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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 complite 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, Jørgensen 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

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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/m^2 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 signicance 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				Karri				Otra				Pomo				Mean			
	GER	MI	G	NG*	GER	MI	G	NG	GER	MI	G	NG	GER	MI	G	NG	GER	MI	G	NG
a[1]	90.8	9.3	8.0	21.2	89.5	14.0	10.0	32.2	96.0	10.0	9.1	31.3	90.5	9.5	7.4	23.5	91.3	10.7	8.7	27.0
b	86.8	57.7	55.7	73.2	82.8	83.5	81.8	88.1	95.8	52.3	47.5	53.3	89.3	58.7	57.9	67.8	88.7	63.8	61.2	70.3
c	89.3	63.2	62.2	70.9	83.0	84.3	83.2	91.1	94.8	78.3	77.5	87.5	90.3	66.7	65.8	76.3	89.3	73.1	72.1	81.5
Mean	88.9	43.3	42.6	55.1	85.1	60.3	58.3	70.5	95.5	46.9	44.7	57.0	90.0	45.0	43.7	55.8			47.3	59.6

* GER = Per cent germination of seed stocks
MI = Mean infection (per cent)
G = Infection level in germinated stocks (per cent)
NG = Infection level in non-germinated stocks (per cent)

F-value: Germination in seed stocks = 11.4**, $LSD_{0.05}$ = 2.15 %
Differences in fungal incidences in germinated and non-germinated seeds were not tested statistically because without exception non-germinated seeds carried higher fungal incidences.

[1] For seed classes see text

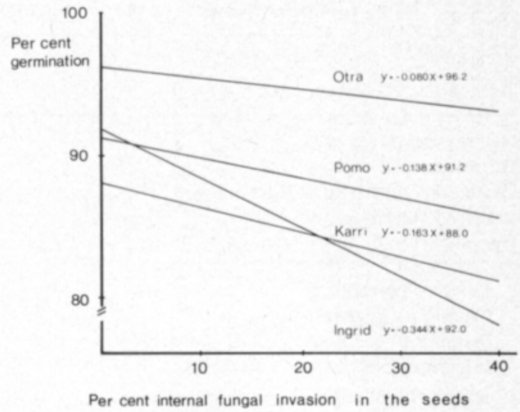

Fig. 1. The effect of fungal invasion on germination of seed stocks of four barley cultivars. Correlation coefficients: Ingrid, r = —0.58[x] Karri, r = —0.62[x], Otra, r = —0.80[xx], Pomo, r = —0.48.

ed. 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

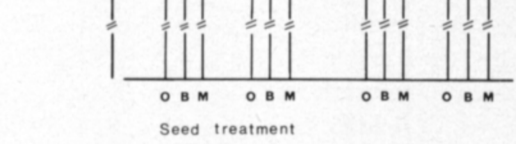

Fig. 2. The effects of seed infection by *Bipolaris sorokiniana* and seed treatment on the grain yield of two barley cultivars in greenhouse experiments. Infection levels in sowing seed were 60 % (cv. Vigdis) and 92 % (cv. Paavo). 0 indicates untreated seed, B and M were treated with benomyl and organomercury respectively. F-values: Seed infection = 86.1[xx], $LSD_{0.05}$ = 10.7 %, Seed treatment < 1.

Table 2. Emergence of barley seed infected with *Bipolaris sorokiniana,* with and without seed treatment.

Treatment	Emergence	
	Greenhouse experiments	Field experiments
Healthy untreated seed	94.5* (7)[1]	55.3[2] (4)
Untreated infected seed[3]	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	Cultivar										
	Ingrid		Karri		Otra		Pomo		Means		
	0[1]	1	0	1	0	1	0	1	0	1	0+1
h[2]	100.0*	103.7	100.0	104.0	100.0	89.5	100.0	98.5	100.0	99.0	99.5
b	102.2	94.7	84.2	83.7	90.5	96.5	79.2	78.7	89.0	88.4	88.7
c	94.5	91.2	93.2	90.5	85.7	76.7	78.0	66.7	87.8	81.3	84.6
Mean	98.9	96.6	92.5	92.8	92.1	87.6	85.7	81.3	92.2	89.6	

[1] 0 = Untreated seed
1 = Organomercury treated seed
[2] h = Healthy seed
b = Embryo infection levels from 4 to 10 %
c = — » — 22 to 36 %
* Relative grain yield; controls for each cultivar = 100.0

F-values: Infection levels = 71.3[xx], $LSD_{0.05}$ = 2.8 %
Cultivars = 10.8[xx], = 9.0 %
Seed treatment < 1

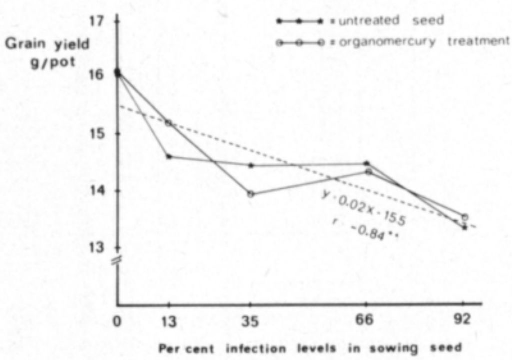

Fig. 3. The effects of seed infection level and seed treatment with organomercury powder on grain yields of barley cultivar Paavo in greenhouse experiments. F-values: Seed infection level = 50.9[xx], $LSD_{0.05}$ = 0.44 g, Seed treatment < 1.

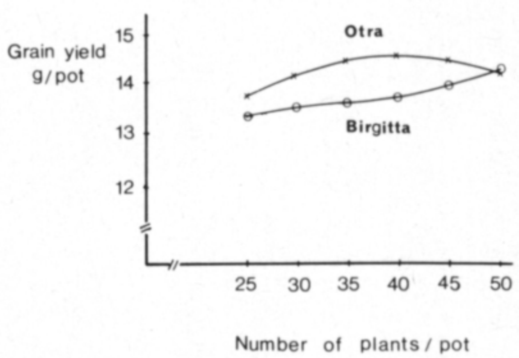

Fig. 4. The effect of plant density on grain yields of barley cvs. Birgitta and Otra. For sowing, pathogen-free seed was used. F-value = 1.8, not significant.

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 Sowing seed			% incidence of *B. sorokiniana* 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^{x}, $LSD_{0.05}$ = 0.316 kg
— » — /infection incidence in grain = 34.2^{xx}, = 5.8 %
Seed treatment/ — » — = 7.4^{xx}, = 7.7 %

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		Vigdis		Karri		Pomo		Ingrid		Mean		
	H[1]	I	H	I	H	I	H	I	H	I	H	I	H+I
None	100.0*	93.5	100.0	89.0	100.0	95.0	100.0	95.0	100.0	96.5	100.0	93.7	96.9
Organo-mercury	96.2	93.2	98.4	93.4	102.5	97.9	101.8	95.1	101.5	96.9	100.0	95.4	97.7
Mean	98.1	93.4	99.2	91.2	101.3	96.5	100.9	95.0	100.7	96.7	100.0	94.5	
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^{xx}, $LSD_{0.05}$ = 4.9 %
Organomercury seed treatment of infected seed (all cultivars) = 1.3
Organomercury or Imazalil treatment (cvs Karri, Otra and Vigdis) < 1

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 %.

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	
		II[1]	EI
Healthy untreated	227.8*	25.5	4.5
Infected — » — [2]	224.2	32.7	10.5
— » — benomyl treated	223.6	36.0	11.5
— » — organomercury treated	223.0	32.3	9.5

[1] II = Infection incidence in the grains (%)
EI = Embryo infection — » —
* Grain yield g/200 heads
[2] Infection level in the seed = 60 %

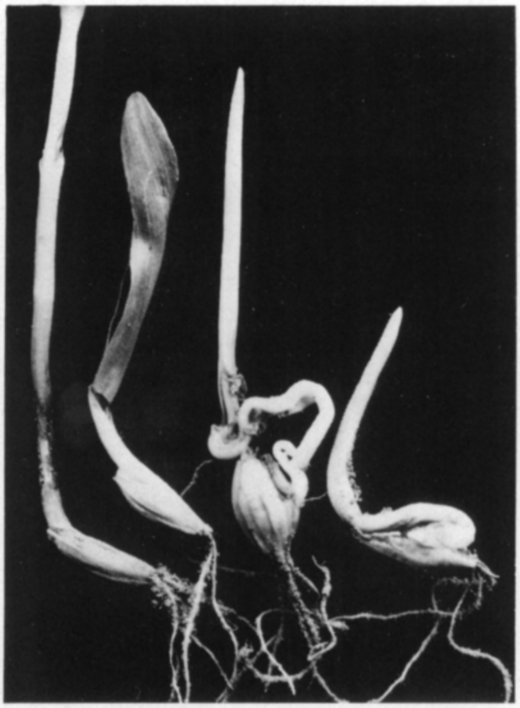

Fig. 5. Seedling blight and pre-emergence blight originating from seed-borne infection in field experiments.

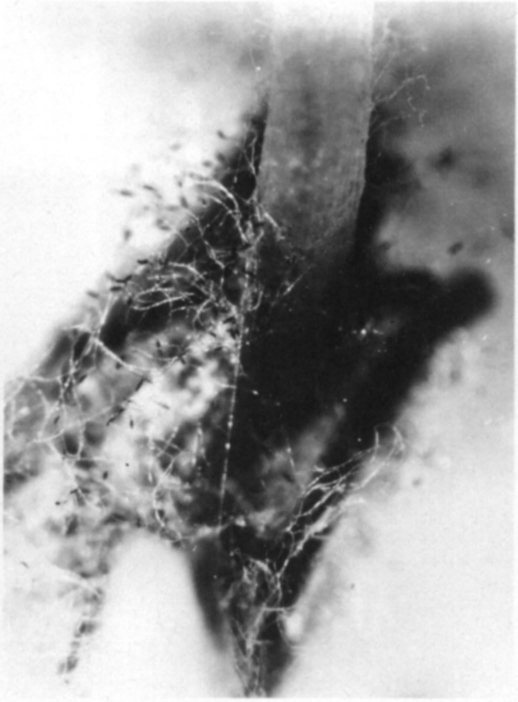

Fig. 6. Fungal growth originating from infected organomercury treated seed.

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 capable 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 deebly 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 phenomen. 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 soilborne 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 Artic 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*, koteloaste *Cochliobolus sativus*) merkitystä ohran siemenlevintäisenä taudinaiheuttajana sekä sienen torjuntaa selvitettiin vuosina 1973—1979 Helsingin yliopiston kasvipatologian laitoksella järjestetyissä tutkimuksissa.

Koesiemenenä käytettiin kenttäkokeella tuotettuja eri asteisesti (19—92 %) infektoituneita, mutta muilta ominaisuuksiltaan vastaavia siemeneriä ja Valtion Siementarkastuslaitokselta tutkimuksiin valittuja siemeneriä. Erien sienitartunta tutkittiin mikroskooppisesti 7 vrk petrimaljoissa idätetyistä siemenistä. Orastuvuus määritettiin virallisen menetelmän mukaisesti. Peittausaineiksi sienen torjuntaan valittiin Ceresan-kuivapeittausjauhe (organoelohopea), Benlate (benomyyli) 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-lehtiasteella. Kenttäkokeista otettiin laskennan yhteydessä myös orasnäytteet (25 orasta/ruutu). Toiset näytteet koeruuduista otettiin hieman ennen maitotuleentumisastetta. Näytteiden avulla pyrittiin varmistamaan *B. sorokiniana*-sienen osuus oireiden esiintymiseen sekä seuraamaan taudin kehittymistä. Kasvustot korjattiin heti niiden tuleennuttua. Jyväsadot kuivattiin välittömästi, punnittiin ja niistä tehtiin sienimääritykset.

Kylvösiemenen sienitartunta alensi ohran itävyyttä ja orastuvuutta; peittaamattomien koejäsenten orastuvuus aleni astiakokeissa keskimäärin 14 % ja kenttäkokeissa 18 %. Itävyyden ja vielä selvemmin orastuvuuden aleneminen riippuivat enemmän jyvien sisäosien sienitartunnasta kuin jyvien määritetystä sienitartunnasta. Sieni esiintyi yleisemmin itämättömissä kuin itäneissä jyvissä. Kylvösiemenen peittaaminen organoelohopeavalmisteella puhdisti pelkästään pinnallisen sienitartunnan saaneet siemenet, mutta ei täysin torjunut sientä sisäosiltaan infektoituneista jyvistä, joiden itäessä kehittyi sairaita oraita. Systeeminen imazalil antoi paremman torjuntatuloksen kuin elohopea, mutta orastuvuus jäi silti alhaisemmaksi.

Sieni aiheutti useimmissa kokeissa merkitseviä satotappioita sekä alensi kenttäkokeissa lisäksi sadon arvoa kylvösiemenenä, mikäli sääolot olivat suotuisat sienen sekundäärilevinnälle. Satotappiot astiakokeissa olivat 7.2—38.5 % ja kenttäkokeissa 5—11 %, keskimäärin 6.3 %. Lajike-erot jäivät vähäisiksi, todennäköisenä syynä kaikkien lajikkeiden kylvösiemenen yhtäläinen, voimakas sienitartunta. Peittaaminen lisäsi satoja jonkin verran, muttei tilastollisesti merkitsevästi. Peittausaineista organoelohopea kohotti satoa enemmän kuin imazalil, vaikkei pystynytkään torjumaan tautia täysin. Benomyyli todettiin peittausaineena täysin tehottomaksi sienen torjunnassa.

Kylvösiemenen sienitartunta johti voimakkaan lehtilaikkutaudin puhkeamiseen kasvustossa tähkälletulon jälkeen kenttäkokeissa Muddusniemen tutkimusasemalla Inarissa, missä sientä ei luontaisena esiinny. Epidemia johti myös jyväsadon sienitartuntaan. Sieni säilyi maassa satojätteissä kahden talvikauden yli ja kykeni infektoimaan maalevintäisesti ohran oraita.

Siemenlevintä on maassamme todennäköisesti *B. sorokiniana*-sienen tärkein säilymis- ja leviämismuoto. Sienen vahingollisuus ja leviämisvoimakkuus riippuvat kuitenkin paljolti kasvukauden säästä. Kuivana kasvukautena siemenlevintä johtaa pelkästään tyvitautiin, kosteana lisäksi lehtilaikkutautiin ja jyväsadon sienitartuntaan.

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