Influence of Seed Treatment with Imazalil on Common Root Rot and the Size of the Subcrown Internode of Wheat

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ABSTRACT

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Common root rot ratings of Neepawa wheat grown at two locations from seed treated with imazalil [1-(B-allyloxy-2, 4 dichlorophenethyl) imidazole] at 0.3 g active ingredient/kg seed averaged 5% and 16% for seedlings and mature plants, respectively, as compared to 32% and 51%, respectively, for those from nontreated seed. Seven other fungicides gave no equivalent reduction in disease ratings. In three tests, using a number of wheat cultivars, soils, and treatment rates in the greenhouse, imazalil lowered disease in the wheat seedlings. The level of control increased with dosage. The internodes of mature plants from treated wheat seed in the field experiment averaged 14.7% larger than those from nontreated seed. In one greenhouse test they averaged 12.8, 27.2, and 37.1% larger, respectively, than those from the nontreated seed, when dosages of 0.1, 0.2, and 0.3 g a.i./kg seed were applied to Neepawa. Similar increases occurred with the Cypress cultivar. The relationship of the differential response of subcrown internode growth to imazalil and the control of common root rot is discussed.

Additional key words: Cochliobolus sativus, Helminthosporium sativum, fungicides, growth responses, control by seed treatment.

Common root rot reduced yield of wheat (*Triticum aestivum* L.) annually by an estimated 816,400 tonnes (30 million bu) between 1969 and 1971 in the Canadian Prairies (8). It caused even more serious losses in barley (12). The disease is incited mainly by *Cochliobolus sativus* (Ito and Kurib.) Drechs. ex Dast., the conidial state of which is *Helminthosporium sativum* Pam. King and Bakke, syn. *Bipolaris sorokiniana* (Sacc. in Sorok.) Shoem. The pathogen is soilborne and the disease is manifest by lesions on the basal stems and subcrown internodes. Numerous studies have been made to develop methods for its control.

One approach to control involves seed treatment with fungicides. Mills and Wallace (9) and Richardson (13) were able to eradicate *C. sativus* from infected barley seed. Some doubt exists however, as to whether such treatments can control common root rot under field conditions. Reports by Babasyan (1), Buga (2), Chukina (4), Das and Srivantava (5), Krotova (6), (Mikhailina and Minbaev (11) indicated that some fungicides and antibiotics, such as arasan, ceresan, granosan, quinolate 15, thiram, azalomyxin C, blasticidin, and trichothecin were effective in the experimental control of common root rot of wheat. Nevertheless, in Western Canada, arasan and ceresan, among others, failed to reduce the disease in adult plants in the field (R. D. Tinline, *personal communication*).

Among those studying systemic fungicides Richardson (13), using Vitavax, F427, and G696 (Uniroyal products) produced healthy barley seedlings from infected seeds but the seeds were planted in compost soil, a medium probably devoid of the pathogen. J. Horricks (*personal communication*) claimed some success with a number of systemic fungicides in controlling common root rot of barley in field tests. In our laboratory, however, routine screening of fungicides from 1960 to 1976 showed little evidence of efficacy.

This paper presents results of a 1977 screening trial in which one fungicide appeared to be outstanding for the control of common root rot of wheat in the field. Furthermore, a striking growth response was observed on the subcrown internodes of plants grown from treated seed. Subsequently, a number of trials were made in the greenhouse to confirm field data.

MATERIALS AND METHODS

Eight fungicides applied as seed treatment were screened for efficacy in controlling common root rot of a spring wheat cultivar, Neepawa. The names, active ingredients (a.i.), and percent a.i. of the eight fungicides were: Baymeb 6447, 1-(4-chlorophenoxyl)-3, 3-dimethyl-1-(IH-1,2,4-triazol-1-yl)-2 butanone, 50%; Bravo W-75 (Daconil), tetrachloroisophthalonitrile, 75%; DPX 14,

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methyl 2-benzimidazolecarbamate, 15% and maneb, manganese ethylenebisdithiocarbamate, 60%; Duter, triphenyltinhydroxide, 19%; EL 222, γ-(2-chlorophenyl)-a-(4-chlorophenyl)-5-pyrimidinemethanol, 12.5%; EL 228, γ-(2-chlorophenyl)-2-(4-fluorophenyl)-5-pyrimidinemethanol, 70%; Imazalil, 1-(β-allyloxy-2, 4-dichlorophenethyl)imidazole, 9.5%; and LFA2043(26,019RP), 1-(isopropylcarbamoyl)-3-(3,5-dichlorophenyl) hydantoin, 50%. The fungicides, some liquids and other wettable powders, were mixed directly with the seed in a jar at the rate of 3.13 g formulation/kg seed (3 oz /bu).

Seeds were planted in the field at Saskatoon and Scott, Saskatchewan 2 and 7 days, respectively, after they were treated. Two 2-m-long rows with 125 seeds/row were sown for each treatment. Depth of seeding was 8 cm at Saskatoon because the soil was particularly dry, and 5 cm at Scott where soil moisture was adequate. The soils at these locations were a clay (pH 6.0) and a loam (pH 5.5), respectively. The populations of conidia of *C. sativus* as determined by the flotation-viability method (3) were 54 and 40 per g of soil at Saskatoon and Scott, respectively.

Ratings of common root rot were made on all the plants in one row for each treatment at the seedling stage (late June) and also at maturity (mid-August) according to the method of Ledingham et al. (7).

The frequency of *C. sativus* in the subcrown internodes was determined for mature plants grown from the imazalil-treated and nontreated seed in the Saskatoon field. The subcrown internodes of one-half of the plants were taken randomly, surface-disinfected with a 10% Javex solution (a.i. 6% NaOCl) for 10 min, rinsed in sterile water, and plated on acidified potato-dextrose agar. The occurrence of *C. sativus* was recorded after 1 wk at room temperature. Diameters at midsection of the subcrown internodes from the remaining half of the mature plants were measured with a microcomparator (Shadowgraph by Nikon, Model 6c).

The efficacy of imazalil in controlling root rot was checked in three greenhouse tests. The first test consisted of determinations made with five soils, two wheat cultivars, and three treatment rates. The soils were obtained from five fallow fields in August 1977. Two fields were adjacent to the field plots at Saskatoon and Scott. Locations, textures, and pH values of the other three soils were: Melfort, silty clay loam, 5.7; Clavet, silty loam,7.4; and Vanscoy, fine sandy loam, 7.4. In addition to the natural infestation of *C. sativus* in the soils, inoculum was added. Sterile straws, four per test tube, were inoculated with *C. sativus* and incubated for about 1 mo. The inoculum was mixed with the soil at the rate of four straws per 5 kg soil. Final populations ranged from 770 to 1,010 conidia of *C. sativus* per g in all five soils. Two wheat cultivars (Neepawa and Cypress) were used. Cypress is more susceptible to *C. sativus* than Neepawa.

The imazalil formation was diluted 10 times with water and applied to the seed at the rate of 0.1, 0.2, and 0.3 g a.i./kg seed. Four replicates of 25 seeds per 1-liter pot were planted and, after 6 wk, the seedlings were harvested and assessed for disease. One replicate was then used to ascertain the frequency of *C. sativus* in the subcrown internodes of the seedlings. The method of isolation was similar to the one used for the mature plants from the field except that the parts were surface-disinfected for only 5 min.

Diameters of the subcrown internodes of seedlings from another replicate were measured as described previously with the exception that the internodes were relatively fresh as compared to those from the field. All seedlings were dried in a forced-air drying oven at 70 C for 24 hr and weighed.

The second greenhouse test consisted of determinations made with soil from Saskatoon and Scott, two wheat cultivars (Neepawa and Cypress) and treatment rates of 0.1, 0.2, 0.3, and 0.4 g a.i./kg seed. The third greenhouse test consisted of determinations made with six bread wheat cultivars (Cypress, Manitou, Neepawa, Park, Thatcher, and Sinton), one utility wheat cultivar (Glenlea), and two durum wheat cultivars (Wakooma and Wascana). Soil from Saskatoon was used and imazalil was applied at 0.2 and 0.3 g a.i./kg seed.

RESULTS

Of the eight fungicides applied to seed, imazalil was very effective in reducing common root rot in seedlings and mature plants at both locations (Table 1). Three of the fungicides appeared somewhat phytotoxic and the remaining four, particularly Duter and LFA2043, reduced the disease slightly.

Cochliobolus sativus was iaolated from 36% and 92%

TABLE 1. Severity of common root rot incited by *Cochliobolus sativus* in seedlings and in mature plants grown from Neepawa wheat seed treated with fungicides in the field at Saskatoon and Scott, Saskatchewan, Canada

		Disease ratings (%)					
		Saskatoon		Scott		Average	
Fungicide[a]	Treatment rate[b] (g a.i./kg seed)	Seedling	Mature plant	Seedling	Mature plant	Seedling	Mature plant
Bravo W-75	2.36	32	32	12	23	22	27
DPX 14	2.36	27	41	17	25	22	33
Duter	0.60	21	36	10	21	15	29
Imazalil	0.30	6	13	4	19[c]	5	16
LFA 2043	1.58	13	43	10	33	11	38
Control		44	67	21	36	32	51

[a]Baymeb 6447 at 1.58, EL 222 at 0.39, and EL 228 at 2.20 g a.i./kg were phytotoxic and data are not presented.
[b]The g a.i./kg seed is based on 3.13 g of formulation/kg seed (3 oz/bu).
[c]Because of a seeding problem many subcrown internodes were too short to assess accurately.

of the subcrown internodes of mature plants that were grown in the field at Saskatoon from imazalil-treated and nontreated seed, respectively. The mean diameter of the subcrown internodes from the plants receiving the treatment averaged 0.941 mm which was 14.7% larger than that from the nontreated control (Fig. 1-A, 2-A).

There was a similar trend in the determinations made in all three greenhouse tests. Only the results from the first test are reported fully. In this test mean disease ratings of seedlings from the treated seed of both Neepawa and Cypress grown in all five soils were lower than seedlings from the nontreated seed (Table 2). The effectiveness of control increased with treatment rate. Disease ratings of seedlings were consistently lower in Neepawa than in Cypress wheat.

With minor exceptions *C. sativus* occurred less frequently in subcrown internodes of seedlings grown from seed treated with imazalil than those not treated (Table 3). At equivalent rates of treatment *C. sativus* occurred less frequently in Neepawa than in Cypress seedlings.

The mean diameters of subcrown internodes were significantly ($P = 0.01$) affected by rate of fungicide, soil, cultivar and by soil × rate, and soil × cultivar interactions but the rate × cultivar interaction was not significant. The mean diameters of the subcrown internodes of Neepawa seedlings grown in the five soils from seed treated at 0.1, 0.2, and 0.3 g a.i./kg seed were 12.8, 27.2, and 37.1% larger, respectively, than those from the nontreated seed (Fig. 1-B, 2-B). Where Cypress seed was used the corresponding increases were 23.1, 37.9, and 40%,

TABLE 2. Severity of common root rot incited by *Cochliobolus sativus* in Neepawa and Cypress wheat seedlings grown in five soils in the greenhouse from seeds treated with imazalil at three rates

Treatment rate (g active ingredient/kg seed)	Mean disease ratings (%)		
	Neepawa	Cypress	Mean
0	17.2	27.2	22.2
0.1	10.1	18.6	14.4
0.2	5.4	11.3	8.4
0.3	2.2	7.8	5.0
S.E. of fungicide tests	0.77	0.77	0.55
Mean	8.7	16.2	12.5
S.E. of cultivar mean	0.37	0.37	

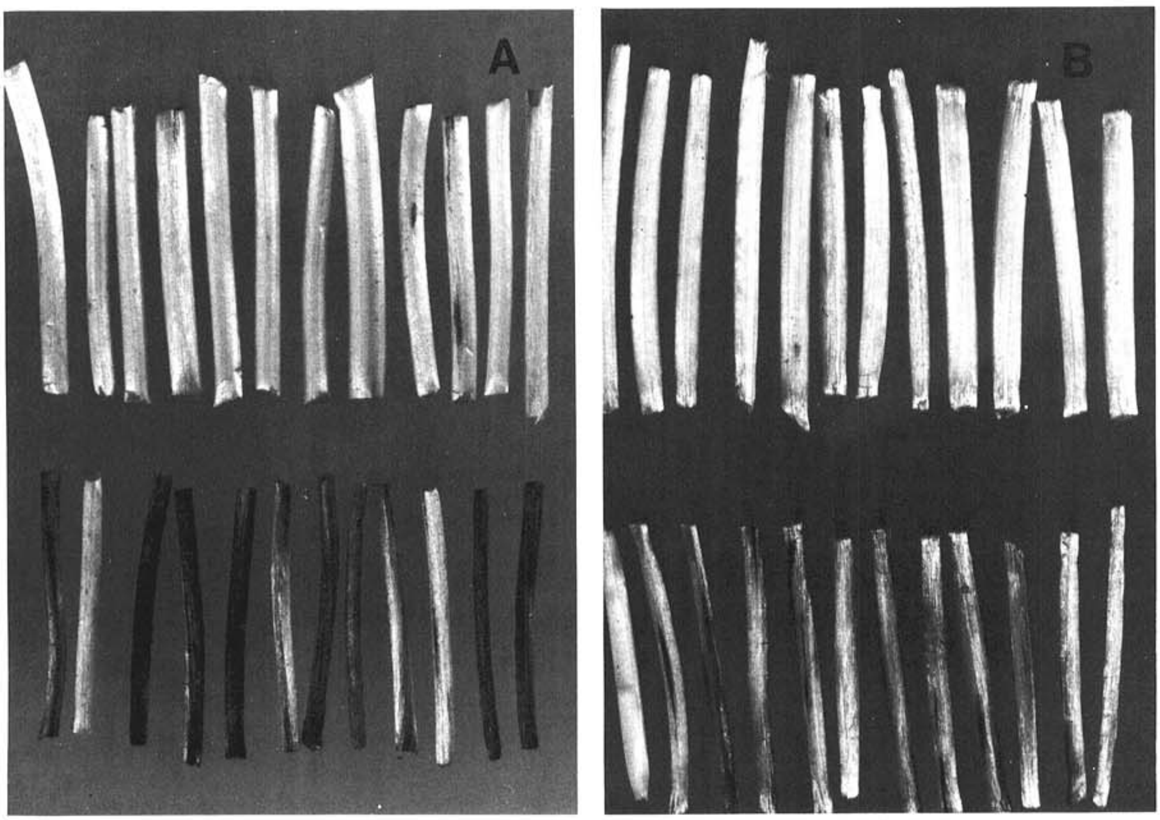

Fig. 1-(A and B). Sizes of subcrown internodes of Neepawa wheat plants grown from nontreated seed and seed treated with imazalil at 0.3 g a.i./kg seed for the control of common root rot incited by *Cochliobolus sativus*. Each space at top of figure represents 1 mm. **A**) From mature plants grown in the field at Saskatoon; upper–treated seed, lower–nontreated seed. **B**) From seedlings grown in Scott soil in the greenhouse; upper–treated seed, lower–nontreated seed. These differences were typical of seedlings of the two cultivars grown in five soils.

respectively (Fig. 2-C). There was also a slight but significant ($P = 0.05$) increase in average dry weight per plant as the amount of imazalil increased. Control plants averaged 92.6 mg whereas plant weights for treatment at 0.1, 0.2, and 0.3 g a.i./kg seed were 93.6, 95.2, and 97.5 mg, respectively (S.E. of mean = 1.27 mg). Results of the second and third greenhouse tests were similar.

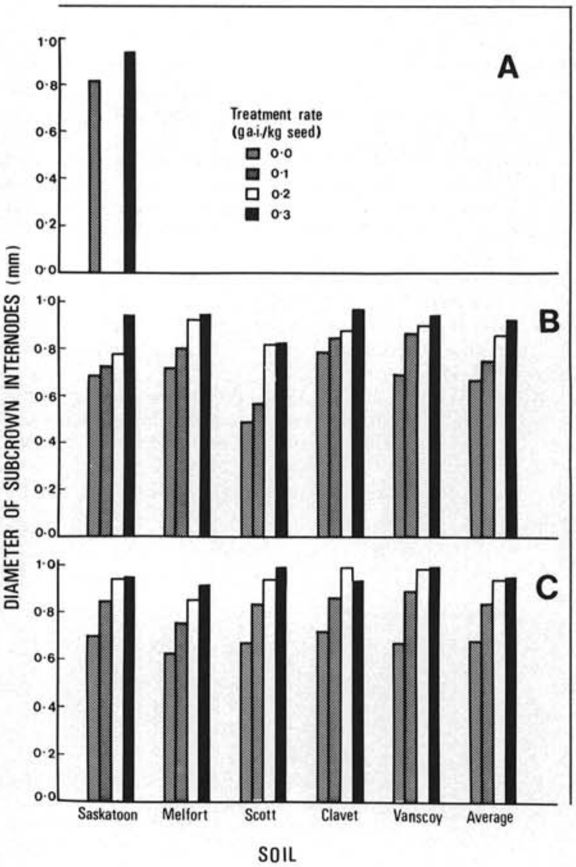

Fig. 2-(A to C). Mean diameters of subcrown internodes of wheat plants grown from seed treated with imazalil. **A)** Mature Neepawa plants grown from nontreated seed and seed treated at 0.3 g a.i./kg seed in Saskatoon field test; **B)** Neepawa seedlings grown from nontreated seed and seed treated at 0.1, 0.2, and 0.3 g a.i./kg seed in five soils in the greenhouse; and **C)** as **B** using cultivar Cypress.

DISCUSSION

Seed treatment with imazalil at 0.3 g a.i./kg seed (3 oz formulation/bu) provided good control of common root rot of Neepawa wheat both in the field and greenhouse. The treatment is probably effective for all wheat as suggested by a similar level of control in all nine wheat cultivars used in one of the greenhouse tests. Raising the rate of treatment from 0.3 to 0.4 g a.i./kg seed in another greenhouse test did not increase control. Probably the 0.2 g rate would be adequate in field application.

Obviously an effective seed treatment to control common root rot would constitute a major step forward. Losses from this endemic disease are large because of the extensive acreage sown to susceptible kinds of crops. Whether yield benefits actually will accrue from the level of control shown in this study with imazalil remains to be determined. If the treatment becomes agronomically feasible, it may not be necessary to treat seed for extended periods. Healthy plants would produce fewer conidia to infest the soil and to infect subsequent cereal crop.

The success of the treatment may be due to the fungitoxic or fungistatic property of imazalil (Chinn, *unpublished*) and the translocation of the chemical from the seed to the subcrown internodes where infection by *C. sativus* commonly occurs. The larger diameters of the subcrown internodes of plants from treated seed were not associated with any visible abnormalities in morphology. Whereas a larger than normal subterranean plant part might be expected to increase the likelihood of its attack by a soilborne pathogen, such was not the case. Investigation is continuing.

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TABLE 3. Percentage frequency of isolation of *Cochliobolus sativus* from subcrown internodes of greenhouse seedlings[a] of two wheat cultivars grown from seed treated with different rates of imazalil in five soils

Cultivar	Treatment rate (g a.i./kg seed)	Subcrown internodes infected with *C. sativus*					
		Saskatoon (%)	Melfort (%)	Scott (%)	Clavet (%)	Vanscoy (%)	Mean (%)
Neepawa	0.0	66.6	60.9	59.1	86.9	86.4	71.9
	0.1	45.5	57.1	47.1	26.1	47.6	44.5
	0.2	40.0	31.8	31.8	4.5	4.0	22.4
	0.3	13.0	18.2	8.7	4.5	0.0	8.8
Cypress	0.0	63.6	81.8	76.0	66.6	82.6	74.1
	0.1	66.6	82.6	52.0	45.8	66.6	62.3
	0.2	66.6	40.9	29.2	20.8	8.3	34.7
	0.3	50.0	45.5	22.7	8.7	4.5	26.1

[a]There were 21 to 25 seedlings in one replicate from each treatment.

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