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PATHOGENIC ABILITY OF *BIPOLARIS SOROKINIANA* IN RELATION TO SPRING BARLEY (*HORDEUM VULGARE*)

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Abstract

The transmission efficiency of *Bipolaris sorokiniana* from grain to the first leaf tips and effect of plant infection time on germination and chemical components of harvested seeds was investigated. Infected grain derived from plants of five spring barley cultivars inoculated by spraying with fungal spore suspension at four development stages: tillering, stem elongation, heading and flowering. The fungus was detected in symptomless first leaf tips of seedling beginning from seven days after sowing and reaching the maximum infection incidence on the second week. Infection incidence subsequently decreased progressively to the end of the trial. Most infected grain were harvested from plants inoculated at the latest terms: heading and flowering. Grain infection by *B. sorokiniana* influenced germination capacity. Grain strongly infected by the pathogen (about 81–86%) germinated worse (75–77%) than less infected (93%). The content of protein, fiber and starch in kernels depended significantly on time of plant inoculation and grain infection by *B. sorokiniana*.

Key words: *Bipolaris sorokiniana*, chemical components, germination, plant, seed infection, transmission efficiency

Introduction

Bipolaris sorokiniana (teleomorph *Cochliobolus sativus*) is a widespread pathogen of many crop plants, especially barley, and it occurs throughout the world (Sivanesan 1987). The fungus is a causal agent of common root rot, leaf spot disease, seedling blight, head blight and black point of grain (Kumar et al. 2002). It is most important in warm climates but is also present and occasionally prevalent in temperate climates. In Western Europe it predominantly infects spring-sown cultivars of barley, however, without causing great injury (de Tempe 1964). Yield

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losses caused by the pathogen have been estimated in average ca 10% in barley and 5% in spring wheat on a long-term, region-wide basis, with losses in individual fields in some years above 30% (Stack 1991). The analyses performed by Łacicowa (1982) showed that the fungus can limit germination drastically, even to 25%, or the diseased grain gives rise to diseased, weak seedlings. The grain yield decrease can reach even 30%, as a result of stem base rot, leaf, ear and grain infection (Baturo-Czajkowska et al. 1998, Łacicowa and Pięta 1993). Some authors stated, that the occurrence of *B. sorokiniana* on grain of barley depended on farming and cultivation system (Baturo 2002, Baturo et al. 2002, Narkiewicz-Jodko et al. 2003). Baturo (2002, 2005) also claimed that organic farming without pesticides, herbicides and mineral fertilisers could be favourable to *B. sorokiniana* development. Due to these reasons seeds from organic farms infected more than in 60% by the pathogen can be a threat, especially when used for sowing.

Clark (1957) studied temperature relations of the host–pathogen complex. For seedling blight and root rot the optimum temperature is ca 20°C, for the development of spot blotch symptoms on leaves it is much higher.

The purpose of this work was to evaluate the potential role of seed-borne *B. sorokiniana*, as primary inoculum for plant infection and the influence of plant inoculation with the pathogen at different development stages on seed infection, germination and chemical components of harvested grain.

Materials and methods

Grain transmission efficiency

Grain transmission was investigated in greenhouse experiments in 2000–2002 using seed lots of five spring barley cultivars: 'Rataj' with *B. sorokiniana* infestation of 41%, 'Rodion' with 48%, 'Rastik' with 34%, 'Rasbet' with 42% and 'Scarlett' with 54%, based on the potato dextrose agar (PDA) medium assay. Each year 2000 grains were sown in multiplot trays containing soil mix (soil:sand:vermiculate 1:1:1), one grain per plot, and maintained in the greenhouse at ca 10/25°C (night/day). At seven-day-intervals, up to 49 days, first leaf tips of 100 symptomless seedlings were cut off separately and aseptically at 1.0 to 1.5 cm height above the soil. Only leaf tips without disease symptoms and spores on the surface were examined, to assure internal plant infection. They were placed without surface disinfection on the potato dextrose agar medium in Petri dishes (10 tips per dish) and incubated for about 14 days at 20–25°C under alternating periods of 12-h NUV light and 12-h darkness. The incidence of the fungus in the first leaves was observed with stereomicroscope. Transmission efficiency of *B. sorokiniana* from grain to the first leaves was estimated from the incidence of leaf tips colonized by *B. sorokiniana* and the incidence of grain infected by the pathogen using the following formula (Carmona et al. 1999):

$$T = \frac{C(\%) \times 100}{S(\%)}$$

where:

T – transmission efficiency,

C – incidence of the first leaf tips colonized by *B. sorokiniana,*

S – incidence of grain infected by the pathogen.

Grain infection assays

The investigations were conducted in 2001–2003 on microplots with barley plants. Grain samples of each of five spring barley cultivars ('Rataj', 'Rodion', 'Rastik', 'Rasbet', 'Scarlett') were sown in the field. A pure culture of *B. sorokiniana* isolate from barley grain was multiplied and spores were suspended in water (Phot. 1). The plants were uniformly sprayed with spore suspension adjusted to 4×10^5 spores per 1 ml, and inoculated at four stages: tillering, stem elongation, heading and flowering; for control uninoculated plants were used. Grain harvested from inoculated and control plants were examined for incidence of *B. sorokiniana*. The examination was carried out on 100 grains of each cultivar placed on PDA medium and incubated for about 14 days at 20°C under alternating periods of 12-h NUV light and 12-h period of darkness. Results were presented as percentage of grain infection. Additionally, thousand kernel weight, germination capacity according to ISTA (International… 2005) and chemical components of grain were estimated. For determination of chemical compounds the Infratec 1255 Food and Feed Analyzer was used.

Phot. 1. Spores of *Bipolaris sorokiniana*

Results

The pathogen was detected in symptomless seedling the first leaf tips beginning at the seventh day after sowing: mean 3.2% (0.7–5.0%, depending on cultivar), and reaching the maximum infection incidence in the second week: mean 3.6% (from 2.7 on 'Rodion' and 'Rastik' to 6.3% on 'Rasbet'). Infection incidence subsequently decreased to the end of the trial (Table 1). Mean transmission efficiency of *B. sorokiniana* from grain to the first leaf tips of symptomless seedlings was about 8% in the first and in the second week of investigation (Fig. 1). It subse-

Table 1

Infected by *Bipolaris sorokiniana* the first leaf tips at intervals of 7 up to 49 days after sowing (mean from three years of investigations) (%)

Cultivar	Week						
	1st	2nd	3rd	4th	5th	6th	7th
'Rataj'	0.7	3.3	2.3	2.0	0.7	1.0	0.0
'Rodion'	2.3	2.7	3.7	0.7	0.7	0.3	0.3
'Rastik'	5.0	2.7	1.0	0.7	1.0	0.0	0.0
'Rasbet'	4.0	6.3	1.0	1.0	1.7	1.3	1.0
'Scarlett'	4.0	3.0	2.0	0.0	0.0	0.3	1.3
Mean	3.2	3.6	2.0	0.9	0.8	0.6	0.5

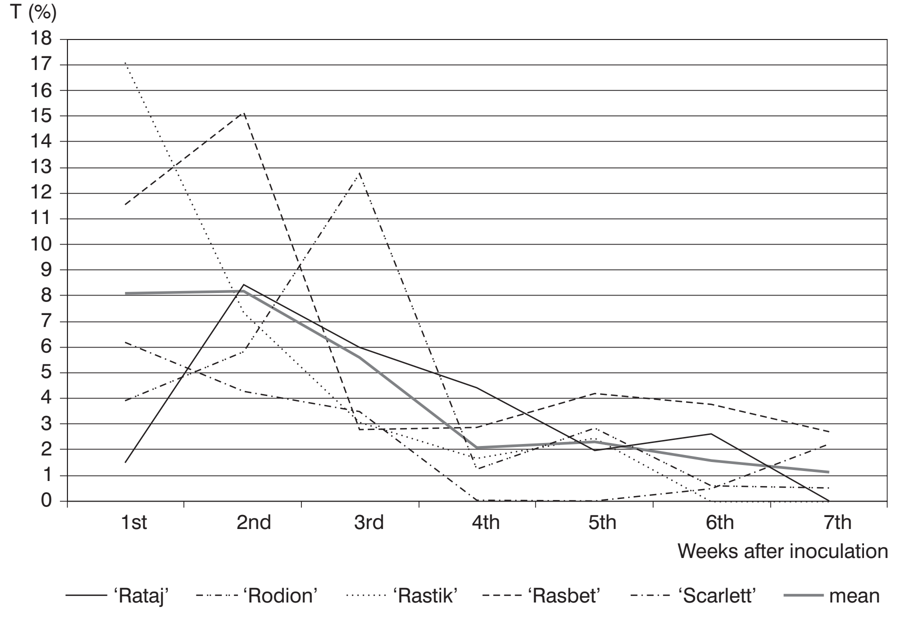

Fig. 1. Transmission efficiency (T) of *Bipolaris sorokiniana* from grain to the first leaf tips (means from three years of investigations)

Phot. 2. Leaf blight symptoms on barley leaf

quently decreased after four to seven weeks and was maintained at a mean level of 2–3%. Abnormal seedlings with root and plumule rot symptoms were observed during the first week of examination and afterwards a few of these plants died. After about 35 days some leaves showed leaf blight symptoms (Phot. 2).

In the harvested grain from uninoculated and inoculated plants the incidence of *B. sorokiniana* ranged from 27.7% in control to 81.2 and 86.1% in grain harvested from plants inoculated during heading and flowering period. Grain infection effected germination capacity. Grain strongly infected with the pathogen germinated worse than those less infected. Germination capacity decreased from 92.9% in control grain to 75.1–76.7% in grain harvested from plants inoculated during heading and flowering. The decrease of germination capacity was related to the increase of abnormal seedlings number (mean 3.7–17.3%). All grain harvested from infected plants had smaller thousand kernel weight (TKW) than grain from control plants (average 32.7–37.9 g and 40.6 g, respectively; Fig. 2). Plant infection in heading stage reduced TKW mean by ca 7.9 g in comparison with control plants.

The content of protein, fiber and starch in kernels depended significantly on time of plant inoculation and grain infection by *B. sorokiniana*. The highest amount of protein – mean 12.7% and fiber – 5.2% and the smallest content of starch – 50.3%, were characteristic of grain harvested from plants inoculated with *B. sorokiniana* at heading (Table 2).

Grain infection with *B. sorokiniana* was highly significantly correlated with all grain characters investigated (Table 3).

Data presented here indicate that *B. sorokiniana* infected grain may be an additional source of inoculum in soil and cause disease in the field. In the performed

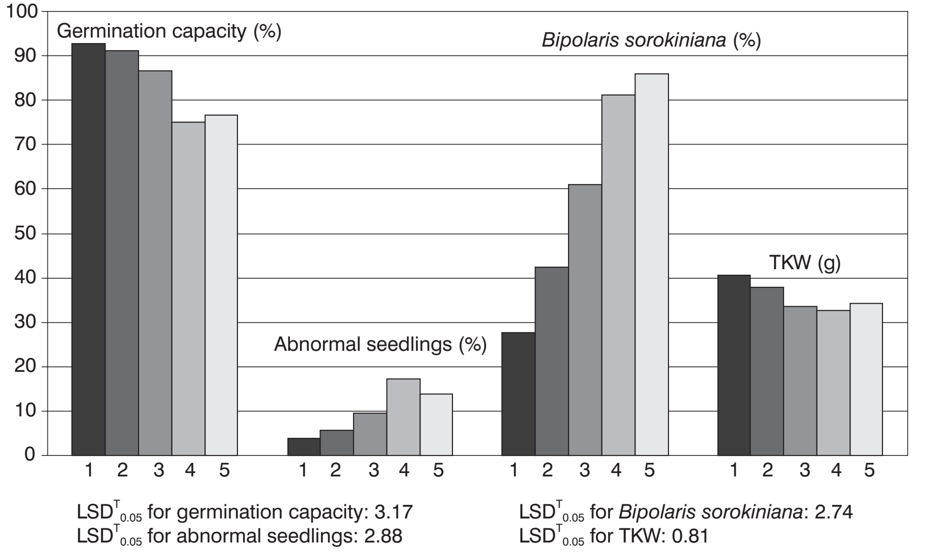

Fig. 2. Influence of plant inoculation time with *Bipolaris sorokiniana* on selected characters of harvested grain (means for five barley cultivars); 1 – uninoculated, 2 – tillering, 3 – stem elongation, 4 – heading, 5 – flowering

Table 2

Influence of plant inoculation time with *Bipolaris sorokiniana* on chemical components of harvested grain

Chemical component	Uninoculated plants	Plants inoculated at				$LSD^{T}_{0.05}$
		tillering	stem elongation	heading	flowering	
Protein	12.1	11.9	11.3	12.7	12.2	0.092
Fiber	4.8	4.9	5.1	5.2	5.1	0.093
Starch	51.4	51.4	51.2	50.3	50.6	0.279

Table 3

Correlation coefficient between grain infection with *Bipolaris sorokiniana* and selected seed characters

Seed character	Correlation coefficient
Germination capacity	–0.57**
Abnormal seedlings	0.52**
Thousand kernel weight	–0.49**
Protein	0.49**
Fiber	0.65**
Starch	–0.58**

**Significant for $\alpha = 0.01$.

experiments the level of grain infection depended on development stage of plant, in which the infection took place: the later plant infection the bigger *B. sorokiniana* incidence in grain.

Discussion

Bipolaris sorokiniana was present in symptomless seedling the first leaf tips beginning from the seventh day after sowing and the infestation incidence subsequently decreased to the end of the trial. Similar results were obtained by Carmona et al. (1999) for *Drechslera teres*. These authors observed that colonization and sporulation of the fungus at first increased, but later decreased probably because of a short life span of coleoptiles and the decline of available nutrients in coleoptile tissues.

High temperatures in May and wet and cool conditions during late May to early July favoured high incidence of *B. sorokiniana* in barley grain the following harvest (Jorgensen 1986). Grain infected by the fungus is one of the main sources of plant infection. The infection is caused both by spores contaminating grain surface and by mycelium in glumes and pericarp (Łacicowa and Pięta 1991). Glume infection was reported in *Cochliobolus sativus* within the parenchyma and sclerenchyma cells of lemma and palea of barley (Stevenson 1981).

The present study showed that grain infection with *B. sorokiniana* effected germination capacity. The decrease of germination capacity was related to the increase of number of abnormal seedlings. Similar relationship between grain infection and germination was reported by Kurppa (1984) who found that barley grain severely infected with *B. sorokiniana* did not germinate or if germinated, seedlings became infected. According to Łacicowa (1982) the presence of seed-borne *B. sorokiniana* often results in abnormality of seedlings.

Bailey et al. (1997) studied the effect of *B. sorokiniana* and other seed-borne cereal pathogens on emergence and yield of the host and found that the effect of seed-borne *B. sorokiniana* on emergence was usually compensated during further development of plants, so that there was no reduction in yield. It could have depended on local conditions and also the levelling effect of a soil-borne inoculum. Spot blotch severity increases with the advancement of growth stages (Chaurasia et al. 2000) and late cultivars are reported to display less disease (Shrestha et al. 1998). On the other hand, in Canada losses due to common root rot of wheat caused by *B. sorokiniana* and *Fusarium* between 1969 and 1971 were 5.7% which is equivalent to $ 42 million (Piening et al. 1976). In Holland it was observed that 3 to 5% seed-borne infections resulted in 1% reduction of both emergence and yield (de Tempe 1958). Grain losses due to spot blotch in South Asia and India were estimated to be 19.6% and 15.5%, respectively (Dubin and van Ginkel 1991). Yield losses of 20–80% were reported by Duveiller and Gilchrist (1994), and may reach 100% under most severe conditions of infection (Srivastava et al. 1971).

The results presented here directly indicate that grain protein, fiber and starch content depended on time of plant inoculation and was related to grain infection by

B. sorokiniana. Berova and Mladenov (1974) claim that the occurrence of head blight on wheat caused by *Fusarium* decreases grain quality and causes protein and gluten reduction, which are essential values for bread-making. The study of Arabi et al. (2001) showed relationship between the occurrence of fungi and chemical compounds of grain: grain infection with *Pyrenophora graminea* dramatically affected the hordein profiles.

No single measure is effective in controlling the disease (Hetzler et al. 1991) and effective control of diseases caused by *B. sorokiniana* can be achieved by introduction of resistant cultivars as a major component of integrated disease management (Łacicowa and Pięta 1991, Mehta 1998).

Streszczenie

PATOGENICZNOŚĆ *BIPOLARIS SOROKINIANA* W STOSUNKU DO JĘCZMIENIA JAREGO (*HORDEUM VULGARE*)

Badano rozprzestrzenianie się grzyba *Bipolaris sorokiniana* z ziarniaków do pierwszych liści jęczmienia oraz wpływ zakażenia roślin na zdolność kiełkowania i skład chemiczny ziarniaków. Porażone ziarniaki zebrano z roślin pięciu odmian jęczmienia jarego inokulowanych w czterech fazach rozwojowych: krzewienia, strzelania w źdźbło, kłoszenia i kwitnienia.

Patogen był izolowany z pierwszych liści bez objawów porażenia, poczynając od siódmego dnia po wysiewie jęczmienia w doświadczeniu szklarniowym. Najwyższy stopień zakażenia liści wystąpił po pierwszym i drugim tygodniu od wysiewu ziarna, a następnie obniżał się aż do końca prowadzenia doświadczenia. Inokulacja roślin grzybem *B. sorokiniana* istotnie wpłynęła na badane cechy ziarna. Analiza zdrowotności wykazała, że im później inokulowano rośliny, tym większe było porażenie ziarna: największe – na ziarnie zebranym z roślin inokulowanych w fazie kłoszenia i kwitnienia. Wraz ze wzrostem porażenia ziarna przez grzyb *B. sorokiniana* do około 80% zdolność kiełkowania zmniejszyła się do 75–77%, co było związane ze wzrostem liczby nasion nienormalnie kiełkujących. Analiza składu chemicznego ziarniaków wykazała istotne różnice w zawartości białka, włókna i skrobi w zależności od fazy rozwojowej roślin, w której nastąpiła ich infekcja przez *B. sorokiniana*.

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