

SEED INFECTION OF BARLEY BY COCHLIOBOLUS SATIVUS AND ITS INFLUENCE ON YIELD¹

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Abstract

Yield losses from planting barley seed heavily infected with *Cochliobolus sativus* were not significant at the normal planting rate. Seed infection reduced seedling emergence by up to 38% in the field, but the reduction in number of plants per row had to be more than 50% of the control before significant yield decreases occurred. Treatment of the seed with mercury fungicides eliminated the reduction in emergence caused by *C. sativus* and also provided an additional increase, due possibly to protection from other soil microorganisms, but there was no significant improvement in seed yields. Treatment of the seed improved kernel weight slightly. Only a few plants grown from infected seed became infected by the causal organism under normal greenhouse conditions. When they were grown under conditions of high humidity, however, almost as many plants were infected as there were infected seeds. Disease development on plants in high humidity was controlled by treatment of the infected seed with mercury fungicides.

Introduction

In 1967 a severe infection of spot blotch and head blight caused by *Cochliobolus sativus* (Ito and Kurib.) Drechs. ex Dastur (imperfect state *Bipolaris sorokiniana* (Sacc. in Sorokin.) Shoemaker (syn: *Helminthosporium sativus* PK & B) occurred on the barley crop in Prince Edward Island. There was considerable concern about what effect the heavily infected seed from this crop might have on the subsequent crop if it were used for planting. Some years ago Greaney and Wallace (1) and Machacek et al. (2) reported that seed infection by this fungus lowered germination but had little effect on yield. They found that chemical seed treatments improved germination of infected seed but had little influence on yield.

The availability of this heavily infected barley seed provided an opportunity to establish further the relationship between yield losses and seed infection by *C. sativus* as well as to determine the benefits obtained from treating this seed with fungicides.

Materials and methods

The barley (*Hordeum vulgare* L.) variety Herta was used in all experiments. Heavily infected seed was obtained from Charlottetown, Prince Edward Island, relatively healthy seed from Saskatoon, Saskatchewan, and commercial grade seed from

Ottawa, Ontario. The amount of internal infection by *C. sativus* was determined by plating aseptically 100 surface sterilized (10 minutes in a Javex solution adjusted to 2% available chlorine as sodium hypochlorite) seeds from each sample on malt agar. Fungus colonies were identified after 10 days' incubation in the dark at 23-24 C.

In field tests, 4-row plots with 100 seeds (unless otherwise specified) per 3-m long rows were planted for each treatment and replicated four times. Seedling emergence was recorded approximately 1 month after seeding. At maturity, the plants were removed from 30 cm at the ends of the two centre rows of each plot and the remainder of the plants in these rows were harvested for seed yield and 1000-kernel weight determinations. Threshing was done with a cyclone thresher.

In greenhouse tests, the seed was planted in steamed soil in flats, 100 seeds per flat. The plants were grown at 25-27 C, in one instance using normal humidification and in another instance using a "mist-bed" where the humidity was maintained at 95-100%. Emergence was recorded 1 month after seeding, and approximately 3 weeks later all plants were harvested and classified as being healthy or infected by *C. sativus*.

Three chemical fungicides⁴ were used to treat the seed at the following rates per 45.5 kg (100 lb) seed: Ceresan M (7.7% ethyl mercury p-toluene sulfonamide), 42 g (1½ oz); Liqui-San 10L (1.97% methyl mercury 2-3 dihydroxypropylmercaptide and 0.42%

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⁴ Ceresan M and Benlate were supplied by Dupont of Canada Ltd., Toronto, Ontario and Liqui-San 10L by Green Cross Products, Montreal Quebec.

methyl mercury acetate), 63 g ($2\frac{1}{4}$ oz), and Benlate (50% benomyl [1-(butyl carbamoyl)-2-benzimidazole carbamic acid, methyl ester]), 112 g (4 oz). Each fungicide was added to a 115 g sample of seed in a 1-litre widemouth Erlenmeyer flask, which was rotated for 5 minutes on a machine designed to give good coverage of the seed. The treated seed was left in the stoppered flasks for at least 48 hr before planting.

Results

Field tests

The percentage of seeds infected by *C. sativus* in the Prince Edward Island sample ranged from 97 to 100 and in the Saskatchewan sample from 2 to 4. The commercial seed from Ottawa was divided into two groups of large or normal sized seed and small seed. The group of large seed was 60% infected by *C. sativus* and the small 55%. Seed from the four samples was treated with the mercury fungicide Liqui-San 10L, the non-mercury systemic fungicide Benlate, and with a combination of the two applied at the same rate as used individually.

Emergence of heavily infected untreated Prince Edward Island seed was 38% lower than that of untreated seed from Saskatchewan (Table 1). Treatment of the seed with Liqui-San 10L increased the emergence of all samples significantly and of the heavily infected seed by 100%. Benlate did not improve emergence over the controls. When Benlate was combined with Liqui-San 10L, emergence of the Saskatchewan seed was somewhat better than with Liqui-San 10L alone; emergence of the other samples

was similar to that with Liqui-San alone. A significant interaction occurred between the seed treatments and the seed sources indicating that the emergence of the seedlings from the various sources did not follow the same pattern with all the fungicides.

Comparable differences in seed yields were not found. There was only a small difference in yield between the heavily and the lightly infected untreated seed. In most instances treatment of the seed with chemicals gave no improvement in yield. Neither Liqui-San 10L nor Benlate improved the yield of any of the seed samples. Liqui-San 10L combined with Benlate gave a small improvement in yield with the Saskatchewan and Prince Edward Island samples.

The data on 1000-kernel weights were not consistent but there was an indication that seed treatment increased them.

In another test, seed from Prince Edward Island and Saskatchewan were combined in different proportions so that two intermediate levels of infection of approximately 33% and 66% could be compared with the original samples. Seed of the four infection levels was treated with Liqui-San 10L and Ceresan M. Ceresan M was found by Mills and Wallace (3) to be the most effective chemical seed treatment for controlling *C. sativus* on barley in the laboratory. The difference between the emergence of the heavily and the lightly infected seed samples (Table 2) was similar to that in the previous test. At the 33% and 66% infection levels emergence was intermediate. Both chemical seed treatments improved the emergence of all levels of infected seed and the 'emergence'

Table 1. Seedling emergence, seed yield and 1000-kernel weight of barley grown in the field from *Cochliobolus sativus* infected seed from three sources and treated with two fungicides

Seed source	Emergence (Plants/row)				Seed yield (g/plot)				1000-kernel weight (g)			
	Control	Liqui-San	Benlate	Liqui-San & Benlate	Control	Liqui-San	Benlate	Liqui-San & Benlate	Control	Liqui-San	Benlate	Liqui-San & Benlate
Saskatchewan	71.2	84.0	71.0	93.2	376.5	370.0	356.5	399.2	37.9	43.0	42.0	41.2
P. E. I.	44.2	88.0	45.5	86.5	366.0	333.5	289.0	398.2	40.4	39.7	39.2	42.8
Ottawa #1 ¹	62.0	90.5	52.0	89.2	396.5	396.0	337.5	380.5	39.5	40.5	39.9	39.5
Ottawa #2 ²	65.7	88.0	67.5	87.7	394.5	358.0	359.7	373.0	41.0	39.3	41.7	42.5
L. S. D. (1%) seed sources			13.2				N. S.					N. S.
fungicides			13.2				N. S.					0.9

¹ large seed

² small seed

of Prince Edward Island seed was increased to the same level as the Saskatchewan seed. There were no significant differences in seed yields among the various infection levels or between treated and untreated seed. Yield from the heavily infected seed was 11% lower than that from the lightly infected seed. Both seed treatments reduced yields at the two lower levels of infection and increased yields at the two higher levels. In general, yields were better with Ceresan M than with Liqui-San 10L. There was an indication that Liqui-San 10L increased the 1000-kernel weight of the subsequent crop.

A third field test was carried out to determine if different numbers of plants per row as well as various levels of seed infection would have any effect on seed yields. This was done by planting untreated and Ceresan M treated seed at the same infection levels as in the previous experiment at 5-, 10-, 15- and 20-cm spacings within the 3-m-long rows. This meant that rows at 10-, 15- and 20-cm spacings had approximately 75%, 50%, and 25% as many plants as rows with seed placed at 5-cm spacings (Table 3). The relative emergence was about the same as in the previous two experiments but, due to the increased size of the experiment, variability was greater. Seed yields were also more variable and there was a greater reduction in the yield from the highly infected seed compared with the lightly infected seed. Treatment of the seed, however, gave no significant improvement in yield. Seed spacing per row and the resulting number of plants per plot did influence yields. Plots planted with untreated seed containing 33% and 98% infection and treated seed with 98% infection and spaced 15 cm apart yielded significantly less than those planted 5 cm apart. There was no significant yield difference between 5- and 10-cm spacings. Therefore there had to be more than a 50% reduction in the number of plants per row before significant yield losses were observed. A significant interaction occurred for yields between seed spacings and infection levels, indicating that significant losses did not occur at all infection levels. However there was no significant third order interaction. The kernel weight data were again inconclusive, but there was a trend toward increased kernel weight as the number of plants per row decreased.

Greenhouse tests

Samples of the same seed that was used in the first two field experiments were grown in the greenhouse. Seedling emergence was similar to that found in the field tests (Tables 4 and 5), except that overall emergence was considerably higher in the greenhouse than in the field and there was less difference between the highly infected Prince Edward Island seed and the lightly infected Saskatchewan seed. The mercury fungicides increased emergence of all samples, while Benlate slightly increased that of two samples but gave a lower emergence with the other two. At the normal humidity level very few *C. sativus*

infected plants were found, even from the heavily infected seed (Table 4). In the greenhouse "mist-bed" (Table 5), where the relative humidity was maintained at approximately 95%, more than 90% of the plants from the heavily infected untreated seed were infected by *C. sativus*. The lower infection levels also produced considerable disease. Plants from the treated seed were relatively free of symptoms and developed about the same amount of disease as those grown in conventional greenhouse conditions.

Discussion

Infection of barley seed by *C. sativus* causes a considerable reduction in seedling emergence especially if the percentage infection is high. The fact that reduction in emergence can be eliminated by the treatment of the seed with mercury fungicides agrees with the findings of Greaney and Wallace (1). Emergence in all seed samples, including those lightly infected, was improved by treatment. The lower emergence in the untreated seed, however, was not followed by a comparable reduction in seed yield, and conversely the increased emergence of the heavily infected treated seed did not give a significant increase in yield. The reliability of yield data from small plots is sometimes questionable. Our yield data had a relatively low coefficient of variation (12.5%, Tables 1 and 2) and thus should be reliable. Our results agree with those found earlier by Machacek et al. (2), and there seems to be no question that the use of seed heavily infected by this fungus has little influence on the eventual yield of the crop.

There appears to be little practical value in treating barley seed infected with *C. sativus* with chemical fungicides. The greenhouse tests showed that under normal conditions of humidity (Table 4) very few of the seedlings from the heavily infected untreated seed were infected by the fungus. Furthermore, even when emergence was reduced in the field, it had to be reduced by more than 50% before there was a significant drop in yield (Table 3); and, on the average, there was no yield benefit from treating the seed. There was a benefit from seed treatment when the humidity in the greenhouse was maintained at a high level (Table 5), as most of the young seedlings from the treated seed were not infected by *C. sativus*, whereas the majority of those from the untreated seed were infected. Occasionally, unusual weather conditions of high relative humidity, dew point, and temperature occur, as in the Maritime Provinces in 1967. In this situation, treatment of the seed with chemicals might have prevented the extensive build-up of the disease that occurred by crop maturity. Such a heavy seed infection would appear to be due primarily to weather conditions rather than to a heavy inoculum load on the seed. The progeny of plants grown from heavily infected seed in these tests were found to be relatively disease-free and the seed produced by them was as disease-free as that produced by plants from clean seed.

Table 2. Seedling emergence, seed yield, and 1000-kernel weight of barley grown in the field from seed infected with four levels of *Cochliobolus sativus* and treated with two fungicides

Infection level (%)	Emergence (plants/row)			Seed yield (g/plot)			1000-kernel weight (g)		
	Control	Ceresan M	Liqui-San	Control	Ceresan M	Liqui-San	Control	Ceresan M	Liqui-San
2	81.5	86.6	88.0	497.5	491.2	450.5	39.0	37.3	40.8
33	69.8	81.3	86.2	509.7	469.0	456.5	39.3	39.1	38.1
66	61.4	95.7	85.7	439.7	566.7	462.2	37.8	38.1	39.6
98	52.8	85.9	85.5	442.7	468.7	491.5	36.8	36.8	40.0
L, S, D, (1%) infection levels		7.5			N. S.			N. S.	
fungicides		7.5			N. S.			N. S.	

Table 3. Seedling emergence, seed yield, and 1000-kernel weight of barley seeded in the field at 5-, 10-, 15-, and 20-cm distances within the row with Ceresan M-treated and untreated seed with four levels of *Cochliobolus sativus*

Seed treatment	Infection level (%)	Emergence (plants/row)				Seed Yield (g/plot)				1000-kernel weight (g)			
		5 cm	10 cm	15 cm	20 cm	5 cm	10 cm	15 cm	20 cm	5 cm	10 cm	15 cm	20 cm
Untreated	2	54.8	27.5	16.8	12.6	503.5	456.7	356.7	425.2	38.2	36.8	37.4	37.8
	33	48.7	25.4	17.0	12.5	513.7	469.0	347.0	323.2	38.6	37.9	38.5	39.2
	66	43.2	19.5	15.1	11.2	400.7	330.0	428.0	283.0	38.4	37.3	38.8	40.8
	98	37.5	17.6	13.4	9.8	504.2	384.7	333.5	294.7	37.0	35.1	39.3	38.9
Treated	2	54.7	26.8	18.0	13.3	463.0	378.7	411.5	375.5	34.4	34.5	38.0	39.4
	33	50.8	23.1	16.5	12.6	454.2	504.5	366.5	383.7	33.6	37.9	35.9	38.6
	66	48.9	24.6	15.7	11.6	398.2	380.7	468.5	342.0	33.7	34.7	40.0	40.2
	98	42.9	21.8	13.9	9.2	444.0	383.0	297.5	284.0	36.8	40.1	40.2	37.0
L.S.D. (1%) treatments			14.0				N. S.					N. S.	
infection levels			14.0				N. S.					N. S.	
seed spacings			14.0				150.0					3.4	

Table 4. Seedling emergence and infection of barley grown at normal greenhouse humidity from *Cochliobolus sativus*-infected seed from three sources and treated with two fungicides

Seed source	Emergence (plants/flat)				Infected plants (%)			
	Control	Liqui-San	Benlate	Liqui-San & Benlate	Control	Liqui-San	Benlate	Liqui-San & Benlate
Saskatchewan	88.5	94.5	92.2	94.5	2.2	4.2	10.9	5.5
P. E. I.	72.7	90.2	57.5	91.5	9.6	6.9	26.5	10.9
Ottawa #1 ¹	74.2	93.0	68.5	94.5	9.7	9.6	12.0	10.5
Ottawa #2 ²	82.0	93.0	83.2	92.5	10.0	5.9	17.3	8.6

¹ large seed

² small seed

Table 5. Seedling emergence and infection of barley grown in a high humidity greenhouse from seed with four levels of *Cochliobolus sativus* and treated with two fungicides

Infection level (%)	Emergence (plants/flat)			Infected plants (%)		
	Control	Liqui-San	Ceresan M	Control	Liqui-San	Ceresan M
2	92.5	97.7	96.7	7.8	2.0	1.2
33	87.5	96.5	95.2	33.0	3.7	5.4
66	81.5	94.5	96.2	56.9	8.8	8.1
98	67.0	94.5	97.5	92.9	10.4	12.2

The mercury fungicides effectively increased emergence and prevented seedling infection from seed-borne inoculum. The manufacturer of Benlate does not recommend this chemical for the control of *C. sativus*, and our findings support this conclusion. The suggestion that it be used with another seed dressing appears satisfactory, as the combination gave the highest average emergence, seed yield, and 1000-kernel weight (Table 1).

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