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Diversity of *Rhynchosporium secalis (Oud.)* J. J. Davis strains in morphological and cultural peculiarities

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Summary

Biological peculiarities of the rye scald fungus *Rhynchosporium secalis* (Oud.) J. J. Davis, in one population of North-West region were examined. Seventy-eight isolates, the causal agent of scald, were taken from infected rye plants. This isolates were analalysed on rate of growth on artificial test medium, structure and color and temperature dependence. Single-spore strains were obtained from each natural isolate. Color and structure of some single-spore isolates remained stable through repeated transfers to fresh PDA medium.

Key words: rye, barley, scald, *Secale cereale, Hordeum vulgare*

INTRODUCTION

Leaf scald, caused by the fungus *Rhynchosporium secalis* (Oud.) J. J. Davis, is a serious leaf disease of barley (*Hordeum vulgare* L.) and rye (*Secale cereale* L.). The disease usually caused 30%-40% yield loss of crop in cool and moist climate areas of Russia.

The disease symptoms of rye and barley are similar and may easily be recognized and distinguished from other cereals blights (Caldwell, 1937, Werres G., Hindorf H., 1991). In the early stages of development appear the dark bluish-gray with water soaked lesions. These lesions are usually 1 to 2 cm in length increase several times before evident collapse of the tissue. This collapse appears very rapidly especially on barley. Developing lesions tend to form stripe. The scalded area soon dries and the center turns to light gray, the margin to dark-brown. However, lesions of the rye have narrow light or brown margin, which is not well identify or is absent at all. Except for stripe form the scalds, which can form concentric brown rings. Leaves are destroyed by severe infection and that results in complete defoliation of the host.

The most part of researches is devoted to the *R. secalis*-barley system. *Rhynchosporium secalis,* causing scald of rye leaves is investigated much worse.

The aim of our research was reveal morphological and cultural peculiarities of *R. secalis* and stability of these peculiarities in single-spores fungus isolates.

MATERIALS AND METHODS

Rye leaves infected by *R. secalis* we collected from shooting stage plants in one field of North-West region of Russia. Leaf pieces with scald lesions were sterilized in 70% ethanol and 1% sodium hypochlorite (1:1) for 30 seconds, rinsed in pure water, transferred to PSA with 1% yeastrel. In total, we obtained 78 isolates one isolate from one scald lesion.

The rate of pathogen growth was studied at isolate cultivation in a range of temperatures from 10°C to 18°C on different artificial media (g/l water):

1. 2% water agar; pure water.
2. Czapek agar: K_2HPO_4 0,5 g, $(NH_4)_2SO_4$ 1g, $MgSO_4$ 0,5 g, KCl - 0,5g, saccharose 20 g, agar 20 g, pH 6,2 $\pm$ 0,2
3. Carrot-sacharose agar: carrots 200g, saccharose 20 g, agar 20 g;
4. Oatmeal agar: oat flakes-30g, agar 15g;
5. potato-saccharose agar (PSŕ): potatoes 200, saccharose 20, agar 20;
6. PSA with 1% yeastrel: potatoes 200, saccharose 20, agar 20; yeastrel 1.

The growth of 20 isolates was studied on each artificial medium.

From every rye isolate we obtained 10 single-spore cultures. These single-spores cultures were transferred for five times on PSA for the testing of stability in display of signs.

RESULTS AND DISCUSSION

It is known that isolation and cultivation of *R. secalis* on artificial medium is complicated, because of fungus growing slowly. So, the colonies of *R. secalis* become visible usually only after 14 days growing on PSA with 1% yeast extract. Small, lights, yeast-like, growing upward colonies were formed on media (Coja, 1998; Konova-lova, 1999).

It has been shown that the medium composition influences the fungus growth (table 1). The result of this study has shown that the best artificial medium for growth of *R. secalis*, isolated from rye leaves is PSA and PSA with 1% yeastrel. On these medium average diameter of colonies reached 14.44 mm to 14.72 mm correspondingly. After 14 days of cultivation the average growth rate of the fungus was 1,03 mm to 1,05 mm per day correspondingly. On water agar and Czapek agar the rye fungus growth was absent. On carrot medium and oatmeal medium, the slow fungus growth was detected.

The comparison of the influence of different media on growth of rye and barley isolates shows that the growth rate of barley isolates on some media differs from that rate of rye isolates (figure 1). For example, the barley isolates grow more rapidly than rye isolates on Czapek and carrot media. Our data confirm the conclusion of other researchers with respect to slow growth of *R. secalis* on all tested media.

Single-spores cultures of *R. secalis*, obtained from initial isolates growing more slowly (figure 2). They reach the same size as initial isolates after 30 days on PSA with 1% yeastrel. According to the rate of growth, after 30 days all single-spores cultures could be divided into 3 groups. After another 30-days we can see the difference of growth rate level and only two groups could be distinguished.

Table 1. Rate of *R.secalis* growth on different artificial media

№ medium	after 14 days of cultivation			after 21 days of cultivation	
	diameter (mm)		rate of growth (mm/day)	diameter (mm)	average growth per 7 days
	limits	average			
1	2,4 – 3,0	2,68 ± 0,37	0,19 ± 0,03	2,9– 3,5	0,07 ± 0,003
2	4,0 – 4,4	4,20 ± 0,03	0,29 ± 0,001	4,4 – 5,3	0,11 ± 0,005
3	10,0 –11,5	10,73 ± 0,11	0,77 ± 0,008	11,1– 12,5	0,15 ± 0,002
4	11,3 –11,5	11,38 ± 0,02	0,81 ± 0,01	12,5 –12,8	0,16 ± 0,01
5	11,8 –17,0	14,44 ± 0,43	1,03 ± 0,03	13,8 –18,0	0,17 ± 0,01
6	12,3 –17,6	14,72 ± 0,38	1,05 ± 0,03	12,5 –18,7	0,19 ± 0,04

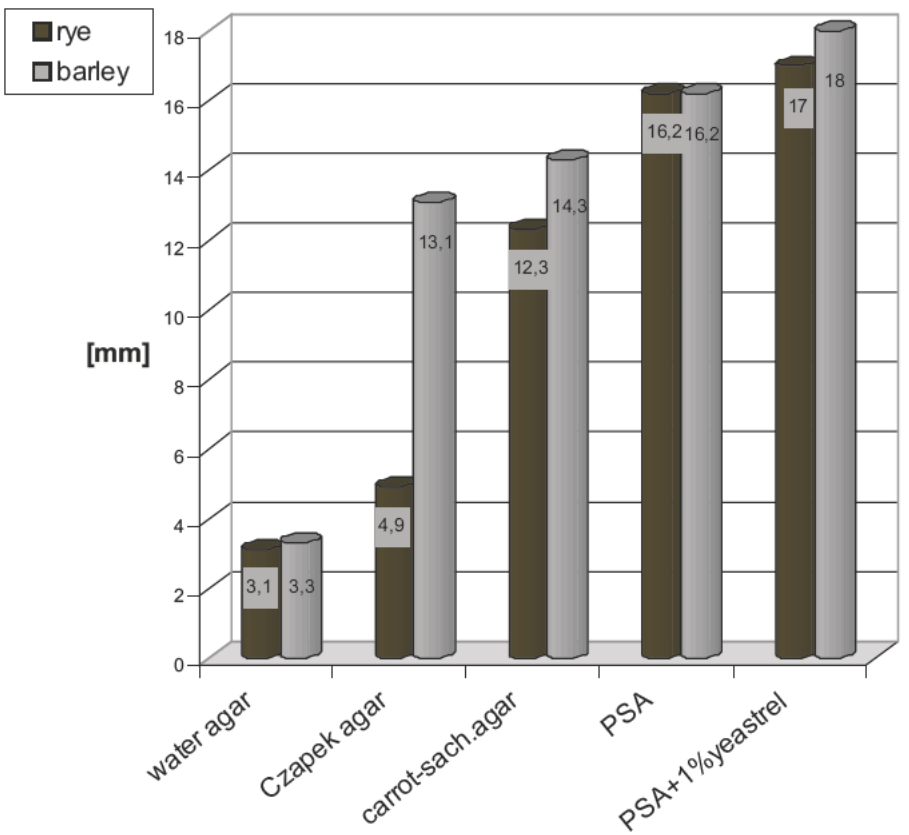

Fig. 1. The comparison of the influence of different media on growth of rye and barley isolates

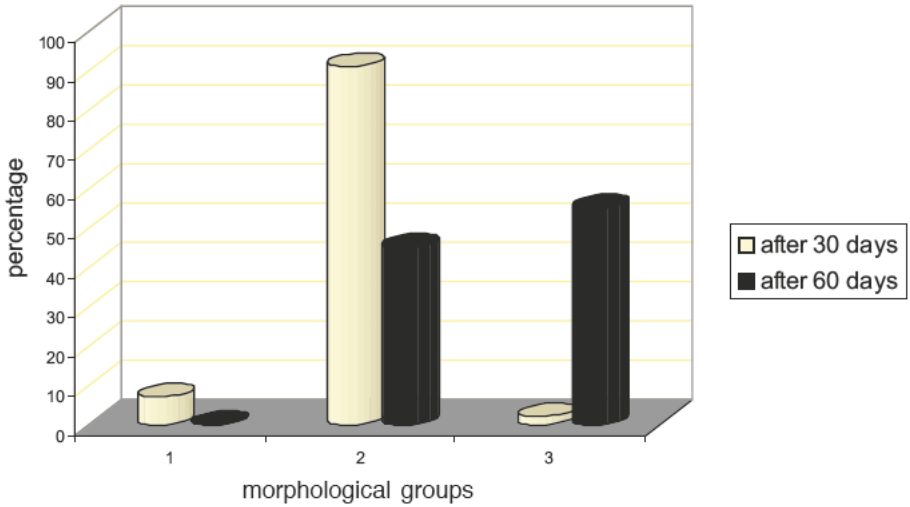

Fig. 2. Growth of single spore cultures of *R. secalis*

The temperature of the incubation is very important for successful growth of the fungus isolate on pure culture (table 2).

The influence of temperature on isolates on PSA-medium was shown. The most quick colony growth we observed at temperature 14°-18°C, average diameter of colonies after 14 days reached 14,3-14,7 mm, and the growth rate was 1,02-1,05 mm per day.

Table 2. The influence of temperature on growth isolates *R. secalis*

temperature ^{0}C	after 14 days of cultivation		after 21 days of cultivation	
	average diameter (mm)	average growth rate per day (mm)	average diameter (mm)	average growth rate 7 per days (mm)
10-12	12,8 ± 0,50	0,91 ± 0,04	13,6 ± 0,50	0,65 ± 0,02
12-14	13,4 ± 0,44	0,96 ± 0,03	14,2± 0,45	0,68 ± 0,02
14-16	14,7 ± 0,38	1,05 ± 0,03	15,8 ± 0,41	0,75 ± 0,02
16-18	14,3 ± 0,35	1,02 ± 0,03	15,2 ± 0,35	0,72 ± 0,02

Under optimum temperature, after 21 days of cultivation the size of colonies was not increased so fast. Our data shown that the optimum temperature for rye fungus isolates is 14-18°C, while for barley isolates it is 15-20°C.

Distinctions of natural rye isolates in morphology (color and the form of colonies) were revealed. All colonies were rounded with margin, had longitudinal or cross radial folds and yeast-like cone-shaped middle. Most of the isolates differed from each other in color, which varied up from light to black (figure 3). All isolates we divided into 7 groups. Numbers of pathogen isolates were approximately identical for each group. However, a few days later part of isolates with light color darkened and changed color into black, brown and black with a white bloom. The isolates became more dense and developed mycelium. Only 2% of all isolates kept beige color and yeast-like structure.

The barley and rye isolates differ according to morphological and cultural characters. Two mycelium types are peculiar to barley isolates (1) thin, weak branch and (2) thick, strong branch and three types of colony color black, pink and beige. It was shown that during the growth, the barley pathogen cultures change their color from pink to black (Coja, 1998; Konovalova, 1999).

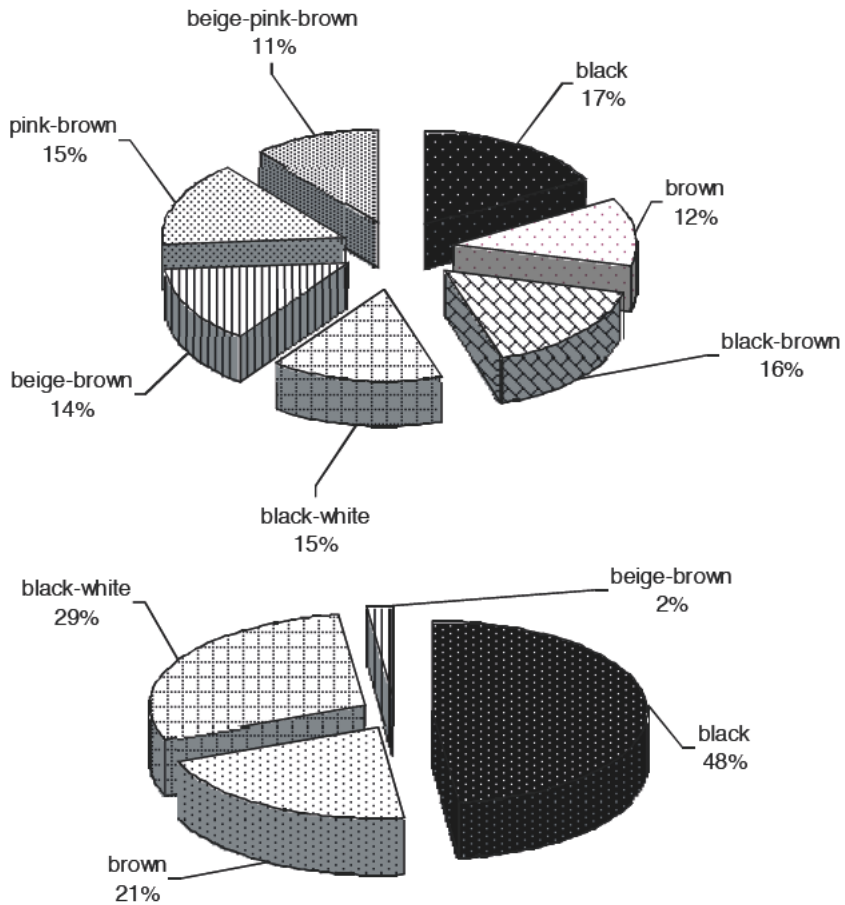

Fig. 3. Colour of natural rye isolates

A after 21 days of cultivation, B after 60 days of cultivation

The changes were explained from the one hand by appearance of dark pigment in some isolates and presence of chlamydospore (single or gathered in chains) and on the other hand by presence of microsclerotium, which increased as the isolate became older (Kajiwara 1968).

The seven morphotypes of rye isolates detected in our study rather differ from barley isolates, dark-colored isolates as usual have mycelia manner of growth but lightly colored isolates have yeast-like type of growth.

The majority of single-spores rye cultures isolated from each group kept structure and color typical for each group. Only small part of light cultures discolored and became dark. Therefore, the high stability allows supposing that the most of single-spore cultures are genetically homogenous.

Thus, the comparison of morphological and cultural properties of rye and barley *Rhynchosporium secalis* isolates show the significant resemblance of studied parameters.

Our data evidence both existence of specialized barley or rye pathogen forms and physiological influence of host-plants.

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Zróżnicowanie szczepów *Rhynchosporium secalis* (Oud.) J. J. Davis pod kątem morfologii i cech wzrostu kultur

Streszczenie

Badano biologiczne właściwości populacji grzyba *Rhynchosporium secalis*, sprawcy rynchosporiozy żyta, w rejonie północno-zachodnim. Bezpośrednio z porażonych roślin uzyskano 78 izolatów sprawcy rynchosporiozy. W przypadku wszystkich izolatów analizowano szybkość wzrostu na podłożach testowych, strukturę, kolor i zależność od temperatury. Jednozarodnikowe szczepy uzyskano z każdego z oryginalnych izolatów. Barwa i struktura części jednozarodnikowych izolatów pozostała bez zmian w trakcie kolejnych pasażowań na świeżą pożywkę PDA.