 | 15.9 | 21.7 |
| 24/10–30 | 99 | 2.4 | 227.2 | 194.6 | 26.6 | 30.7 |
| LSD | 3 | 0.2 | 30.9 | 21.7 | 5.8 | 5.9 |
| 6–24/10 | 90 | 1.8 | 72.2 | 69.4 | 11.8 | 14.8 |
| 6–24/20 | 98 | 2.3 | 169.5 | 132.5 | 17.4 | 22.8 |
| 6–24/30 | 98 | 2.8 | 189.2 | 167.6 | 19.8 | 20.2 |
| LSD | 3 | 0.2 | 30.9 | 21.7 | 5.8 | 5.9 |

[a] After inoculation hosts were misted in a lighted chamber at 10, 20, and 30 C (temperature) for 6, 12, and 24 hr (wet periods), fan dried, and kept at the respective 10, 20, and 30 C until all were at 24 hr after inoculation, then placed on a glasshouse bench at 16–23 C for 24 hr. Then the top leaf was placed into alcoholic lactophenol 48 hr after inoculation for observation (LSD $P = 0.05$).

[b,c] Each number is the average of 120 observations (40 conidia × three replications) for b and 720 (40 × three replications × three temperatures or three wet periods) for c.

[d] This LSD compares values between two wet periods at the same temperature.

observation time, wet period × observation time, and wet period × temperature were nonsignificant.

Colonization of host cells was successful when no papilla formed around the infecting hypha as it entered the host epidermal cell and spread among other epidermal and mesophyll cells or when the hypha grew (rarely) through the papilla and spread. The percentage of infecting hyphae successfully colonizing host cells was not significantly different in ND495 and BH1146 over wet periods, observation times, or temperatures (Tables 1 and 3). Percentage of colonization at 20 C increased significantly between 6 hr wetting (8%) and 12 hr (39.6%), did not increase with 24 hr (35.9%), and significantly decreased with 48 hr (28.6%) or 66 hr (28.1%) (Table 1). Percent colonization appeared to increase with increasing dry period after 12 hr wetting but was variable in the dry periods following 6, 24, and 48 hr wetting (Table 1). It significantly increased with increasing wet period over 6–24 hr at 10–30 C. It increased with increasing temperature from 10 to 20 C but not 20 to 30 C (Table 3). The interactions wheat selection × wet period, wheat selection × temperature, and wheat selection × observation time were nonsignificant. The interaction wet period × observation time was significant, reflecting the drop in colonization after 48 and 60 hr wetting (28.6 and 28.1, Table 1), without a similar drop at 48 and 72 hr observation times (31.3 and 32.4, Table 1). The interaction wet period × temperature was significant, reflecting the significant increase in colonization with increasing wet period and the lack of increase with temperature rising from 20 to 30 C (Table 3).

Lesions per square centimeter of leaf were not significantly different on ND495 and BH1146 in the several wet periods and temperatures (Tables 2 and 4). However, in the average of 6–60-hr wet periods, the number of lesions on BH1146 was just significantly higher than on ND495 (Table 2). No lesions were produced from a 6-hr wet period at 10 C and few from 20 or 30 C or from a 12-hr wet period at 10 C (Table 4). The number of lesions increased with increasing wet period and rising temperature but not always significantly (Tables 2 and 4). The interactions wheat selection × wet period, wheat selection × temperature, and wet period × temperature were nonsignificant. The number of lesions per square centimeter was positively correlated with the number of penetrations (percent colonization) ($r = 0.86$).

Mycelial longitudinal growth in lesion, lesion length, lesion size (length × width), and percent area affected (severity of tan spotting) were greater in ND495 than in BH1146 and grew with lengthening wet period (Tables 2 and 4). Lesion size and percent leaf area affected grew with rising temperature (Table 4). Lesion length and mycelial longitudinal growth in lesion were positively correlated ($r = 0.89$). Growth of the lesion beyond the mycelium (lesion length minus mycelial longitudinal growth) did not differ significantly with wheat selection or wet period (Table 2). Lesion width was significantly greater in ND495 than in BH1146 and greater as wet period increased from 6 to 24 hr but not greater as wet period increased from 24 to 60 hr (Table 2).

For mycelial longitudinal growth in the lesion the interaction wheat selection × wet period was not significant. For lesion length the interaction wheat selection × wet period was not signficant. For lesion length minus mycelial growth the interaction wheat selection × wet period was not significant. For lesion width the interaction wheat selection × wet period was not significant. For lesion size the interaction wheat selection × wet period was significant, reflecting a more significant growth in size in the susceptible ND495 at shorter wet periods; wheat selection × temperature was significant, reflecting the faster rise in lesion size in ND495 with rising temperature, and wet period × temperature was significant, reflecting the significant rise in size from 12 to 24 hr wetting and the nonsignificant rise from 20 to 30 C. For percent leaf area affected the interactions wheat selection × temperature and wet period × temperature were not significant. The interaction wheat selection × wet period was significant, reflecting the faster significant rise with increasing wet period in ND495. Percent leaf area affected was positively correlated with lesions per square centimeter ($r = 0.84$) and lesion size ($r = 0.82$).

Percent leaf area affected (severity of tan spot) was greatly inhibited through curtailment of lesion numbers by the wet period × temperature range of 6 hr wet × 10–30 C to 12 hr wet × 10 C. Severity was steadily increased through greater lesion numbers and size with increasing wet period × temperature over the range of 12

TABLE 4. Effect of wet period × temperature on disease development in ND495 (susceptible) and BH1146 (resistant) hard red spring wheat leaves inoculated with *Pyrenophora tritici-repentis*[a]

| Wet period (hr)/ temperature (C) | Lesions ($cm^2$) | | | Lesion size ($mm^2$) | | | Leaf area affected (%) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 495 | 1146 | LSD | 495 | 1146 | LSD | 495 | 1146 | LSD |
| 6/10 | 0.0 | 0.0 | 4.2 | 0.0 | 0.0 | 0.5 | 0.0 | 0.0 | 11.7 |
| 6/20 | 1.1 | 0.4 | 4.2 | 2.1[b] | 0.4 | 0.5 | 2.4 | 0.2 | 11.7 |
| 6/30 | 1.1 | 0.2 | 4.2 | 2.1 | 0.5 | 0.5 | 2.2 | 0.1 | 11.7 |
| 12/10 | 1.6 | 1.0 | 4.2 | 2.1 | 0.6 | 0.5 | 3.5 | 0.7 | 11.7 |
| 12/20 | 6.1 | 9.1 | 4.2 | 2.9 | 0.9 | 0.5 | 18.2 | 8.3 | 11.7 |
| 12/30 | 8.2 | 6.2 | 4.2 | 3.9 | 0.9 | 0.5 | 31.7 | 5.5 | 11.7 |
| 24/10 | 10.0 | 9.5 | 4.2 | 2.9 | 1.0 | 0.5 | 28.4 | 9.7 | 11.7 |
| 24/20 | 13.1 | 11.1 | 4.2 | 3.6 | 1.4 | 0.5 | 47.0 | 16.1 | 11.7 |
| 24/30 | 13.6 | 14.0 | 4.2 | 3.9 | 1.4 | 0.5 | 53.9 | 19.9 | 11.7 |
| LSD[c] | 4.2 | 4.2 | | 0.5 | 0.5 | | 11.7 | 11.7 | |
| 6–24/10–30 | 6.1 | 5.7 | 1.4 | 2.6 | 0.8 | 0.2 | 20.8 | 6.7 | 3.9 |
| | 495 & 1146 | | | 495 & 1146 | | | 495 & 1146 | | |
| 6/10–30 | 0.5 | | | 0.9[d] | | | 0.8 | | |
| 12/10–30 | 5.4 | | | 1.9 | | | 11.3 | | |
| 24/10–30 | 11.9 | | | 2.4 | | | 29.2 | | |
| LSD | 1.7 | | | 0.2 | | | 4.8 | | |
| 6–24/10 | 3.7 | | | 1.1 | | | 7.0 | | |
| 6–24/20 | 6.8 | | | 1.9 | | | 15.3 | | |
| 6–24/30 | 7.2 | | | 2.1 | | | 18.9 | | |
| LSD | 1.7 | | | 0.2 | | | 4.8 | | |

[a] After inoculation hosts were misted in a lighted chamber at 10, 20, and 30 C (temperature) for 6, 12, and 24 hr (wet periods), fan dried, and kept at the respective 10, 20, and 30 C until all were at 24 hr after inoculation, then placed on a glasshouse bench at 16–23 C. Observations were made 8 days after inoculation on the top leaf of 28-day-old seedlings. The results were analyzed by SAS ANOVA procedure (LSD $P = 0.05$).

[b] Each number is the average of 240 observations (10 lesions × 8 seedlings × 3 replications).

[c] This LSD compares values between two wet periods at the same temperature.

[d] Each number is the average of 1,440 observations (10 leaves × 8 seedlings × 3 replications × 3 wet periods or temperatures × 2 sections).

hr wet × 20–30 C to 24 hr wet × 30 C and 60 hr wet × 20 C (Tables 2 and 4).

## DISCUSSION

Lengthening the postinoculation wet period and/or raising the temperature increased conidial germination, number of germ tubes per conidium, length of germ tubes, appressoria production, papillae production, and percentage of appressoria colonizing host cells. At differing wet periods and temperatures (see Results) some of these increases plateaued or declined. Of these prepenetration and penetration activities only percent papillae formation was significantly higher on resistant BH1146 than on susceptible ND495. However, percent colonization was not significantly different on the two wheats. Apparently, BH1146 was not able to produce enough papillae to effectively resist the fungal attack. These results were in agreement with those earlier reported for a single wet period and temperature (24). None of these activities increased with increasing dry periods following wet periods (Table 1; 24), indicating that they stopped with differing degrees of completion at the end of the wet period.

Increased production of appressoria and penetrations with respectively longer wet periods correlated with lengthening germ tubes and increased number of lesions. Politowski and Browning (34) found a similar correlation between duration of wet period, production of appressoria, penetrations, and number of pustules produced by *Puccinia coronata* Cda. var. *avenae* Fraser & Led. on oats. They also reported the suppressing effect of dry conditions on fungal growth. Levy and Cohen (26) also observed that the percentage of corn leaf area blighted by *Exserohilum turcicum* (Pass.) Leonard & Suggs was proportional to the percentage of conidia producing appressoria.

Because the number of lesions per unit of leaf area was not significantly different on the two wheats for individual wet periods and temperatures and just significantly higher on BH1146 for the combined wet periods in only one of the two studies (Tables 2 and 4), resistance was not related to number of lesions. This differed from the findings of da Luz (5) in which resistant selections had fewer lesions as lesions increased from 12 to 24–28 C and decreased at 32 C. BH1146 had only one or two lesions per square centimeter at 20 C after a 30-hr wet period. In our study, BH1146 had 11 lesions per square centimeter at 20 C after a 24-hr wet period (Table 4). With three wheat selections infected with *C. sativus*, resistant selections had fewer lesions at 12 C, but as the temperature rose to 28 C the lesions on all selections increased to similar numbers (5,6). Resistant barley genotypes infected with *P. teres* had smaller lesions than susceptible genotypes, but lesion numbers on some resistant genotypes were higher than on susceptible and others lower (31).

Plateauing of increasing lesion width with lengthening wet period at 24 hr wetting (Table 2) could be due to blocking of hyphal growth by the large vascular bundles in the wheat leaf. Hyphae did not grow into these bundles (24).

The differences observed between longitudinal hyphal growth and length of the lesions indicated that part of the marginal expansion of the lesions was induced by toxic substances produced by the invading fungus and diffusing beyond the limits of the hyphae. The distance of diffusion was similar in BH1146 and ND495 (Table 2), suggesting that the smaller lesion size in BH1146 (Tables 2 and 4) was not due to a suppression of fungal toxin. The distance of diffusion was not significantly altered by lengthening wet period, indicating that wet period did not act on the toxin to increase lesion size.

The effect of postinoculation wet periods and/or temperature on infection and disease development has been studied in several host-parasite relationships (1,2,5,6,9,12–15,29,30,34,37). It has been shown that duration of leaf wetness can be used as a tool to identify resistant genotypes to several fungal diseases (1,2,4,9,12–17,21,31). In this study, a wet period of at least 24 hr was necessary to consistently detect clear differences in resistance between ND495 and BH1146. Whereas differences existed after a 12-hr wet period, after 24 hr wetting the size differences in lesions were different enough for the eye to clearly distinguish. Increasing the wet period at a given temperature had the greatest effect of either factor (wet period or temperature) on fungal and disease development. At a constant temperature of 20 or 30 C, increasing the wet period above 6 hr significantly enhanced fungal and lesion development. These observations agreed with those by Levy and Cohen (26) with *E. turcicum* on corn and those by Nelson and Tung (29,30) with *Helminthosporium maydis* Nisik. on corn. However, other diseases differ. Increasing temperature from 24 to 28 C resulted in the dramatic rise of severity of spotting by *C. sativus* on resistant wheats equal to the severity in a susceptible wheat. It was suggested that this resulted from an increase in appressoria and in successful colonizations (5,6). "Size increase of lesions caused by *Colletotrichum lagenarium* on cucumbers in the field was determined mostly by the length of time following inoculation and was less affected by variation in environmental conditions." In the greenhouse, size increase of lesions was initially greater at 20–28 C but later was greater at 16 C. Lesions size was "two to four times lower" in two resistant cultivars compared with a susceptible one (38).

The suppressing effect of 10 C on leaf penetrations by *P. tritici-repentis* and lesion development was overcome to a great extent by increasing the length of the wet period to 24 hr. This indicated that under field conditions, temperatures around 10 C would play a major role in restricting tan spot severity only in wet period shorter than 24 hr. Severity of spotting (percent leaf area spotted) increased on both susceptible and resistant wheat as temperature rose to 30 C (Table 4). This differed from the findings of da Luz (5), in which severity declined at 28 and 32 C from a peak at 24 C. He too found that over a range of temperatures (15–32 C) severity was always greater on a susceptible wheat.

The expansion of tan spot lesions with lengthening wet period and rising temperature in ND495 and BH1146 and similar reports with other fungal diseases (1,4,9,34,37) support the concept that in some fungal diseases not only infection but also disease development following infection are dependent on external factors. Moisture and temperature are evidently two of these factors.

The effect of a lengthening wet period from 6 to 60 hr on increasing tan spot lesion development (size) also extends into the following dry period, with lesions growing bigger for 6–8 days after inoculation. This extended effect is also evident with other wet period dependent fungal leaf spot diseases (13–16).

Resistance to *P. tritici-repentis* in BH1146 is expressed at a molecular level in the leaf cells and results in reduced mycelial and leaf spot development (23,24). It persists during mycelial and lesion growth with lengthening duration of free moisture on the leaves and rising temperature. It is unrelated to toxin advance ahead of the mycelium. It persists as lesser lesion growth as lesions grow in the days after a wet period. It is similar to a much greater resistance restricting fungal growth to a few leaf cells in Lodi oats (23,24). What appears to be a second form of resistance, papella formation, is sparse in ND495, greater but ineffectual in BH1146, and more prevalent and effective in Lodi oats (23,24). Resistance has been reported as polygenic (3,28) and monogenic (22) and of intermediate to high heritability (3,28). Leaf glaucousness is unrelated to resistance (36).

Virulence or growth of *P. tritici-repentis* is promoted by lengthening wet period and rising temperature in both susceptible and resistant wheat. Virulence may involve many fungal genes (7). It includes a toxin advancing ahead of the mycelium among the host cells, but also involves resistance inhibited molecular factors unrelated to toxin spread. Further investigation is warranted.

Augmenting either of the host defense systems (papilla formation and restriction of mycelial spread among leaf cells) through resistance genes or other means should help to control tan spot. Determining the actual molecular nature of these resistances might lead to elimination of tan spot.

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