The pathogenicity and importance of seed-borne infection by *Bipolaris sorokiniana* on barley in Finland

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**Abstract.** Seed-borne infection by *Bipolaris sorokiniana* decreased the percentage germination of barley seeds and the emergence of seedlings. Infection levels were higher in non-germinated than in germinated seeds. Seed treatment with organomercurial fungicide or imazalil improved the percentage emergence but a low number of diseased seedlings still remained in the crops. The fungus caused a reduction in grain yields in most experiments and also decreased their value as sowing seed, if the weather conditions were favourable for complete disease expression. Yield losses in greenhouse experiments varied from 7.2 to 38.5 % and in the field from 5 to 11 %, and showed a strong correlation with the infection levels in the seed stocks. Higher losses were associated with the six-row cultivars. Organomercury seed treatment resulted in a slight but insignificant increase in yields but it was able to prevent an occurrence of secondary infection in the crop resulting in a lower seed infection levels of the grain. In field experiments in Inari (69° N.L.) seed-borne inoculum could be demonstrated clearly to be the only source of a severe disease outbreak. The inoculum remaining in the soil was capable of initiating soil-borne infection of barley seedlings during the following two growing seasons.

**Introduction**

*Bipolaris sorokiniana* (Sacc. ex Sorok.) Shoem. (syn. *Helminthosporium sativum* Pamm., King & Bakke), perfect state *Cochliobolus sativus* (Ito & Kurib.) Dastur has been recently reported to be increasingly common in commercial barley seed stocks in North Western Europe (de TEMPE 1964, JORGENSEN 1974, HEWETT 1975, OLOFSSON 1976, KURPPA 1984). Estimates concerning its economic importance have been variable but the latest information assumes losses in yield of up to 15 % due to a high level of seed infection (WHITTLE & RICHARDSON 1978). Diseased plants from infected seeds have also been found serving as important sources for spore liberation during later developmental stages of the crop, and their residues remain

Index words: *Bipolaris sorokiniana, Helminthosporium sativum, Cochliobolus sativus*, common root rot, kernel blight
objects for further sporulation (Chinn 1977, Reis & Wünsche 1984).

The persistence of B. sorokiniana in barley seed for 10 years or more precludes ageing as practical method for obtaining seeds free from the pathogen (Machacek & Wallace 1952, Couture & Sutton 1980). Seed treatment with organomercurial or systemic fungicides (Hewett 1975, Chinn 1978, Whittle & Richardson 1978) has resulted in satisfactory control of the disease.

Materials and Methods

Determination of percentage germination in seed stocks including examination for fungal growth was carried out in petri dishes as described by Kurppa (1984). Percentage emergence was determined by the official method used at State Seed Testing Institute, Helsinki. Field experiments at Viikki, Helsinki and Muddusniemi Research Station, Inari, and pot experiments at Viikki were designed to study disease development, varietal differences and the effects of seed-borne disease and seed treatment on grain yield and infection levels. Seed stocks used for sowing in the experiments originated from a field experiment at Viikki (Kurppa 1985 b.), or were selected from commercial stocks examined at State Seed Testing Institute. This material included stocks with various levels (19—92 %) of seed and embryo infection, and was classified according to the latter parameter. Class a indicates low, b moderate and c high infection levels.

In greenhouse experiments barley was grown in plastic 25 × 25 cm pots filled with non-sterilized loamy field soil, which was previously determined to be free of the pathogen. In field experiments a plot size of 5 × 1.33 m was used. Experimental soils were fertilized with a commercial N-P-K (15-20-15) fertilizer, 500 kg/ha in field experiments or 3 g/pot in greenhouse experiments. For seed treatment an organomercuric fungicide (Ceresan 2 g/kg seeds), benomyl (Benlate 1 g/kg and imazalil (experimental sample 0.3 g a.i./kg) were used. In the field barley was sown in a density of 400 (six-row cvs) or 450 (two-row cvs) seedlings/m² and in greenhouse experiments 50 seeds were sown/pot. The pots were watered as required with an equal volume per pot of tap water.

The seedling population was counted at the 3—4 leaf stage and samples (25 seedlings/plot) were collected in the field. The second sampling occurred close to the yellow ripening stage. Basal stems of the plants showing disease symptoms were surface-sterilized with Na-hypochloride and plated on potato dextrose agar (PDA). After 7 days of incubation at room temperature the samples were examined for the presence of B. sorokiniana. Pots and field plots were harvested when ripe, grain yields were drained immediately, weighed, and samples were taken for grain analysis.

Analysis of variance and linear regression equations with correlation coefficients was used to test the significance of the results.

The weather conditions at Viikki during research period were as follows: The growing seasons of 1973 and 1975 were warm and dry, the season of 1979 was near average and the rest were cooler than average with high rainfall (Anon 1973—1979).

Results

B. sorokiniana significantly decreased percentage germination in all stocks of seed infected at various levels (Table 1). Non-germinated seeds carried higher levels of infection in all seed classes of all barley cultivars tested. Reduction in percentage germination was particularly related to embryo infection by the fungus (Fig. 1). The fungus had a stronger effect on field emergence than on laboratory germination of the seeds (Table 2). A high number of young infected seedlings failed to emerge and those capable doing so showed seedling blight symptoms (Fig. 5). The mean percentages of reduction in emergence of seedlings in 14 pot experiments and 18 field experiments were record-
### Table 1. Germination of barley seed stocks infected at various levels by Bipolaris sorokiniana and infection levels in germinated and non-germinated seeds.

| Seed class | Cultivar | Ingrid | MI | G | NG |
|------------|----------|--------|----|----|----|
| a)         | GER      | 90.8   | 9.3 | 8.0 | 21.2 |
|            | MI       | 89.5   | 9.0 | 10.0 | 32.2 |
|            | G        | 82.8   | 5.7 | 5.7 | 57.7 |
|            | NG       | 82.8   | 5.7 | 5.7 | 57.7 |
|            | Mean     | 88.9   | 4.3 | 4.3 | 42.6 |

| Seed class | Cultivar | Pomo   | MI | G | NG |
|------------|----------|--------|----|----|----|
| a)         | GER      | 90.5   | 9.5 | 9.5 | 23.5 |
|            | MI       | 89.3   | 8.7 | 10.7 | 27.0 |
|            | G        | 88.7   | 7.4 | 7.4 | 23.5 |
|            | NG       | 88.7   | 7.4 | 7.4 | 23.5 |
|            | Mean     | 89.3   | 9.3 | 9.3 | 25.6 |

| Seed class | Cultivar | Otra   | MI | G | NG |
|------------|----------|--------|----|----|----|
| a)         | GER      | 96.0   | 9.0 | 10.0 | 32.2 |
|            | MI       | 95.8   | 9.5 | 9.5 | 32.2 |
|            | G        | 95.8   | 9.5 | 9.5 | 32.2 |
|            | NG       | 95.8   | 9.5 | 9.5 | 32.2 |
|            | Mean     | 95.8   | 9.5 | 9.5 | 32.2 |

| Seed class | Cultivar | Porno | MI | G | NG |
|------------|----------|-------|----|----|----|
| a)         | GER      | 89.3   | 9.3 | 9.3 | 23.5 |
|            | MI       | 89.3   | 9.3 | 9.3 | 23.5 |
|            | G        | 88.7   | 7.4 | 7.4 | 23.5 |
|            | NG       | 88.7   | 7.4 | 7.4 | 23.5 |
|            | Mean     | 88.7   | 7.4 | 7.4 | 23.5 |

*GER = Per cent germination of seed stocks

**MI** = Mean infection level in germinated and non-germinated seeds

**NG** = Infection level in non-germinated stocks

\* For seed classes see text

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In pot experiments, an improvement in emergence of 7 per cent was recorded with organomercury seed treatment, while benomyl and imazalil caused improvements of 3 and 5 per cent respectively. In the field improvements of 16 and 5 per cent were reached with organomercury and imazalil treatments but benomyl had no positive effect (Table 2). Seedlings from seeds with embryo infection frequently showed disease symptoms in spite of the treatments. In greenhouse experiments, infection levels in sowing seed were 60% (cv. Vigdis) and 92% (cv. Paavo). 0 indicates untreated seed, B and M were treated with benomyl and organomercury respectively. F-values: Seed infection = 86.14, LSD<sub>0.05</sub> = 10.7 %, Seed treatment < 1.
Table 2. Emergence of barley seed infected with *Bipolaris sorokiniana*, with and without seed treatment.

| Treatment                                      | Greenhouse experiments | Field experiments |
|------------------------------------------------|------------------------|-------------------|
| Healthy untreated seed                         | 94.5* (7)              | 55.3* (4)         |
| Untreated infected seed¹                       | 80.5 (7)               | 45.1 (4)          |
| Organomercury treated infected seed            | 86.4 (7)               | 52.2 (4)          |
| Benomyl treated                                | 82.7 (4)               | 44.9 (2)          |
| Imazalil treated                               | 84.7 (2)               | 47.3 (2)          |

* Per cent emergence
¹ Number of experiments
² Number of seedlings/drill m
³ Infection levels from 19 to 92 %

In all individual experiments analyzed statistically, organomercury seed treatment of infected seed significantly increased germination and emergence at P = 0.05 level.

Table 3. The effects of seed infection levels of *Bipolaris sorokiniana* and of seed treatment on grain yields of four barley cultivars in greenhouse experiments.

| Seed class | Ingrid | Karri | Otra | Pomo | Means |
|------------|--------|-------|------|------|-------|
| h²         | 100.0* | 103.7 | 100.0| 104.0| 100.0 |
| b          | 102.2  | 94.7  | 84.2 | 83.7 | 79.2  |
| c          | 94.5   | 91.2  | 93.2 | 90.5 | 85.7  |
| Mean       | 98.9   | 96.6  | 92.5 | 92.8 | 92.1  |

¹ 0 = Untreated seed
² 1 = Organomercury treated seed
³ h = Healthy seed
⁴ b = Embryo infection levels from 4 to 10 %
⁵ c = " → " 22 to 36 %

F-values: Infection levels = 71.3, LSD₀.05 = 2.8 %
Cultivars = 10.8, = 9.0 %
Seed treatment < 1

Fig. 3. The effects of seed infection level and seed treatment with organomercury powder on grain yields of barley cultivar Paavo in greenhouse experiments. F-values: Seed infection level = 50.9, LSD₀.05 = 0.44 g, Seed treatment < 1.

Fig. 4. The effect of plant density on grain yields of barley cvs. Birgitta and Otra. For sowing, pathogen-free seed was used. F-value = 1.8, not significant.
of previous seed treatment and their further development typically resulted in root rot with low grain yield (Fig. 6).

The diseases on barley originating from seed-borne infection by *B. sorokiniana* resulted in significant losses in grain yield in most experiments and also in a decrease in its value as sowing seed, if the weather conditions were favourable for complete disease expression. A mean yield reduction of 38.5% was reached in one series of pot experiments (Fig. 2) but in another (Table 3) it was lower with the following means for the cultivars: Ingrid 7.2, Karri 8.1, Otra 18.8 and Pomo 27.6%.

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**Table 4.** The effects of seed infection and seed treatment on yield and infection incidence in grain on barley cv. Vigdis in a field experiment.

| Seed treatment          | Grain yield kg/plot | % incidence of *B. sorokiniana* |
|-------------------------|---------------------|---------------------------------|
|                         | Sowing seed         | Sowing seed                     | Sowing seed | Sowing seed |
|                         | Healthy             | Infected*                       | Mean        | Healthy     | Infected   | Mean     |
| None                    | 3.415               | 3.090                           | 3.252       | 3.3         | 17.5       | 10.4     |
| Benomyl                 | 3.225               | 3.082                           | 3.153       | 8.3         | 22.5       | 15.4     |
| Organomercury           | 3.300               | 3.507                           | 3.403       | 4.0         | 7.5        | 5.7      |
| Mean                    | 3.313               | 3.226                           | 5.2         | 15.8        |            |          |

* Seed infection level = 60.0 %

F-values: Sowing seed/yield = 4.7, LSD$_{0.05}$ = 0.316 kg

*infection incidence in grain = 34.2, = 5.8 %

Seed treatment/ = 7.4, = 7.7 %

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**Table 5.** Pathogenicity of seed-borne infection of *Bipolaris sorokiniana* on five barley cultivars in field experiments and the effect of seed treatment to control the disease.

| Seed treatment          | Cultivar         |            |            |          |          |          |          |          |          |
|-------------------------|------------------|------------|------------|----------|----------|----------|----------|----------|----------|
|                         | Otra             | H I        | Vigdis     | H I      | Karri    | H I      | Pomo     | H I      | Ingrid   |
| H1                      | 100.0*           | 93.5       | 100.0      | 89.0     | 100.0    | 95.0     | 100.0    | 95.0     | 100.0    |
| Organomercury           | 96.2             | 93.2       | 98.4       | 93.4     | 102.5    | 97.9     | 101.8    | 95.1     | 101.5    |
| Mean                    | 98.1             | 93.4       | 99.2       | 91.2     | 101.3    | 96.5     | 100.9    | 95.0     | 100.7    |
| Imazalil                | 94.9             | 92.3       | 95.9       | 90.6     | 95.1     | 94.0     |          |          |          |
| Mean                    | 97.0             | 93.0       | 98.1       | 91.0     | 99.2     | 95.6     |          |          |          |

1 H = Healthy seed; I = Seed infected at levels from 60 to 84 %

* Relative mean grain yield of three experiments; controls for each cultivar = 100.0

F-values: Seed infection/grain yield (all cultivars) = 31.4, LSD$_{0.05}$ = 4.9 %

Organomercury seed treatment of infected seed (all cultivars) = 1.3

Organomercury or Imazalil treatment (cvs Karri, Otra and Vigdis) < 1

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**Table 6.** The effects of seed infection and seed treatment on estimated grain yield and its infection incidence in barley cv. Vigdis in a field experiment in Inari (69° N.L.).

| Seed                     | Grain yield | Incidence of *B. sorokiniana* in grains |
|--------------------------|-------------|----------------------------------------|
| Healthy untreated        | 227.8*      | 25.5                                   |
| Infected — » — 2         | 224.2       | 32.7                                   |
| — » — benomyl treated    | 223.6       | 36.0                                   |
| — » — organomercury treated | 223.0   | 32.3                                   |

1 II = Infection incidence in the grains (%)

* EI = Embryo infection — —

* Grain yield g/200 heads

2 Infection level in the seed = 60 %
An experiment with seed stocks of cv. Paavo containing various infection levels presented a significant correlation between infection level and grain yield (Fig. 3). Organomercury or benomyl seed treatment had a minor effect on yield improvement in pot experiments (Table 3, Figs 2 and 3), although a delay in disease expression was observed. A variation in population from 25 to 50 seedlings per pot in the healthy crop had no significant effect on yield (Fig. 4).

Seed-borne infection by the fungus resulted in a relatively low but in most cases significant reduction in yields in field experiments. A 60% level of seed infection caused a mean reduction in yield of 9% on barley cv. Vigdis without seed treatment (Table 4). Organomercury seed treatment, however, significantly improved the yield but benomyl failed to do so. Infection levels in grain yields from the plots sown with infected untreated or benomyl treated seed were significantly higher than from the rest of the plots.

Field experiments with five barley cultivars over three years resulted in a mean reduction of 6.3% (ranging from 5—11%) in the yields from the plots sown with moderately or highly infected seed without seed treatment (Table 5). Organomercury seed treatment increased the yield somewhat but the treatment remained insignificant. Secondary infection frequency in the crops during the experimental years (1975—1977) remained low and no outstanding foliar disease or grain infection occurred.

The effect of seed-borne infection on the foliar disease outbreak could be clearly demonstrated with field experiments in Inari, Lapland, where the fungus was naturally absent. The fungus, which initiated from primarily diseased seedlings, sporulated intensively and caused an epidemic in the experimental crops, resulting also in high incidences in infection in the grain (Table 6). A high level of fungal inoculum remained in the soils with the crop residues and was ca-
pable of infecting barley seedlings in the following two growing seasons.

Discussion

The seed-borne inoculum of Bipolaris sorokiniana is unquestionably of great importance in initiating root rot and foliar diseases on barley grown in cool and temperate regions with relatively high rain fall during growing seasons. This significance is supported by the lifecycle of the fungus as well as by the high incidences of seed infection on barley reported by de Tempe (1964), Jørgensen (1974), Whittle (1977) and Kurppa (1984).

There is a lack of agreement on the effect of the fungus on seed germination. Reports published by de Tempe (1964) and Hewett (1975) deny its importance but a number of studies including the present support it. A study by Clark and Wallen (1969) reports a reduction in germination of as high as 38%. Comparable data on the reduction in emergence has been reported by Olofsson (1976). In the present study, yield losses in field experiments due to the fungus were similar to those reported by Whittle and Richardson (1978), but the increase in the yield as a result of seed treatment remained lower. A high incidence of fungus located deeply embedded in the grain embryos probably decreased the effectiveness of control obtained with seed treatment. Due to this factor a sufficient number of diseased plants existed in the crop able to initiate secondary infection of the fungus when weather conditions were favourable.

Among the fungicides used for seed treatment the organomercurial compound was fairly effective eradicating the fungus from superficially infected seeds but was not capable of controlling disease initiating from internally infected seeds. Severe outbreaks of the disease in barley originating from organomercury treated seed as reported by Whittle (1977) could probably be explained by this phenomenon. The yields from the plots sown with imazalil treated seeds remained lower than those from organomercury treated seeds although this fungicide was highly effective on the fungus as also reported by Chinn (1978). Possibly the dose of 0.3 g a.i./kg seeds was too high, causing toxic effects on young barley seedlings. A number of systemic fungicides effective against B. sorokiniana have been shown to be phytotoxic to barley by Couture and Sutton (1978). The ineffectiveness of benomyl treatment against the fungus agrees with the observations of Richardson (1972).

The differences in yield losses between cultivars remained less significant than expected, regarding the earlier information by Kurppa (1985 a., b.) on varietal susceptibility to soil-borne and secondary infection. The differences were, however, comparable to those in the previous studies. The lack of outstanding varietal differences is probably due to the high levels of infection in the seed stocks of all cultivars tested. However, a few specific cultivars have always been associated with a high seed infection level.

Seed-borne infection of B. sorokiniana in barley was clearly demonstrated to be an important or even the only way for the fungus to initiate the disease in remote areas. The present study reports for the first time the ability of B. sorokiniana to overwinter in soil and then to induce disease in barley north of the Arctic Circle.

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**SELOSTUS**

*Bipolaris sorokiniana*-sienen merkitys ohran siemenlevintäisenä taudinaiheuttajana

Suomessa

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Ohran tyvi- ja lehtilaikkua aiheuttavan *Bipolaris sorokiniana*-sienen (syn. *Helminthosporium sativum*, keteloaaste *Cochliobolus sativus*) merkitystä ohran siemenlevintäisenä taudinaiheuttajana sekä sienen torjuntaa selvittävissä tutkimuksissa.

Koesiemenenä käytettiin kenttäkokeella tuotettuja eri asteisesti (19 — 92 %) infektoituneita, mutta muita ominaisuuksiltaan vastaavia siemeniä ja Valtion Siemen-tarkastuslaitoksella tutkimuksiin valittuja siemeniä. Eriin sientartunta tutkittiin mikroskooppisesti 7 vrk petraljoissa idätytystä siemenistä. Orastuvuus määritettiin virallisen menetelmän mukaisesti. Peittauaineiksi sieniä torjutaan valitettiin Ceresan-kuivapeittausjauhe (organoelohopea), Benlate (benomyli) ja Kemira Oy:n koe-erä imazalil-valmistetta. Tutkimukseen sisältyneet astiakokeet tehtiin Viikissä ja kenttäkokeet Viikissä sekä Inarissa Muddusniemen tutkimusasemalla.

Orastuvuus astia- ja kenttäkokeissa laskettiin ohran 3—4-lehtitekstile. Kenttäkokeista otettiin laskennan yhteydessä myös orasnäytteet (25 orasta/tuutu). Toiset näytteet koeruuudasta otettiin hieman ennen maatutulentumisastetta. Näytteiden avulla pyrittiin varmistamaan B. sorokiniana-sienen osuu oireiden esiintymiseniin sekä seuraamaan tuudin kehittymistä. Kasvustot korjattiin heti niiden tuleennuttua. Jyväsadot kuivattiin välittömästi, punnittiin ja niistä tehtiin sientäsyntyväiset.

Kylvösiemenen sientartunta alensi ohran itävyyttä ja orastuvuutta; peittaamattomien koejäsenten orastuvuus alensi astiakokeissa keskimäärin 14 % ja kenttäkokeissa 18 %. Itävyyden ja vielä selvemmin orastuvuuden alennin riippuivat enemmän jyvien sisäosien sientartunnasta kuin jyvien määritetystä sientartunnasta. Sieni esiintyi yleisemmin itämyöntäisissä kuin itäisissä jyvissä. Kylvösiemenen peittaaminen organelohopeavalmis-teella puhdisti pelkästään pinnallisen sientartunnan saaneet siemenet, mutta ei täysin torjunut sientä sisä-osiinä infektoituneista jyvistä, joiden itäessä kehittyivät sairaita oraita. Systeeminen imazalil antoi paremman torjuntarykksen kuin elohopea, mutta orastuvuus jäi silti alhaisemmaksi.

Sieni aiheutti useimmilla kokeissa merkitseviä sato-tappioita sekä alensi kenttäkokeissa lisäksi sadon arvoa kylvösiemenenä, mikäli sääolot olivat suotuisat sienen sekundääriquevinnalle. Satotappiot astiakokeissa olivat 7.2—38.5 % ja kenttäkokeissa 5—11 %, keskmäärin 6.3 %. Lajike-erot jäivät vähäisiä, todennäköisenä syyyn kaikkien lajikkeiden kylvösiemenenä yhtäläinen, voimakas sientartunta. Peitteenä lisäsi satoja jonkin verran, muttei tilastollisesti merkitsevästi. Peittausaineista organoelohopea kohotti satoa enemmän kuin imazalil, vaikkei pystynytään torjumaan tai autua täysin. Benomyliä todettiin peittaustaineneen täysin tehottomaksi sienin torjunnassa.

Kylvösiemenen sientartunta johti voimakkaan lehtilaikkutaudin puhkeamiseen kasvustossa táhkkäletalon jälkeen kenttäkokeissa Muddusniemen tutkimusasemalla Inarissa, missä sientä ei luontaista esiinny. Epidemia johti myös jyväson sientartuntaan. Sieni säilyi maas-satojätteissä kahden talvikauden yli ja kykeni infektoimaan maalevintäisesti ohran oraita.

Siemenlevintä on maasamme todennäköisesti *B. sorokiniana*-sienen tärkein säilymis- ja levimismuoto. Sienen vahingollisuus ja levimisvoimakkuus riippuu kuitenkin paljolti kasvukauden säästä. Kuivana kasvu- ja jäätemäärä on laittenlevynä voimakkaasti tyytäviin, kosteana lisäksi lehtilaikkutautiin ja jyväson sientartuntaan.

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