Pathogenic Variation in *Rhynchosporium secalis* from Southern Ontario

G. XUE, Research Associate, and R. HALL, Professor, Department of Environmental Biology, and D. FALK, Assistant Professor, Department of Crop Science, University of Guelph, Guelph, Ontario, Canada N1G 2W1

ABSTRACT

Xue, G., Hall, R., and Falk, D. 1991. Pathogenic variation in *Rhynchosporium secalis* from southern Ontario. Plant Dis. 75:934-938.

Among 352 isolates of *Rhynchosporium secalis* collected from barley (*Hordeum vulgare*) in Ontario during 1987, 1988, and 1989, 20 races were identified on the basis of virulence to five barley differentials, cvs. La Mesita, Turk, Trebi, Abyssinian, and OAC Elmira. The races were labeled SO1 to SO20 in order of increasing number of differentials matched. SO1, the most common race, composed 55% of the population and was virulent to the barley breeding line GW8614 and two Ontario winter barley cultivars, OAC Acton and OAC Halton, but to none of the differentials. The remaining 19 races were virulent on one to five differentials. None of the races was virulent to cv. Atlas or Atlas-46. Of the cultivars grown commercially in Ontario, OAC Acton and OAC Halton were susceptible to all races and OAC Elmira was resistant to nine races. The frequency of races was inversely related to the number of differential cultivars matched.

In Ontario, scald, caused by *Rhynchosporium secalis* (Oudem.) J.J. Davis, is a severe disease of winter barley (*Hordeum vulgare* L.) and also occurs on spring barley (19). Both OAC Acton and OAC Halton, cultivars of winter barley commonly grown in Ontario, can develop high levels of scald in the field. Resistance to scald has been reported in barleys (2,7,10,15–17), but the effectiveness of this resistance against Ontario isolates of the pathogen is unknown. This information would assist in breeding barleys resistant to scald in Ontario. The purpose of the present investigation was to characterize the variation in pathogenicity to barleys that occurs in *R. secalis* from southern Ontario.

MATERIALS AND METHODS

Collection and isolation of the fungus. Of 352 isolates of *R. secalis* from naturally infected barley in southern Ontario, 17 were collected from the University of Guelph Research Station, Elora, in 1987; 143 were collected from 17 counties in 1988; and 192 were collected from 23 counties in 1989 (Fig. 1). Of 220 isolates obtained from commercial fields, 84 were from cv. OAC Halton, 89 from cv. OAC Acton, and 47 from unidentified cultivars of spring barley. A further 132 isolates were obtained from breeders' plots in Wellington, Oxford, Perth, Middlesex, and Kent counties. The fungus was recovered in early May and late June from winter and spring barleys in the southwestern and southern portions of Ontario and in late June from spring barley in the southeastern portion, generally at or near growth stage 11 (milky ripe to ripe for harvesting) on the Feekes scale (13).

To isolate the fungus, a 4-mm^2 segment cut from a scald lesion was surface-sterilized for 90 sec in 0.5% aqueous NaOCl, rinsed in sterile distilled water, and placed in petri dishes on the surface of wheat germ agar (WGA), adapted from Elliott (8) by substituting wheat germ for wheatmeal. The dishes were incubated at 17 C with constant illumination from fluorescent and longwave ultraviolet lamps (50 $\mu E \cdot m^{-2} \cdot s^{-1}$) for 5 days to permit the development of a small, pink colony of *R. secalis* around the leaf piece. Spores were scraped from the surface of the colony with a wire loop, suspended in a drop of water, streaked onto plates of WGA, and incubated as above. Single germinated conidia were transferred to WGA and incubated at 17 C for 2 wk to establish the single-spored cultures used to assess pathogenicity. Cultures were stored on porcelain beads (12) at −18 C until required for inoculation.

Test plants. Each isolate of the fungus was tested against five plants of each of nine cultivars (Atlas, Atlas-46, La Mesita, Turk, Trebi, Abyssinian, OAC Elmira, OAC Acton, and OAC Halton) and one breeding line (GW8614) of barley. Genes for resistance to scald have been identified in the first six members of the set (Table 1). Each barley was grown as groups of five plants in 7-cm-diameter plastic pots containing commercial potting mix (Pro-Mix BX, Plant Products Co. Ltd., Brampton, Ont.) in a growth room at 20 C with a photoperiod of 14 hr and a light intensity of 150 $\mu E \cdot m^{-2} \cdot s^{-1}$ provided by fluorescent and incandescent lamps. Pots were watered from the bottom twice a week.

Preparation of inoculum and inoculation. To prepare inoculum, 1 ml of a spore suspension (10^7 spores per milliliter) from a culture derived from a single spore was spread over the surface of WGA medium in 9-cm petri dishes. The dishes were incubated at 17 C for 2 wk in continuous light. Each dish then received 5 g of glass beads 3 mm in diameter and 8 ml of water, then was shaken at 150 rpm for 10 min on a rotary shaker to dislodge the spores. The resulting spore suspension was filtered through two layers of cheesecloth and adjusted to 2×10^5 spores per milliliter. At the three-leaf stage, the five seedlings in each pot were sprayed with a total of 2 ml of spore suspension with a DeVilbiss model 15 atomizer. Two pots each of five plants of line GW8614 sprayed with dis-

Accepted for publication 26 February 1991 (submitted for electronic processing).

tilled water were included with each group of differentials as a check against extraneous airborne inoculum. Inoculation of the susceptible line GW8614 and the susceptible cultivars OAC Acton and OAC Halton in each trial provided a check that inoculum and environmental conditions were suitable for infection. After the inoculum dried for 1 hr, the plants were transferred to a saturated atmosphere in the growth room for 48 hr, then returned to the growth room bench. Preliminary experiments showed that maximum disease developed at an infection temperature of 20 C, an incubation temperature of 20 C, a wetness period of 48 hr, and a spore concentration of 5×10^5 spores per milliliter. Isolates collected in 1987 were tested immediately and again after 8 mo of storage on beads. Isolates collected in 1988 and 1989 were tested once.

Disease severity was rated after 2 wk on the second and third leaf according to the following scale: 0 = no visible symptoms, 1 = small brown or gray spots confined to leaf margins and tips, 2 = small brown or gray spots scattered over the leaf surface, 3 = large lesions covering more than 50% of the leaf area, and 4 = large, coalescing lesions, leaf withering (7). Values were averaged for the five plants and the two leaves per plant for each combination of isolate and cultivar or line. Disease scores of 0, 1, and 2 were classified as resistant responses and scores of 3 and 4 were considered as susceptible responses.

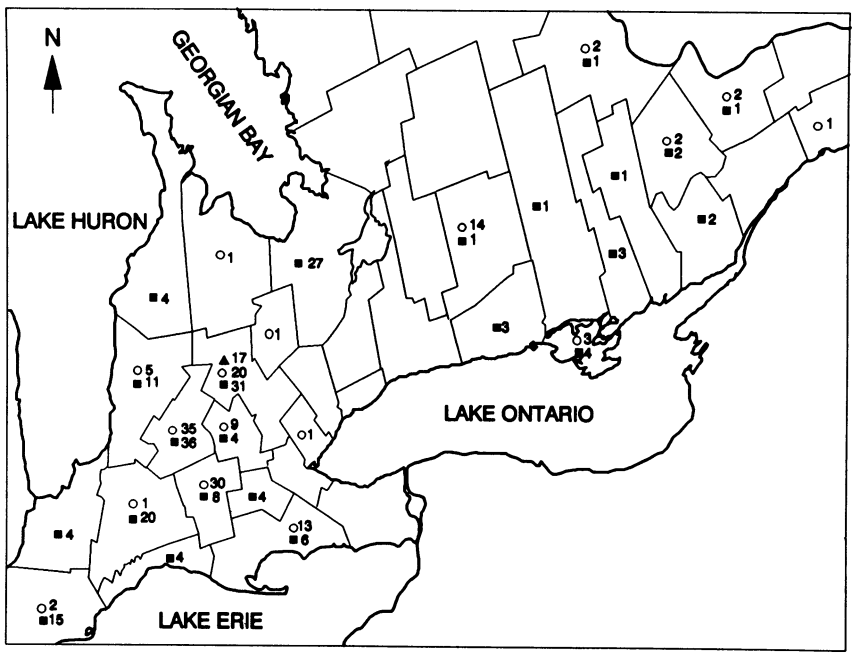

Fig. 1. Number of isolates of *Rhynchosporium secalis* collected per county in southern Ontario in 1987 (▲), 1988 (○), and 1989 (■).

Table 1. Relative effectiveness of resistance of barley cultivars to *Rhynchosporium secalis* isolates collected from southern Ontario in 1987, 1988, and 1989

Cultivar	CI no.[a]	Genes for scald resistance[b]	Matching races: Races (no.)	Matching races: Isolates (no.)	Matching races: Percentage of sample
Atlas	4118	*Rh2* (7)	0	0	0.0
Atlas-46	7323	*Rh2*, *Rh3* (7) *Rh* (10)	0	0	0.0
La Mesita	7565	*Rh4* (7) *Rh4*, *Rh10* (10)	6	10	2.8
Turk	14400	*Rh3*, *Rh5* (7) *Rh*, *rh6* (10) *Rh3* (16)	10	27	7.7
Trebi	936	*Rh4* (7) Unnamed *rh* (15)	10	61	17.3
Abyssinian	668	*Rh9* (2)	9	62	17.6
OAC Elmira			11	92	26.1
OAC Acton			20	352	100.0
OAC Halton			20	352	100.0
GW8614			20	352	100.0

[a] Accession number of Cereal Crop Research Branch, U.S. Department of Agriculture, Agricultural Research Service, Beltsville, Maryland.
[b] Literature citations of papers describing scald resistance gene(s) enclosed in parentheses.

RESULTS

Among 352 isolates tested on 10 barleys, resistant reactions (0–2) per isolate/barley combination averaged 0.15 (SE = 0.51, n = 2,213) and most (92%) were 0 (Fig. 2). Susceptible reactions (3 and 4) averaged 3.76 (SE = 0.18, n = 1,307) and most (78%) were 4. Contrast analysis showed that the resistant and susceptible groups were significantly different at $P < 0.0001$. Throughout the study, lesions usually were either absent or small or large and involving more than 50% of the leaf. For this reason, the reactions of the barleys to the isolates were considered to be qualitative, and the 352 isolates were classified into 20 races on the basis of reactions of the 10 members of the test set (Table 2). The races were labeled SO1 to SO20 in order of the number of differentials matched, the prefix SO indicating southern Ontario. Among races, resistant reactions (0–2) averaged 0.40 (n = 94) and susceptible reactions (3 and 4) averaged 3.63 (n = 106).

In the preliminary study of 17 isolates from one location in 1987, the same four races were identified when isolates were tested immediately and after 8 mo of storage. All of these races were detected again in 1988 and three of them in 1989 (Table 3). The 1988 and 1989 collections contained a much larger number of isolates from a wider area of the province and can be more easily compared with each other. The number and abundance of races differed considerably between these two years. Seven races were detected in 1988 and 17 in 1989. Only four of these races (SO1, SO3, SO4, and SO17) were detected in both years. SO1 was the dominant race in both 1988 and 1989 and composed 55.4% of the total sample of 352 isolates. SO3, SO4, and SO17 were much more common in 1988 (12.6, 7.7, and 4.2%, respectively, of the sample) than in 1989 (1.6, 1.1, and 0.5%, respectively). Thirteen races were found only in 1989, and SO2 made up 27.1% of the sample.

GW8614, OAC Acton, and OAC Halton were susceptible to all races, and Atlas and Atlas-46, both containing resistance gene *Rh2*, were resistant to all races. The races could be distinguished by the reactions of five cultivars: OAC Elmira, Abyssinian, Trebi, Turk, and La Mesita (Table 2). This group was designated the differential set for the 20 races. Differentials ranked in order of susceptibility to an increasing proportion of the pathogen collection were La Mesita, Turk, Trebi, Abyssinian, and OAC Elmira (Table 2).

The races obtained from research plots were more numerous and had a wider range of virulence than those obtained from commercial fields. The 11 races obtained from commercial fields matched 0–2 differentials, whereas the 16 races obtained from research plots matched

0–5 differentials (Tables 2 and 3).

Within the total sample, the frequency of the 20 races was inversely related to the number of differentials matched (Fig. 3). SO1, the most common race (55.4% of the pathogen collection), was virulent to GW8614, OAC Halton, and OAC Acton but avirulent to all differentials. Five races (29.3% of the collection) were virulent to one differential, six races (8.2% of the collection) to two differentials, four races (3.1% of the collection) to three differentials, three races (3.4% of the collection) to four differentials, and one race (0.6% of the collection) to all five differentials.

DISCUSSION

The purposes of this study were to determine the extent of pathogenic variability in *R. secalis* from southern Ontario and the responses of locally adapted cultivars of winter barly to local isolates of the pathogen. However, there is no standard set of host differentials and no standard race nomenclature for this pathogen. Moreover, there is confusion in the literature concerning the numbers and identities of genes for resistance to scald. We therefore attempted to assemble a set of differentials that could be used to describe pathogenic variation among Ontario isolates of *R. secalis*. In preliminary tests we inoculated 11 barleys used as differentials in previous studies (3,4,11,18) with three isolates of race SO1 and one isolate each of races SO2, SO3, and SO4 of *R. secalis* collected in 1987. Osiris (CI1622), Jet (CI967), and Kitchin (CI1296) were resistant to all six isolates and Nigrinudum (CI11549) and Modoc (CI7566) were susceptible to all six and were excluded from further testing. Extensive testing was restricted to four previously used cultivars that gave differential reactions to the isolates from southern Ontario (Turk, Trebi, Abyssinian, and La Mesita), two previously used cultivars that proved to be resistant to all southern Ontario isolates (Atlas and Atlas-46), and the three cultivars of winter barley most widely grown commercially in Ontario.

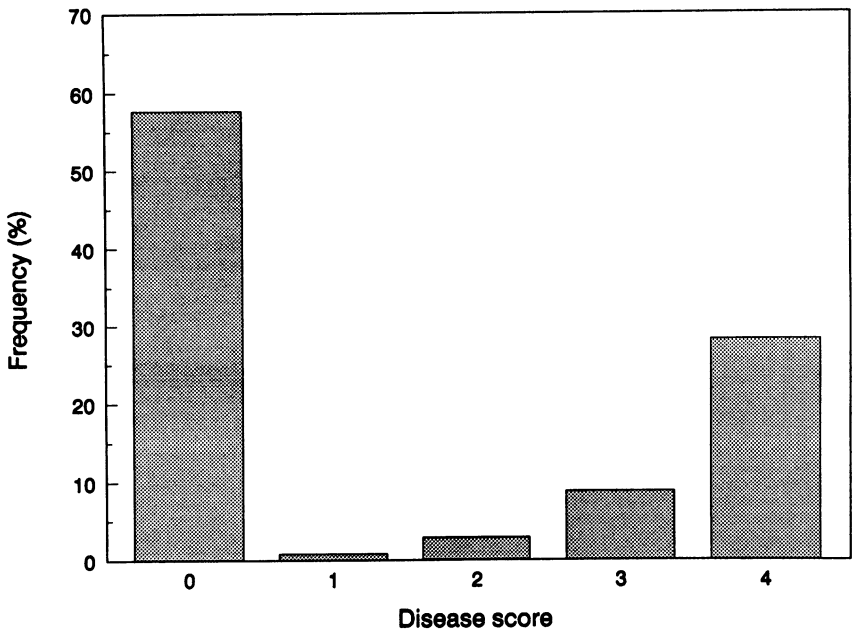

Fig. 2. Frequency of disease scores (0–2 = resistant, 3 and 4 = susceptible) produced by 352 isolates of *Rhynchosporium secalis* on 10 barley cultivars.

Table 2. Disease reactions of five barley cultivars to 20 races of *Rhynchosporium secalis* collected from southern Ontario in 1987, 1988, and 1989

	Cultivars[a]				
Race	La Mesita	Turk	Trebi	Abyssinian	OAC Elmira
1	R	R	R	R	R
2	R	R	R	R	S
3	R	R	R	S	R
4	R	R	S	R	R
5	R	S	R	R	R
6	S	R	R	R	R
7	R	R	R	S	S
8	R	R	S	R	S
9	R	S	R	R	S
10	S	R	R	R	S
11	R	R	S	S	R
12	R	S	S	R	R
13	R	R	S	S	S
14	R	S	R	S	S
15	R	S	S	R	S
16	S	S	R	S	R
17	R	S	S	S	S
18	S	S	S	R	S
19	S	S	S	S	R
20	S	S	S	S	S

[a] All races were avirulent to cvs. Atlas and Atlas-46 and virulent to cvs. OAC Acton and OAC Halton and line GW8614. R = resistant, S = susceptible.

Pathogenic variability was considerable among isolates of *R. secalis* from southern Ontario. Because differences between susceptible and resistant reactions were generally large, we allocated isolates to races on the basis of the responses of the differentials. Of the 32 possible races identifiable by the five differentials, 20 were found. Similarly, high variability in the pathogen has been reported from South Australia, Western Australia, and Tasmania (1), California (9,11), and Italy (4). However, considerably less variability has been reported from Britain (18), Victoria, Australia (3), and New Zealand (6). Because different sets of differentials were used in these studies, comparisons are difficult.

A nomenclature for races of *R. secalis* was first proposed by Schein (16). Four races from Argentina were designated A.1 to A.4 and seven races from the United States were labeled U.S.1 to U.S.7. This system has been used by other workers to name two British races U.K.1 and U.K.2 (18), 35 Australian pathotypes Aust.1 to Aust.35 (1), and 17 Italian races RC1 to RC17 (4). Thus, we have labeled the 20 races from southern Ontario SO1 to SO20.

The virulence patterns of the populations appeared to change considerably between 1988 and 1989. Similarly, Crandall (5) found large changes between 1983 and 1984 in the pathotype composition of Californian populations. Moreover, more than 75% of the pathotypes found in California in 1983 and 1984 differed from those found in an earlier study using the same differentials (11). Although it is premature to assess the pace and direction of change in virulence of *R. secalis* in Ontario, it seems likely that virulence characteristics of the pathogen can change widely and rapidly.

Various factors appear to affect pathogenic variability in *R. secalis* (14). In Ontario, the inverse relationship between the frequency of races from barley and their virulence indicates that selection against unnecessary virulence may occur.

On the other hand, nearly half (44.6%) of the pathogen population contained greater virulence than that needed to cause disease in the cultivars of winter barley grown commercially in the province. This appears to indicate that unnecessary virulence occurs in the populations. And since not all known resistance genes were tested in this study, considerable, apparently unnecessary virulence may remain to be detected. However, we have observed scald symptoms on diverse barleys in breeders plots, in commercial crops of spring barley, and on grasses. Directional selection pressure toward greater virulence in the pathogen population may arise from unidentified resistance in these host plants.

When the two commercial cultivars of winter barley presently grown in Ontario were released, OAC Halton was considered to have some "tolerance" to scald and OAC Acton was considered to have more resistance than OAC Halton. However, the demonstrated susceptibility of these cultivars to all races of *R. secalis* in Ontario helps explain why scald is widespread and severe on these cultivars in the province. Cultivars Atlas and Atlas-46 are presently resistant to Ontario races. These cultivars were also found to be resistant to populations in New Zealand (6), Australia (1,3), and Italy (4) but susceptible to populations in California (5,11) and Idaho and Oregon (9). The resistance in these two cultivars is known to be race-specific (7,11). OAC Elmira was developed recently as a scald-resistant cultivar for use in Ontario. Its source of scald resistance is not known, but we have shown that it has resistance to the most common race (SO1) and eight other races of *R. secalis* in Ontario. It would be instructive to track changes in frequencies of races of the pathogen should OAC Elmira become more widely used. In view of the capacity for pathogenic variability in the pathogen population over a small area, race-specific resistance may be short-lived. Although it appears that gene *Rh2* confers resistance to the Ontario population of *R. secalis*, further studies are needed to determine the nature and durability of the resistance in Atlas and Atlas-46 to scald and the possible occurrence of non-race-specific resistance in barleys to the Ontario population of the pathogen.

Table 3. Numbers of isolates of races of *Rhynchosporium secalis* collected from barleys in southern Ontario in 1987, 1988, and 1989

Race	Number of isolates				Percentage of sample	Source[a]			
	1987	1988	1989	Total		OAC Halton	OAC Acton	Spring barley	Breeder plot
SO1	2	96	97	195	55.4	X	X	X	X
SO2			52	52	14.8	X	X	X	X
SO3	3	18	3	24	6.8	X	X	X	X
SO4	11	11	2	24	6.8	X	X		X
SO5	1	1		2	0.6				X
SO6			1	1	0.3			X	
SO7			10	10	2.8	X	X	X	
SO8			4	4	1.1	X	X		
SO9			4	4	1.1	X		X	X
SO10			1	1	0.3			X	
SO11		8		8	2.3	X	X		X
SO12			2	2	0.6				X
SO13			6	6	1.7	X	X		X
SO14			1	1	0.3				X
SO15		3		3	0.9				X
SO16			1	1	0.3				X
SO17		6	1	7	2.0				X
SO18			2	2	0.6				X
SO19			3	3	0.9				X
SO20			2	2	0.6				X
Total	17	143	192	352					

[a] Indicated by X.

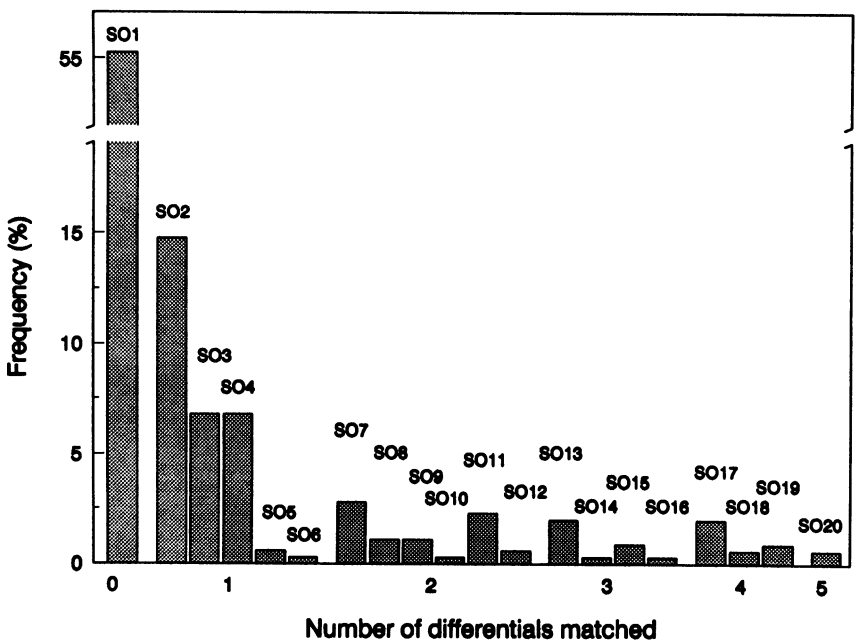

Fig. 3. Frequency of races of *Rhynchosporium secalis* from barley in southern Ontario in relation to number of differential cultivars matched.

ACKNOWLEDGMENTS

We gratefully acknowledge the financial assistance provided by the Canadian International Development Agency through the Black Dragon River Consortium, by the Natural Sciences and Engineering Research Council, and by the Ontario Ministry of Agriculture and Food.

LITERATURE CITED

1. Ali, S. M., Mayfield, A. H., and Clare, B. G. 1976. Pathogenicity of 203 isolates of *Rhynchosporium secalis* on 21 barley cultivars. Physiol. Plant Pathol. 9:135-143.
2. Baker, R. J., and Larter, E. N. 1963. The inheritance of scald resistance in barley. Can. J. Genet. Cytol. 5:445-449.
3. Brown, J. S. 1985. Pathogenic variation among isolates of *Rhynchosporium secalis* from cultivated barley growing in Victoria, Australia. Euphytica 34:129-133.
4. Ceoloni, C. 1980. Race differentiation and search for sources of resistance to *Rhynchosporium secalis* in barley in Italy. Euphytica 29:547-553.
5. Crandall, B. 1987. Studies on the interaction of *Rhynchosporium secalis* on barley composite cross populations and pureline cultivars from California and Montana. Ph.D. thesis. University of California, Davis. 122 pp.
6. Cromey, M. G. 1987. Pathogenic variation in *Rhynchosporium secalis* on barley in New Zealand. N.Z. J. Agric. Res. 29:95-99.
7. Dyck, P. L., and Schaller, C. W. 1961. Inheritance in barley of resistance to several physiologic races of the scald fungus. Can. J. Genet. Cytol. 3:153-164.
8. Elliott, R. F. 1975. Viability of fungous cultures dried and stored over silica gel. N.Z. J. Sci. 18:577-583.
9. Goodwin, S. B. 1988. Analysis of gene-for-gene interactions, and variability for isozyme and pathogenicity markers in *Rhynchosporium secalis* (Oud.) Davis. Ph.D. thesis. University of California, Davis. 126 pp.
10. Habgood, R. M., and Hayes, J. D. 1971. The inheritance of resistance to *Rhynchosporium secalis* in barley. Heredity 27:25-37.
11. Jackson, L. F., and Webster, R. K. 1976. Race differentiation, distribution, and frequency of *Rhynchosporium secalis* in California. Phytopathology 66:719-725.
12. Lange, B. J., and Boyd, W. J. R. 1968. Preservation of fungus spores by drying on porcelain beads. Phytopathology 58:1711-1712.
13. Large, E. C. 1954. Growth stages in cereals:

Illustration of the Feekes scale. Plant Pathol. 3:128-129.
14. McDonald, B. A., McDermott, J. M., Goodwin, S. B., and Allard, R. W. 1989. The population biology of host-pathogen interactions. Annu. Rev. Phytopathol. 27:77-94.
15. Riddle, O. C., and Briggs, F. N. 1950. Inheritance of resistance to scald in barley. Hilgardia 20:19-27.
16. Schein, R. D. 1958. Pathogenic specialization in *Rhynchosporium secalis*. Phytopathology 48:814-815.
17. Wells, S. A., and Skoropad, W. P. 1963. Inheritance of reaction to *Rhynchosporium secalis* in barley. Can. J. Plant Sci. 43:184-187.
18. Williams, R. J., and Owen, H. 1973. Physiologic races of *Rhynchosporium secalis* on barley in Britain. Trans. Br. Mycol. Soc. 60:223-234.
19. Xue, G., and Hall, R. 1989. Virulence of *Rhynchosporium secalis* in Ontario to barley. Can. Plant Dis. Surv. 69:32.