Pathogenic Variation of *Rhynchosporium secalis* in Denmark and Sources of Resistance in Barley

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**ABSTRACT**


Thirty-eight Danish isolates of *Rhynchosporium secalis*, 36 from barley and two from rye, were inoculated together with 23 cultivars of barley, one cultivar of rye, and nine species of noncereal grasses. The isolates were divided into 28 different races (DK1-DK28) according to their reaction with the barley cultivars. These 28 races represent a very broad virulence spectrum. Race 1 caused susceptible reactions in 19 of the 23 cultivars, whereas race 26 caused susceptible reactions in only six cultivars and race 27 in only one cultivar. All barley cultivars were resistant to race 28, represented by the two isolates from rye. Fifteen cultivars of barley with race-specific resistance were included in the present set of differentials. Of these cultivars, only two, Atlas and Osiris, were resistant to all races tested, whereas the rest was susceptible to two or more races. Of eight commercial cultivars, Corgi, Digger, and Hasso were resistant to 27, eight and eight races, respectively. Four other cultivars were susceptible to all but two of the races: Race 27, virulent on only cultivar Magda and race 28, comprising isolates from rye. The last cultivar was only resistant to race 28. These results suggest that known sources of race-specific resistance to *R. secalis* may not be effective in Denmark if not used in combination with race-nonspecific resistance. One isolate from barley caused minor infections in rye but not in any of the noncereal grasses. The two isolates from rye caused minor infections in some barley cultivars but the noncereal grasses were not infected. This suggests not only that *R. secalis* is rather strongly specialized to the host species from which it is isolated, but also that it may infect other species as well.

Additional keywords: cross inoculation, host specialization, physiologic specialization, stabilizing selection, virulence spectrum

**MATERIALS AND METHODS**

**Collection and isolation of *R. secalis***

Infected leaves of barley and rye were collected during the spring and summer of 1990 and 1991, air dried and subsequently stored in a refrigerator. Single spore isolates were obtained by placing dry leaves with lesions at 100% relative humidity and 18°C in the dark. After 48 h, conidia from a sporulating lesion were placed in a drop of sterile water and streaked onto lima bean agar (Difco, Detroit, MI) plates. After 48 h incubation in the dark, germinating single conidia were removed and cultured on lima bean agar slants. Figure 1 shows the collection sites of the isolates.


The first 15 barley cultivars in Table 1 were selected because they have been used in similar studies (4,11,15,41) and represent a major portion of the most well known sources of resistance to leaf blotch. The last eight barley cultivars were selected from the Danish variety list (5). Lenka was included as the susceptible control whereas the rest had a high level of resistance in the field. The noncereal grasses were selected from among species reported as hosts of *R. secalis* (6,7,34). In addition, *D. glomerata*, normally considered a nonhost of *R. secalis* (10), was included to test its reaction to inoculation. Seeds of *B. hordeaceus*, *B. inermis*, *E. repens*, *H. lanatus* and *P. arundinacea* were collected from wild populations of these species whereas *D. glomerata*, *L. perenne*, *L. multiflorum* and *P. pratensis* were selected from among cultivars on the Danish variety list (5,20).

Plants were grown in plastic pots, 7 cm in diameter, containing the soil mix “Weibulls Enhetssjord” (K jord, W. Weibull AB, Landskrona, Sweden) (80% peat moss, 20% clay, with NPK and micro nutrients added). Plants were grown on a greenhouse bench (20-25°C)

Leaf blotch or scald caused by *Rhynchosporium secalis* (Oud.) Davis is an important disease of barley (*Hordeum vulgare* L.) in many parts of the world. In Denmark, leaf blotch has become increasingly important since the middle of the 1960s (35, C. Stapel, unpublished) chiefly because of an increased and often continuous growing of barley together with changed cultivation practices, i.e., choice of cultivars, harvest methods, increased fertilization (35). Danish plant breeders have, however, become interested in the disease only during the past few years.

Before an effective breeding program can be initiated, knowledge of the pathogenic variation within the fungal population must be available. Although numerous reports indicate that *R. secalis* isolated from barley exhibits a high degree of pathogenic variability on barley (4,11,15,17,36,40), there are other reports demonstrating only a limited virulence spectrum (8,12,41).

Several investigators have studied whether isolates of *R. secalis* obtained from one host species were capable of infecting other species. In a few, often limited studies, strict host specialization was found, i.e., isolates infected only the species from which they were originally isolated (9,24,30). However, in the majority of investigations, less host specialization was observed, i.e., isolates from one species were able to infect one or a few other species in addition to the one from which they were originally isolated (10,19,26,28,32). In a very few cases, little host specialization was found (6,33).

Lack of host specialization within *R. secalis* suggests that variation in the fungal population found on barley could be introduced from alternative hosts for the pathogen. If so, fungal variation originating from alternative hosts should be considered in future breeding programs.

This study was undertaken to examine the variability within the Danish population of *R. secalis* on a number of barley cultivars, a rye cultivar (*Secale cereale* L.), and a number of noncereal grasses. Unfortunately, it was impossible to obtain isolates of *R. secalis* from hosts other than barley and rye. A second objective was to identify the most effective sources of resistance to leaf blotch among the barley cultivars used as test plants.

Accepted for publication 15 December 1994.

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under natural daylight conditions. Twenty-four hours before inoculation, plants were transferred to a growth chamber (23).

Inoculum and inoculation. Inoculum of the various isolates was produced on lima bean agar plates in the dark at about 18 C. The spore concentration was determined with a hemacytometer and adjusted to 200,000 conidia per milliliter of distilled water. One drop of Tween 80 was added per 150 ml of suspension. In all tests, each cereal cultivar was represented by 5–6 seedlings, whereas noncereal grasses were represented by 10–30 seedlings. Cereal seedlings were inoculated when they had two fully emerged leaves and the third was expanding. Noncereal grasses were inoculated when they were 5- to 6-wk-old and had developed 5–10 leaves. Inoculum was atomized onto the plants until run-off by applying about 150 ml of inoculum to the whole set of test plants. Control seedlings were sprayed with distilled water to which one drop of Tween 80 per 150 ml had been added. Incubation was as previously described (23), with the exception that plants were maintained at 100% relative humidity for 48 h only. All plant-isolate combinations were tested at least twice.

Assessment of disease. Leaf blotch was assessed on the lamina of the second developed leaf of the cereals and on all leaves of noncereal grasses, using the following scale (modified from 15,17,38):

0 = no visible symptoms; 1 = one to a few small discrete lesions, often with a dark margin, and covering less than 10% of the lamina; 2 = larger lesions covering about 10–40% of the lamina, often irregular wilted grayish-green areas without a dark margin; 3 = partial or total collapse of the lamina, about 40–100% covered with grayish-green wilted areas without dark margins.

Disease ratings 0 and 1 were considered resistant reactions; ratings 2 and 3 were classified as susceptible. Disease was assessed 11 and 20 days after inoculation. Only data for the second assessment are presented here.

RESULTS

Virulence of isolates of *Rhynchosporium secalis* on cultivars of barley. Twenty-eight different races, arranged in decreasing order of virulence, could be distinguished among the 36 isolates from barley and two from rye tested for virulence on 23 cultivars of barley (Table 1). The races are assigned the prefix DK to designate their geographic origin. Many races are composed of only one isolate. However, races 25 and 26 are represented by four isolates each, race 21 by three isolates and races 2 and 28 by two isolates each. The races represent a broad virulence spectrum. Race 1 caused susceptible reactions in 19 of the 23 cultivars whereas race 26 caused susceptible reactions in only 6 cultivars and race 27 in only one cultivar. All cultivars were resistant to race 28 (the isolates from rye), reacting with no symptoms or small inconspicuous and nonexpanding lesions. The remaining 24 races caused susceptible reactions in 7–18 cultivars. No obvious trends were observed for virulence pattern and geographic origin.

Identification of the most effective sources of resistance in barley. Atlas and Osiris were resistant to all races tested. Atlas 46 was susceptible to two races, La Mesita to three races, and Abyssinian to four races. The rest of the cultivars were susceptible to 10 or more races, with Pioneer susceptible to all races derived from barley except race 27. Of the cultivars from the Danish variety list (16–23), Corgi was resistant to all races except DK9. Digger and Hasso were resistant to eight races each. The remaining cultivars were susceptible to all races from barley except race 27, which was only virulent on cultivar Magda.

Cross inoculation test. Except for one out of 36 barley isolates (race 15), which caused small inconspicuous lesions (type 1) on rye, no other barley isolates caused visible symptoms on rye or any of the noncereal grass species tested. The two isolates from rye readily infected rye (reaction type 3) and caused small lesions (type 1) on several cultivars of barley (classified as resistant), but did not cause symptoms on any of the noncereal grass species.

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**Fig. 1.** Collection sites for *Rhynchosporium secalis* in Denmark. Each dot on the map represents a barley field from which *R. secalis* was isolated. The numbers indicate the DK race to which the isolate belongs.

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DISCUSSION

Virulence spectrum of isolates on barley. The present investigation has revealed a pathogenicity spectrum broader than in several other investigations (3, 4, 11, 40). The Danish population of R. secalis even appears to be relatively more variable in virulence than the one in Canada, where 45 races were identified among 111 isolates tested on 10 barley cultivars (36) and the one in California, where 75 races were distinguished among 175 isolates tested on 14 barley cultivars (17).

No other studies of the virulence spectrum of R. secalis have been conducted in Denmark. Åström (41) tested 15 isolates from the southern part of Sweden. This part of Sweden is comparable to Denmark with respect to cultivation and conditions for the growth of barley. However, Åström (41) found it possible to divide the isolates into only 2 races.

No satisfactory explanation has been found as to why the Danish population of R. secalis appears to be considerably more variable than populations in other countries. Considering the rather limited geographical size of Denmark, it is surprising that such a broad virulence spectrum has been found. Denmark is divided into 3 major parts (Jutland, Zealand, and Funen) and many smaller islands, all parts being separated by water. Therefore, it could be expected that each part of the country has its own separate sub-population of R. secalis with different virulence spectra. However, no obvious pattern between geographical distribution of races and specific virulence patterns from various parts of the country was found.

Selection pressure on the population of R. secalis from cultivated barley in Denmark may have influenced the level of variability in the pathogen. Although only a few barley cultivars with considerable levels of resistance have been widely grown (Table 1), the fungal population appears sufficiently variable to overcome the resistance of a large part of the currently known sources of resistance to leaf blotch sources that have not been used in cultivars grown in Denmark. Our data, and others (15, 17) suggest that the stabilizing selection theory (races with unnecessary virulence are less fit), proposed by Van der Plank (37), does not apply to the barley-leaf blotch system. Other conflicting results, however, suggest that the theory of stabilizing selection does apply to the barley-leaf blotch system (2, 18, 39).

Seedborne inoculum as a source of primary infections of R. secalis has been reported (25, 30), but since Denmark imports only limited amounts of seed grains of barley, this source of genetic variation in R. secalis is considered to play only a minor role.

Selection pressure on R. secalis from alternative hosts in America has been demonstrated for Hordeum species in Australia (3, 4). However, alternative hosts do not appear to be important for creating genetic diversity in R. secalis in Denmark.

No attempt has been made to compare specific races of R. secalis on barley found in this study with races identified in other studies of virulence diversity. As suggested by several authors (4, 8, 11, 36, 41), such comparisons would probably be arbitrary because the various investigations have not been performed in exactly the same way. Thus, differences in the environment under which the various experiments have been done may influence the performance of individual cultivars (1, 29, 38). Williams and Owen (38) considered it very likely that differences in results among different studies could be attributed to environmental variation.

Variation in the size and composition of the set of test plants employed will influence the number and nature of races distinguished (11, 17, 36). Furthermore, different resistance genes may exist in cultivars with the same name when a common seed source has not been used (8). Hence, Åström (41) doubted whether the Nigrinumud she used was genetically identical to CI 2222-Nigrinumud used in other studies. In the present work, CI 2222 was also used, but the name Nigrinumud was not attached to it. However, the CI-number indicates that it is the same cultivar as used in previous studies. The cultivar Turk used by Brown (8) and in this study was CI 14400. Cultivar Turk used in previous studies has been designated as CI 5611-2. It is not known whether these are actually the same cultivar.

The scoring scale used to assess the disease can also strongly influence the results. This is because no distinct borderlines exist between the different categories in each investigation, different

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<th>Table 1. Disease ratings of 23 barley cultivars inoculated with Danish isolates of Rhynchosporium secalis</th>
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<td><strong>Cultivar</strong></td>
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<td><strong>Abbysinian</strong></td>
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<td><strong>Atlas</strong></td>
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<td><strong>Atlas 46</strong></td>
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<td><strong>Brier</strong></td>
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*Accession number, Cereal Crops Research Branch, ARS, USDA, Beltsville, Maryland.

*Isolates from rye. DK prefix designates geographic origin.

*Resistant reaction, ratings 0–1; susceptible reaction, ratings 2–3.
people have used the scales, and disease ratings might often have been grouped into only two categories (susceptible and resistant). Therefore, a cultivar showing an intermediate reaction and classified as resistant in one study may have been classified as susceptible in another study.

Comparisons of the virulence spectra among different investigations should be done with caution, especially because varying numbers (often rather small numbers) of isolates of *R. secalis* have been tested on varying numbers of differentials. Åström (41) stated that the reason why so few races were discovered in her study could have been that only 15 isolates were tested. Likewise, Owen (29) suggested that testing of additional isolates (he tested 10) would reveal isolates with distinct pathogenicity.

**Sources of resistance.** In the present work, only Atlas and Osiris were resistant to all isolates of *R. secalis.* However, taking the diversity of the fungal population into consideration, it would not be surprising if isolates virulent to these cultivars could be found. In Canada, Tekauz (36) also found that none of the differential cultivars used (nine with race-specific resistance and one universal suspect) were resistant to all 111 isolates evaluated.

The present results indicate the Danish population of the fungus is so highly variable that there is a risk that race-specific resistance would not confer an effective and durable protection to new cultivars. Tekauz (36) also considered it risky to base a breeding program on single genes for resistance, although he suggested that incorporation of multiple race-specific resistances possibly would be effective against all races of *R. secalis* in Canada.

That *R. secalis* may overcome race-specific resistance has been indicated in at least two cases. The cultivar Atlas 46 was introduced in California in 1947, at which time it was considered resistant. In 1953, it was infected at several localities, and by 1956, it was extremely susceptible in all parts of the state (16). Furthermore, there are indications that the seedling resistance of the cultivar Digger has been overcome in the United Kingdom. Digger had shown a high level of resistance (22), and it was suggested that it had a resistance gene(s) not present in other differentials employed in routine tests for virulence (21). However, it was less resistant to several isolates in 1990/1991, indicating that erosion of its resistance had taken place (22).

Race-non-specific resistance (12,13,14) may offer a lasting, but not completely effective protection, against leaf blotch. However, in combination with race-specific resistance, it may confer the best possible protection against *R. secalis.*

Cross inoculation test. In this experiment, strict host specialization was observed. One barley isolate caused minor infections on rye and both rye isolates caused minor infections on some barley cultivars. These reactions were, however, classified as resistant. Cross infections between barley and rye could be of great epidemiological importance because the disease-reducing effect of crop rotations might not be effective. Noncereal grasses were not infected by any isolate and it was not possible to obtain samples of *R. secalis* from hosts other than barley and rye. Other grasses are not normally infected by *R. secalis* in Denmark (B. Welling, personal communication). Leaf blotch has, however, been observed on couch grass (*Elytrigia repens* (L.) Nevski) by the authors. Further, Rostrup (31) reported that leaf blotch was found on barley, rye, and some wild grasses, but did not specify which species. Lind (27) reported the disease on barley, couch grass, wood millet (*Mimum effusum* L.), and oats (*Avena sativa* L.). However, it is unlikely that *R. secalis* has been the cause of symptoms on wood millet and oats because these species are not normally infected by *R. secalis* (34). *Poa pratensis*, *H. lanatus*, and *B. hordeaceus* ssp. *hordeaceus* were included among the species to be tested for their reaction to *R. secalis* in this study. They have apparently only been reported as hosts of *R. secalis* by Bartels (6) and Brooks (7), respectively. *Dactylis glomerata* was also included, though, as both it is considered a nonhost of *R. secalis* (10).

It is not possible to draw any general conclusions about host specialization from the present investigation because the cross-inoculation tests were limited. Many isolates of *R. secalis* from barley were tested, but only on one accession of rye and each of the noncereal grasses. Furthermore, only two isolates from rye were tested on rye and the same accessions of noncereal grasses. By using only one or a few accessions of each grass species and only a few isolates of *R. secalis,* the choice of whether resistant or susceptible interactions are studied is left to chance. This is due to the fact that rye and noncereal grasses are apt to vary in resistance to pathogens as much as barley does. This has been shown by Ali (3) for barley grass (*Hordeum murinum* L. ssp. *leporinum* (Link) Arcangeli). However, although Owen (28) and Kajiwara (24) performed only limited cross-inoculation studies, they considered *R. secalis* to be so strongly specialized to a particular host species that it could be divided in "formae specialis" or "formae." This was done even though other reports had already been published showing that strict host specialization was not always found (6,32,33). These investigations also demonstrated that there was no consistent pattern as to which species were infected in addition to the one from which an isolate originated.

In spite of the limited extent of most cross-inoculation studies, the present results and those of most other investigations (6,10,19,26,28,32,33) indicate that *R. secalis* has a preference for a particular species. However, it is possible that isolates of *R. secalis* from other hosts may adapt to barley and vice versa when optimal conditions for the fungus are present. This was suggested by Kay and Owen (26) on the basis of laboratory experiments. These experiments showed that isolates of *R. secalis* from rye, couch grass, and wall barley (*Hordeum murinum* L. ssp. *murinum*) increased their aggressiveness to barley after serial passages through barley leaves. In Australia, isolates of *R. secalis* may readily infect both barley and barley grass (3,4).

In the present study, rye was only slightly infected by barley isolates and vice versa, which indicates that adaptation to other host species may take place in Denmark also. However, in Denmark, adaptation to other hosts possibly includes only barley and rye because *R. secalis* does not easily infect other species.

**ACKNOWLEDGMENTS**

The authors are indebted to the following institutions and companies for providing seeds for the experiments: Nordic Gene Bank, Alnarp and Svalöf Weibull AB in Sweden as well as Danko Planteforudlings A/S, J. AsmusSENS EfF. A/S, Pajbjergstr. and Sejet Planteforudlings. Furthermore, thanks are extended to C. Tornøe, E. de Neergaard, and N. Lademark Petersen for reviewing the manuscript.

**LITERATURE CITED**


cultivars to Rhynchosporium secalis. Trans. Br. 
cence spectrum of Rhynchosporium secalis in 
 Norway and sources of resistance in barley. 
 Phytopathol. Z. 76:303-313.
 Newly determined races of the barley scald 
 differentiation, distribution, and frequency of 
 Rhynchosporium secalis in California. Phyto 
pathology 66:719-725.
dynamics of a controlled population of Rhyn 
chosporium secalis, changes in race composition 
 and frequencies. Phytopathology 66:726-728.
 and gruels as possible sources of Rhynchospo 
rion secalis for barley in California. Plant 
chosporium of barley. U.K. Cereal Pathogen 
chosporium of barley. U.K. Cereal Pathogen 
23. Jørgensen, H. J. L., de Neergaard, E., and 
 Smedegaard-Petersen, V. 1993. Histological 
 examination of the interaction between Rhyn 
chosporium secalis and susceptible and resistant 
 42:345-358.
24. Kajiwara, T. 1968. Comparative studies on the 
 pathogenicity of barley and rye scald fungus, 
 Rhynchosporium secalis (Oud.) Davis. Bull. 
 Rhynchosporium secalis on barley grain. 
 60:413-422.
 Pages 484-487 in: Danish Fungi as Represented 
 in the Herbarium of E. Rostrup. Gyldendal 
 Fogtland - Nordisk Forlag, Copenhagen.
 41:99-108.
sporium secalis on cultivated barley. Trans. Br. 
31. Rostrup, E. 1899. Oversigt over landbrugs 
 planternes sygdomme i 1897. Tidsskrift for 
 Landbrugets Plantecv. 5:113-137.
 Reaccion de algunas cebadas con respecto a 
 "Rhynchosporium secalis" en Argentina. Rev. 
 Invest. Agric. 1:243-260 (+ 3 plates).
33. Schein, R. D. 1958. Pathogenic specialization 
 in Rhynchosporium secalis. Phytopathology 
 48:477-480.
34. Sprague, R., and Fischer, G. W. 1952. Check 
 List of the Diseases of Grasses and Cereals in 
 the Western United States and Alaska. Wash. 
35. Stapel, C., Jørgensen, J., and Hermansen, J. 
 E. 1976. Sædekorsets sygdomme i Danmark, 
 deres udbredelse, betydning og bekæmpelse ved 
 afvandingsarbejde, især i perioden 1906-1975. 
chosporium secalis on barley in Canada. Can. 
37. Van der Plank, J. E. 1968. Disease Resistance 
38. Williams, R. J., and Owen, H. 1973. Physiologic 
 races of Rhynchosporium secalis on barley in 
39. Williams, R. J., and Owen, H. 1975. Suscep 
tibility of barley cultivars to leaf blotch and 
 aggressiveness of Rhynchosporium secalis races. 
 variation in Rhynchosporium secalis from 
41. Åström, B. 1983. Virulenseradskockning av 
kornets sköldfläcksjurka (Rhynchosporium 
 Institutionen för Växt- och Skogskydd, 
 Sveriges Lantbruksuniversitet.