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Effects of surface wetness duration, temperature, and inoculum concentration on infection of winter barley by *Rhynchosporium secalis*

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The effects of surface wetness duration, temperature, and inoculum concentration on development of scald in winter barley (*Hordeum vulgare*) inoculated with race SO1 of *Rhynchosporium secalis* from southern Ontario, Canada were examined. On barley line 'GW8614' sprayed with a spore suspension (2×10^5 conidia mL⁻¹), wet periods of 2-48 h and constant temperatures of 10-25°C during the wet and dry periods, 10-25°C during the wet period and 20°C during the dry period, or 20°C during the wet period and 10-30°C during the dry period allowed scald to develop 8.3-11.5 d after inoculation. The disease developed most rapidly and most severely when the wet period after inoculation was 48 h and the temperature of the wet period and subsequent dry period was 20°C. Scald did not develop within 14 d following temperatures of 30°C during the wet period or of 5°C during the wet or dry periods. At inoculum densities of 10^2 - 10^6 conidia mL⁻¹, the disease severity index values (0-100 scale) increased from 53 to 100 in line 'GW8614' and from 0 to 90 in cultivar OAC Acton and the latent periods decreased from 13.3 to 7.8 d in line 'GW8614' and from more than 14 to 8.5 d in cv. OAC Acton. This information should facilitate screening of barley for resistance to scald.

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Nous avons étudié les effets de la durée d'humectation, de la température et de la concentration de l'inoculum sur le développement de la rhynchosporiose chez l'orge d'automne (*Hordeum vulgare*) inoculée avec la race SO1 du *Rhynchosporium secalis* du sud de l'Ontario (Canada). Sur la lignée d'orge GW8614 vaporisée avec une suspension de spores (2×10^5 conidies mL⁻¹), des périodes d'humectation de 2-48 h et des températures de 10-25 °C pendant les périodes humides et sèches, de 10-25 °C pendant la période humide et de 20 °C pendant la période sèche, ou 20 °C pendant la période humide et 10-30 °C pendant la période sèche ont permis le développement de la rhynchosporiose 8,3-11,5 jours après l'inoculation. La maladie s'est développée plus rapidement et plus intensément quand la période humide après l'inoculation était de 48 h et quand la température de la période humide et de la période sèche subséquente était de 20 °C. La rhynchosporiose ne s'est pas développée à l'intérieur de 14 jours suite à des températures de 30 °C pendant la période humide ou de 5 °C pendant les périodes humides ou sèches. À des concentrations d'inoculum de 10^2 - 10^6 conidies mL⁻¹, l'indice de gravité (échelle de 0-100) a augmenté de 53 à 100 pour

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la lignée GW8614 et de 0 à 90 pour le cultivar OAC Acton et les périodes latentes ont diminué de 13,3 à 7,8 jours pour la lignée GW8614 et de plus de 14 à 8,5 jours pour le cultivar OAC Acton. Ces renseignements devraient faciliter le criblage de cultivars d'orge résistants à la rhynchosporiose.

INTRODUCTION

Scald of barley (*Hordeum vulgare* L.), caused by *Rhynchosporium secalis* (Oudem.) J.J. Davis, causes significant yield losses in many barley production areas (Shipton *et al.* 1974) and is often severe on winter barley in Ontario (Xue and Hall 1991). The disease is controlled principally by the use of genetic resistance in the host plant. When plant germplasm is screened in controlled environments, conditions generally include a high inoculum dose (2.5×10^5 conidia mL⁻¹), wetness periods after inoculation of 12-48 h, and incubation temperatures of 15-25°C (Ali *et al.* 1976; Ayres and Owen 1971; Jackson and Webster 1976; Williams and Owen 1973; Xue *et al.* 1991). However, few reports quantify the effects of pathogen, host, and environmental variables on the development of the disease.

Severity of scald has been reported to increase (Carr *et al.* 1971) or to be unaffected (Ayres and Owen 1971; Schein 1960) as inoculum dose is raised but little information is available on interactions between barley genotype and inoculum dose of *R. secalis*. Skoropad (1957) and Polley (1971) examined the effects of temperature and duration of the wet period, respectively, on severity of scald, but in both studies temperatures fluctuated by several degrees centigrade during the test period. Only Ryan and Clare (1975) have reported data relating development of scald to duration and temperature of the wet period under controlled conditions. There appears to be no report on the effect of temperature on scald development when temperature is held constant between inoculation and disease assessment or between the end of the wet period and disease assessment. To rationalize methods used to screen winter barley germplasm for resistance to Ontario races of *R. secalis*, this study examined the effects of surface wetness duration, temperatures during the wet and subsequent dry periods, and inoculum density, on

disease severity and latent period in winter barley inoculated with the most common race of *R. secalis* from southern Ontario.

MATERIALS AND METHODS

Inoculum production and maintenance

Isolate Rs018 of race SO1 of *R. secalis* (Xue 1990) was stored on unpolished porcelain beads (Lange and Boyd 1968) at -18°C. To produce inoculum, the beads were transferred to sterile distilled water in a glass tube and shaken at 150 r min⁻¹ for 10 min on a Burrel shaker to dislodge conidia. Aliquots (1 mL) of the resulting spore suspension (10^7 - 10^8 conidia mL⁻¹) were spread over the surface of wheat germ agar medium containing 100 mg L⁻¹ streptomycin in 9-cm-diam petri dishes (Xue 1990). The inoculated dishes were incubated at 18°C for 3 wk with constant illumination from cool white fluorescent and near ultraviolet lamps. Conidia were harvested as described by Xue *et al.* (1991), suspended in sterile distilled water, and adjusted to the desired concentration with the aid of a hemocytometer.

Plant production and inoculation

The susceptible barley breeding line 'GW8614' and the moderately resistant cultivar OAC Acton were grown as groups of five plants in 7-cm-diam pots containing Pro-Mix BX potting mix (Plant Products Co. Ltd., Bramalea, Ontario). Plants were produced in a growth room at 20°C with a photoperiod of 14 h and a light intensity of 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ provided by fluorescent and incandescent lamps, and were watered from the bottom of the pots twice a week. At the 3-leaf stage, 14 d after seeding, plants were sprayed with a spore suspension at the rate of 0.4 mL of spore suspension per plant using a DeVilbiss model 15 atomiser (The DeVilbiss Co., Somerset, Pennsylvania). Four replicate pots were used per treatment and all inoculated leaves were assessed on each plant.

Surface wetness duration, temperature and inoculum concentration

The effects of surface wetness duration after inoculation and temperatures of the wet period and subsequent dry period on disease development were examined in three experiments using barley line 'GW8614'. Temperatures of 5, 10, 15, 25, and 30°C ± 1°C were provided by growth cabinets and a temperature of 20°C ± 1°C was provided by the growth room. The growth cabinets provided the same photoperiod and light intensity as the growth room. In each experiment, plants were inoculated with a spore suspension adjusted to 2 × 10⁵ conidia mL⁻¹ and placed immediately in a mist chamber located beneath the growth room bench or within individual growth cabinets for periods ranging from 1 to 48 h. Surface wetness was maintained by a mist produced by a vaporiser. Plants were transferred from the growth room to mist chambers in the growth cabinets 6 h before inoculation and the lights were turned off during the wet period. At the end of the wet period surface moisture was evaporated from the plants within 15 min with a stream of air at room temperature.

In the first experiment, temperature was held constant at 5, 10, 15, 20, 25 or 30°C throughout the wet and dry periods. In the second experiment, the temperature of the wet period ranged from 5 to 30°C and the temperature of the dry period was held constant at 20°C. In the third experiment, the temperature of the wet period was 20°C and the temperature of the dry period ranged from 5 to 30°C. In the fourth experiment, spore suspensions were adjusted to concentrations of 10², 10³, 10⁴, 10⁵, 2 × 10⁵, 5 × 10⁵ and 10⁶ conidia mL⁻¹ and sprayed onto line 'GW8614' and cv. OAC Acton as described above. Inoculated plants were exposed to a wet period of 48 h and a constant temperature of 20°C between inoculation and disease assessment.

Symptom rating and statistical analysis

Following the wet period, plants were examined each day for symptoms of scald. The time from inoculation to onset of symptoms is referred to here as the latent period (Butt and Royle 1980). Fourteen days after inoculation, the plants were

rated for disease severity on a scale of 0-4, in which 0 = no visible symptoms, and 1, 2, 3 and 4 represent <5%, 5-15%, 16-30% and >30% of leaf area with visible symptoms, respectively. Symptoms included grey-green lesions that were water-soaked, pale brown lesions with a dark brown border, and extensive necrosis and desiccation of the lamina. Disease severity scores were converted to a disease severity index (DSI) from the equation

$$DSI = 100 \times (\sum \{ \text{severity score} \times \text{no. plants in category} \}) / (\text{total no. plants} \times 4).$$

Analysis of variance was conducted (SAS Institute Inc. 1987) and treatment means were separated by the least significant difference test at a probability level of 0.05.

RESULTS

Temperature of wet and dry periods

The effects of surface wetness duration and constant temperatures during the wet and dry periods on disease severity and latent period are shown in Table 1. No disease occurred at 5 or 30°C (data not shown) or following a surface wetness duration of 1 h. The minimum duration of surface wetness required to produce disease ranged from 2 h at 20°C to 48 h at 25°C. Disease occurred in the temperature range 10-25°C and symptoms first appeared 8.3-10.8 d after inoculation. Disease developed most rapidly at 15-20°C and was most severe (DSI = 100) at a constant temperature of 20°C.

Temperature of the wet period

The effects of surface wetness duration and temperature of the wet period on disease severity and latent period are shown in Table 2. No disease occurred if the wet period was 1 h or if the temperature of the wet period was 5 or 30°C. The minimum duration of surface wetness required to produce disease ranged from 2 h at 15 and 20°C to 9 h at 25°C. Disease occurred in the temperature range of 10-25°C and symptoms first appeared 8.3-10.8 d after inoculation. Disease developed most rapidly at 15-20°C and was most severe (DSI = 100) at a wet period temperature of 20°C.

Table 1. Effect of surface wetness duration and constant temperature during the wet and dry periods on disease severity index and latent period of scald of barley^a

Surface wetness duration (h)	Disease severity index ^b				Latent period ^c (d)			
	Temperature (°C)				Temperature (°C)			
	10	15	20	25	10	15	20	25
1	0	0	0	0	-. ^d	-	-	-
2	0	0	5	0	-	-	10.8	-
4	0	0	6	0	-	-	10.3	-
6	6	6	11	0	10.3	8.5	9.5	-
9	4	9	11	0	10.5	9.8	9.3	-
12	8	10	79	0	10.1	8.8	9.0	-
48	29	80	100	15	10.3	9.5	8.3	10.0
LSD ($P = 0.05$) ^e	9.2				1.4			

^a Plants of barley line 'GW8614' were inoculated with race SO1 of *Rhynchosporium secalis* and then exposed to surface wetness durations of 1-48 h. Plants were exposed to constant temperatures of 10, 15, 20 and 25°C during the wet and subsequent dry periods.

^b Disease severity index (0-100 scale) was determined 14 d after inoculation.

^c Latent period defined as period (d) from inoculation to onset of symptoms.

^d - No disease. No disease occurred at 5°C or 30°C at any period of surface wetness tested.

^e LSD values refer to comparisons among all disease severity index values and among all latent periods, respectively.

Table 2. Effect of surface wetness duration and temperature of the wet period on disease severity index and latent period of scald of barley^a

Surface wetness duration (h)	Disease severity index ^b				Latent period ^c (d)			
	Temperature (°C)				Temperature (°C)			
	10	15	20	25	10	15	20	25
1	0	0	0	0	-. ^d	-	-	-
2	0	6	4	0	-	10.5	10.8	-
4	0	4	6	0	-	10.5	10.3	-
6	2	3	10	0	10.5	10.3	9.3	-
9	3	5	9	3	10.3	9.0	9.5	10.0
12	4	70	81	31	9.0	9.3	9.0	9.0
24	12	94	99	43	8.8	8.5	8.3	8.3
36	17	91	98	29	8.5	8.8	8.3	8.3
48	13	90	100	19	8.8	8.8	8.3	8.3
LSD ($P = 0.05$) ^e	7.8				1.4			

^a Plants of barley line 'GW8614' were inoculated with race SO1 of *Rhynchosporium secalis* and then exposed to surface wetness durations of 1-48 h. Plants were exposed to 5-30°C during the wet period and incubated at 20°C during the subsequent dry period.

^b Disease severity index (0-100 scale) was determined 14 d after inoculation.

^c Latent period defined as period (d) from inoculation to onset of symptoms.

^d - No disease. No disease occurred at 5 or 30°C at any period of surface wetness tested.

^e LSD values refer to comparisons among all disease severity index values and among all latent periods, respectively.

Table 3. Effect of surface wetness duration and temperature of the subsequent dry period on disease severity index and latent period of scald of barley^a

Surface wetness duration (h)	Disease severity index ^b					Latent period ^c (d)				
	Temperature (°C)					Temperature (°C)				
	10	15	20	25	30	10	15	20	25	30
1	0	0	0	0	0	- ^d	-	-	-	-
2	0	6	6	0	0	-	10.3	10.8	-	-
4	4	4	6	0	0	11.3	11.3	10.3	-	-
6	4	6	13	3	0	11.3	10.5	9.0	9.3	-
9	6	10	12	4	1	11.3	9.5	9.0	9.5	9.0
12	19	39	81	4	5	10.8	9.5	9.0	8.5	9.0
48	71	88	100	34	10	11.5	9.0	8.3	8.3	8.5
LSD ($P = 0.05$) ^e	7.5					1.4				

^a Plants of barley line 'GW8614' were inoculated with race SO1 of *Rhynchosporium secalis* and then exposed to surface wetness durations of 1-48 h. Plants were exposed to 20°C during the wet period, and exposed to temperatures of 5-30°C during the subsequent dry period.

^b Disease severity index (0-100 scale) was determined 14 d after inoculation.

^c Latent period defined as period (d) from inoculation to onset of symptoms.

^d - No disease. No disease occurred at 5°C at any period of surface wetness tested.

^e LSD values refer to comparisons among all disease severity index values and among all latent periods, respectively.

Table 4. Effect of inoculum concentration on disease severity index and latent period of scald of barley^a

Inoculum concentration (conidia mL ⁻¹)	Disease severity index ^b		Latent period ^c (d)	
	GW8614	OAC Acton	GW8614	OAC Acton
	1 × 10 ²	53	0	13.3
1 × 10 ³	73	8	11.5	13.8
1 × 10 ⁴	84	8	10.3	13.5
1 × 10 ⁵	87	39	7.8	11.0
2 × 10 ⁵	100	79	7.8	10.0
5 × 10 ⁵	100	86	8.0	8.8
1 × 10 ⁶	100	90	7.8	8.5
LSD ($P = 0.05$) ^e	7.3		0.8	

^a Plants of barley line 'GW8614' and cultivar OAC Acton were inoculated with race SO1 of *Rhynchosporium secalis* and then exposed to surface wetness for 48 h. Plants were exposed to 20°C for the total period between inoculation and disease assessment.

^b Disease severity index (0-100 scale) was determined 14 d after inoculation.

^c Latent period defined as period (d) from inoculation to onset of symptoms.

^d - No disease.

^e LSD values refer to comparisons among all disease severity index values and among all latent periods, respectively.

Temperature of the dry period

The effects of surface wetness duration and temperature of the dry period on disease severity and latent period are shown in Table 3. No disease occurred if the temperature of the dry period was 5°C (data not shown) or if the wet period was 1 h. The minimum duration of the wet period required to produce disease ranged from 2 h at 15 and 20°C to 9 h at 30°C. Disease occurred in the temperature range 10-30°C and symptoms first appeared 8.3-11.5 d after inoculation. Disease developed most rapidly at 20°C and was most severe (DSI = 100) at a dry period temperature of 20°C.

Inoculum concentration

The effects of inoculum concentration on disease severity and latent period are shown in Table 4. As inoculum concentration (conidia mL⁻¹) increased from 10² to 10⁶, the disease severity index increased from 53 to 100 in line 'GW8614' and from 0 to 90 in cv. OAC Acton. In the same range of inoculum concentrations, latent period declined from 13.3 to 7.8 d in line 'GW8614' and from more than 14 to 8.5 d in cv. OAC Acton. Maximum disease severity occurred at 2 x 10⁵ conidia mL⁻¹ in line 'GW8614' and at 10⁶ conidia mL⁻¹ in cv. OAC Acton.

DISCUSSION

Following inoculation of barley line 'GW8614' with race SO1 of *R. secalis*, scald developed most rapidly and most severely when plants were provided with a leaf wetness period of 48 h and were held at a constant temperature of 20°C during the wet and dry periods. Although each experiment was conducted only once these optimum conditions appeared in all four experiments and the data obtained for these conditions were very similar; disease severity indices were consistently 100 and latent periods ranged from 7.8 to 8.3 d. A surface wetness duration of 48 h may not be necessary at optimum temperature, since a wet period of 24 h at a constant temperature of 20°C permitted maximum disease development. At wet period temperatures above and below 20°C, disease severity declined as surface wetness duration was increased from 24 to 48 h (Table 2).

When the temperature of the dry period was 20°C, disease severities were greatest and almost identical following 48-h wet period temperatures of 15 and 20°C and were considerably less at 10 and 25°C (Table 2). In contrast, under similar conditions (2 x 10⁵ conidia mL⁻¹, 44-h wet period, at 20°C day and 16°C night temperatures during the dry period), Ryan and Clare (1975) concluded that the disease became most severe when the temperature of the wet period was 25°C. Moreover, they found a broader range of wet period temperatures (10-25°C) that provided comparable levels of disease severity (21.3-26.4% leaf area affected 28 d after inoculation). Both studies, however, found that very little or no disease developed following a wet period temperature of 30°C. Our results are more similar to those of Skoropad (1957), who found that disease severity was greatest and latent periods were shortest (11 d) when the temperature of the wet period (duration 48 h) was 15-18°C and the temperature of the subsequent dry period was 24°C. In addition, Skoropad (1957) showed that appreciable disease developed at a wet period temperature of 6-14°C provided that the dry period temperature was 24°C. Post-inoculation temperatures of 6-12°C or greater than 24°C greatly inhibited lesion development. It is not clear from the report whether the post-inoculation period referred to is the wet period, dry period, or both. The reports of Ryan and Clare (1975) and Skoropad (1957) together with this study indicate that temperatures at or near 20°C for both the wet period and dry period are favourable for scald development, but that the precise optimum temperature for specific combinations of barley and *R. secalis* may range from 15 to 25°C.

When the temperature of the wet period was 20°C, disease severities at dry period temperatures above and below the optimum (20°C) were greater (Table 3) than when the temperature was held constant throughout the wet and dry periods (Table 1). Apparently, by favouring establishment of the fungus in the leaf, a temperature of 20°C during the wet period permitted earlier and more severe expression of disease symptoms when less favourable temperatures were applied subsequently. In particular, a temperatu-

re of 30°C prevented infection but did not preclude expression of symptoms from established infections. Mayfield and Clare (1985) reported that scald could continue to develop if high temperatures (35 and 40°C) were applied for short periods (2-6 h) at various intervals after an initial wet period of 14 h at 20°C. It appears that high temperatures following successful establishment of infection do not prevent expression of symptoms.

Wet periods as short as 2 h permitted infection at optimum temperature. This matches the report of Ryan and Clare (1975) of a minimum wet period of 2 h at 20°C. Polley (1971) reported that, under glasshouse conditions where temperatures fluctuated from 12 to 21°C and averaged 15°C, the minimum wet period permitting symptom development was 9 h. Low disease severities were also observed in this study at wet periods of up to 9 h at 15°C.

The effect of inoculum concentration on disease severity and latent period depended on the genotype of barley tested. Line 'GW8614' is more susceptible to scald than cv. OAC Acton in the field (G. Xue, personal observation) and in controlled environments (Xue *et al.* 1991). The present study shows that the higher susceptibility is expressed across a wide range of inoculum concentrations and is particularly evident at low inoculum levels. This suggests that inoculum doses lower than those commonly used in screening trials in controlled environments (2-5 x 10⁵ conidia mL⁻¹) may be useful in detecting quantitative resistance in barley to scald.

In summary, infection and disease can occur within the range 10-25°C for many combinations of barley and *R. secalis* genotypes. The optimum temperatures for infection and disease development vary widely within the pathosystem, ranging from 15 to 25°C. Disease can occur after 2 h of surface wetness but maximum disease occurs after 24-48 h. Disease severity increases with inoculum concentration but the dose-response curve depends on the genotype of the host.

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