Influence of Fungicide Sprays on Sporulation of Cochliobolus sativus on Cypress Wheat and on Conidial Populations in Soil

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ABSTRACT

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Seven fungicides were sprayed on the basal stems of wheat (cultivar Cypress) in field experiments at Saskatoon and Scott, Saskatchewan. Duter, LFA 2043, and Panogen 15 were the most effective in reducing sporulation of *Cochliobolus sativus* on the stems. None of the fungicides influenced sporulation on the subcrown internodes. Results were similar whether the fungicides were applied in mid-July, late July, or early August. At Saskatoon, the sporulation indices on the basal stems following sprays with Duter and water were 1.8 and 4.6, respectively, and conidial numbers per gram of soil were 996 and 4,045, respectively. Of the seven fungicides, Duter influenced the viability of the conidia most adversely. Sporulation indices on basal stems and conidial populations in soil were much higher at Saskatoon than at Scott. The potential of fungicide sprays to control common root rot of wheat by reducing sporulation of the pathogen is discussed.

Additional key word: Helminthosporium sativum.

Cochliobolus sativus (Ito and Kurib.) Drechsl. ex Dastur [conidial state *Helminthosporium sativum* P. K. and B. *Bipolaris sorokiniana* (Sacc. ex Sorokin) Shoemaker] is the primary cause of common root rot of cereals in Western Canada. This pathogen is perpetuated by soilborne conidia formed mainly on the basal stems and lower leaves and to a lesser extent on the subcrown internodes of infected host plants (S. H. F. Chinn, *unpublished*). These conidia subsequently are liberated and distributed throughout the soil by cultivation and wind movement. Chinn et al. (4) found populations of *C. sativus* ranging from 8 to 893 (average 188) conidia/g of soil in 100 cultivated fields in Saskatchewan.

Many fungicides have been tested for control of common root rot of wheat. Mills and Wallace (15, 16) and Richardson (17) applied various fungicides to eradicate *C. sativus* from wheat and barley seeds. Although successful in laboratory tests, these fungicides did not control the disease in the field. The ineffectiveness of seed-treatment fungicides may be attributed to the fact that control of *C. sativus* on or around the seeds does not necessarily prevent infection of the basal stems and subcrown internodes. Chinn (1) was able to control common root rot by mixing 5 and 10 mg Panogen PX per kilogram of infested soil. The treatment, however, is impractical because of cost and residues.

Horsfall and Rich (9, 10) reported antisporulant activity for several chlorinated aliphatic compounds in vitro and more recently Garraway (6) showed that thiamine at 1 μg/ml inhibited sporulation but not mycelial growth of *Helminthosporium maydis*. These authors suggested that inhibiting sporulation of fungal pathogens might provide a means of reducing the inoculum level.

Preliminary results with 11 fungicides in field experiments in 1973 indicated that, although none prevented infection of wheat (*Triticum aestivum* L.) plants by *C. sativus*, four reduced sporulation of *C. sativus* on the basal stems. This paper confirms and expands these preliminary findings.

MATERIALS AND METHODS

Seven fungicides were assessed for effect on sporulation of *C. sativus* on the basal stems and subcrown internodes of a root rot-susceptible, hard red spring wheat cultivar, Cypress. The wheat was seeded in late May 1974 in experimental fields at Saskatoon and Scott, Saskatchewan. A test at each location comprised 28 plots separated laterally by 60-cm and distally by 120-cm pathways. Plots were 180 cm long and contained four rows 30 cm apart. Seeding rate was 125 seeds per row sown 5 cm deep.

The names, active ingredients (a.i.), percent a.i. of the seven fungicides, and sources were: Benlate (benomyl), methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate, 50%, Du Pont of Canada Ltd.; Cyprex 65-W (dodine),

dodecylguanidine acetate, 65%, Cyanamid of Canada Ltd.; Duter, triphenyltinhydroxide, 19%, CIBA-GEIGY, Canada Ltd.; LFA 2043 (26,019 RP), 1-(isopropylcarbamoyl)-3-(3,5-dichlorophenyl) hydantoin, 50%, May and Baker Ltd. England; Manzate D (maneb), manganese ethylenebisdithiocarbamate, 80%, Du Pont of Canada Ltd.; NC 5936, 2,3,5-trichloromuconononitrile, 50%, Fisons (Canada) Ltd.; and Panogen 15, methylmercury dicyandiamide, 2.2%, Green Cross Products, Canada, Ltd. Duter, Manzate D, NC 5936, and Panogen 15 had been used in the preliminary tests; only NC 5936 appeared ineffective. Benlate and Cyprex 65-W were selected because they inhibited conidiation and ascospore development of *Venturia inaequalis* on infected apple leaves (14, 19). LFA 2043, an experimental fungicide, was included since it was reported to have high activity against *Cochliobolus* spp. (13). The dosage rates of the fungicides (Table 2) were based partially on preliminary tests but were mainly chosen arbitrarily.

Each fungicide was mixed with 500 ml of distilled water and sprayed on the basal 10-cm of stems of the four rows of plants in a plot using a 1.2-liter pneumatic hand sprayer (Green Cross Ltd. of Canada). Some of the spray probably reached the crowns and subcrown internodes. The seven fungicidal spray treatments and a control (water only) treatment were applied to random plots on each of three dates. Four plots were untreated. To determine the initial date of application, a few plants were taken from the untreated plots at 2- to 4-day intervals beginning in the third week of June. The basal stems and leaves arising from the primary axes of the two-to-five tillers that commonly occurred on each plant and the subcrown internode were examined with a stereoscopic microscope at ×54 for signs of conidiophore development and production of conidia of *C. sativus*. Examination of the basal stems generally consisted of the first 5-6 cm above the crown because no fungal structures were seen beyond that distance. The basal stems and leaves of each plant were grouped together as a unit and referred to as basal stems. The first spray was applied when the first conidium was seen. Dates for subsequent sprayings were set arbitrarily. At Saskatoon, the three dates of spraying were 15 and 29 July, and 6 August; and at Scott 7 and 19 July, and 5 August.

The effectiveness of a fungicide spray in reducing sporulation was based on an estimate of the number of conidia present on the basal stems and subcrown internodes. Five plants were taken randomly between 5 and 10 September at Saskatoon and between 15 and 20 September at Scott from the outer rows of each plot, examined, and indexed from 1 to 5 as follows:

Estimated number of conidia			sporulation index of
	0	=	1
	1 to 5	=	2
	6 to 100	=	3
	101 to 1,000	=	4
	over 1,000	=	5

Estimating or counting the number of conidia on basal stems and subcrown internodes with sporulation indices of 1, 2, and 5 was not difficult. Often borderline cases were encountered with those given sporulation indices of 3 and

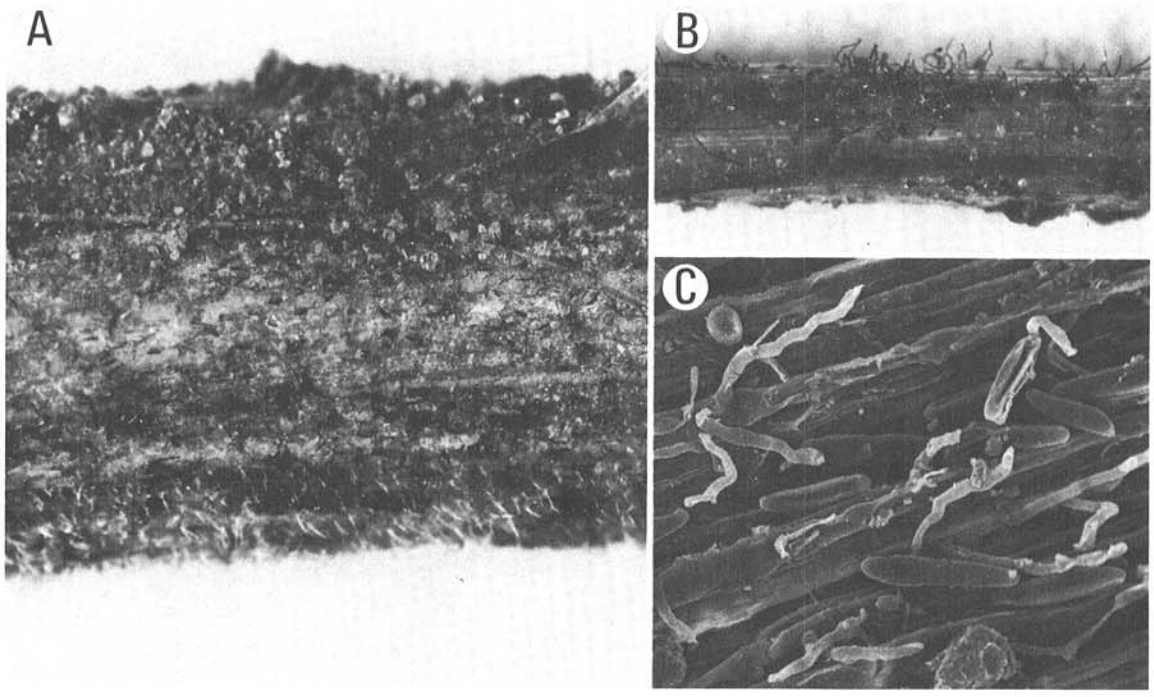

Fig. 1-(A to C). Conidia and conidiophores of *Cochliobolus sativus* on basal stem or subcrown internode of wheat cultivar Cypress. **A)** on basal stem (×22), **B)** on subcrown internode (×22), and **C)** on basal stem under a scanning electron microscope (×210).

4. Figure 1 shows conidiophores and conidia on sections of basal stem or subcrown internode.

Because the dependent variables are polychotomous (i.e., qualitative or categorical data) the data were analyzed by the maximum likelihood procedures described by Fienberg (5) and Goodman (8), and used more recently by Gavora et al. (7). Since the data are in the form of a table of counts or a tridimensional contingency table with the categories being fungicide treatment, date of application, and sporulation index, maximum likelihood estimates of the expected frequencies were carried out for a number of models. The appropriate $\log_e$ likelihood ratio statistic was calculated for each of these models. This statistic approximately follows the chi-square distribution and was used to test hypotheses on how well the various models described the data.

Common root rot ratings on wheat plants were made according to the procedure of Ledingham et al. (12) using 100 plants taken in early September from the outer rows of the plots treated on 15 July at Saskatoon and on 7 July at Scott.

Determinations were made on the populations of *C. sativus* conidia in the soil adjacent to the basal stems and subcrown internodes as follows: Soon after maturity in September, the crops were swathed and the swath removed; then in early October, soil and stubble were taken from each of the two center rows of the treated plots and processed as previously reported (3). The soil so obtained is referred to as the "plant-row" soil. One determination of the total and viable population of *C. sativus* conidia was made of each plant-row soil on a dry weight basis according to the flotation-viability method (4). Average number of conidia in the two center rows was recorded. If determinations could not be made immediately, soil was stored at 2 C. Counts were completed in February 1975. Determinations also were

TABLE 1. Multi-dimensional contingency table analysis of numbers of plants with different sporulation indices of *Cochliobolus sativus* on basal stems or subcrown internodes pooled over three dates of application of fungicide sprays on Cypress wheat at Saskatoon and Scott, Saskatchewan

Tissue and treatment	Saskatoon		Scott	
	df	$-2 \times$ LLR[b]	df	$-2 \times$ LLR[b]
Basal stems				
All treatments	28	139.54**[a]	21	67.11**
Seven fungicides	24	114.19**	18	55.93**
Control vs. seven fung.	4	25.35**	3	11.18*
Subcrown internodes				
All treatments	21	20.77	14	11.72
Seven fungicides	18	16.29	12	17.49
Control vs. seven fung.	3	4.48	2	0.23

[a]The asterisks indicate (**) statistically significant difference, $P = 0.01$; and (*) statistically significant difference, $P = 0.05$.
[b]The abbreviation $-2 \times$ LLR stands for the negative of two times the log likelihood ratio.

TABLE 2. Effect of fungicide sprays on sporulation indices of *Cochliobolus sativus* on basal stems of Cypress wheat at Saskatoon and Scott, Saskatchewan

Location	Fungicide	Dosage rate[a] (g)	Average sporulation index on five basal stems from plots sprayed on			
			15 July	29 July	6 August	Average
Saskatoon	Benlate	2.0	4.4	4.8	4.4	4.5
	Cyprex 65-W	2.6	3.4	3.8	2.8	3.3
	Duter	1.6	2.4	1.4	1.6	1.8
	LFA 2043	2.0	2.0	2.0	1.2	1.7
	Manzate D	3.2	3.4	3.8	2.6	3.3
	NC 5936	0.4	4.4	3.6	4.2	4.1
	Panogen 15	0.18	1.8	2.0	1.8	1.9
	Avg.		3.1	3.1	2.7	2.9
	Control		4.2	4.8	4.8	4.6
			7 July	27 July	5 August	Average
Scott	Benlate	2.0	4.0	3.6	2.8	3.5
	Cyprex 65-W	2.6	3.0	3.4	2.6	3.0
	Duter	1.6	1.6	2.0	2.4	2.0
	LFA 2043	2.0	2.2	1.8	1.8	1.9
	Manzate D	3.2	3.2	2.6	2.2	2.7
	NC 5936	0.4	3.2	3.0	2.4	2.9
	Panogen 15	0.18	1.6	1.4	2.0	1.7
	Avg.		2.7	2.5	2.3	2.5
	Control		3.4	3.6	3.2	3.4

[a]Gram of active ingredient of fungicide applied per plot, each consisting of four 180-cm rows.

made on total populations of *C. sativus* from eight soil samples taken randomly along the pathways in September 1974. The data were analyzed and differences between means were determined by Duncan's multiple range test.

RESULTS

The average sporulation index on basal stems sprayed with fungicide was significantly different from those sprayed with water (control) at both Saskatoon and Scott (Table 1). Duter, LFA 2043, and Panogen 15 were the most effective fungicides (Table 2). In general, date of spraying did not influence the sporulation index on this part of the plant. No difference in average sporulation index occurred on the subcrown internode; it ranged between 1.7 and 2.5 at Saskatoon, and between 1.3 and 1.8 at Scott regardless of treatment and date of application. With minor exceptions, sporulation indices on the basal stems and subcrown internodes that received similar treatments were higher at Saskatoon than at Scott.

Averages of 56 and six conidia per gram of soil, respectively, were found in the eight soil samples from the pathways at Saskatoon and Scott. Since these conidia had probably been produced in previous years they were considered residual and were subtracted from the population in the plant-row soil to give the number produced by the crop in the current year.

In general, the lowest conidial population of *C. sativus* in plant-row soil occurred after spraying with LFA 2043, Panogen 15, and Duter followed by Manzate D (Table 3). Cyprex 65-W and NC 5936 had no appreciable effect and slightly higher values were associated with Benlate treatment. Average viabilities of conidia in the plant-row soil following spraying with water were 87.5 and 93.2%, whereas those with the seven fungicides ranged between 57.7 and 91.1%, and 81.7 and 96.1% at Saskatoon and Scott, respectively. Of the seven fungicides, Duter influenced viability most markedly, especially at Saskatoon. With few exceptions, date of spraying did not influence number or viability.

Conidial populations of *C. sativus* in plant-row soil following similar spray treatment were higher at Saskatoon than at Scott. Populations ranged from eight times higher following spraying with Duter to 16 times higher with water and 17 times higher with NC 5936.

Incidence of infection of plants sprayed with water or fungicides on 15 July at Saskatoon ranged between 84 and 99, averaging 91.7% and on 7 July at Scott between 92 and 100, averaging 95.3%. Disease ratings ranged between 36.4 and 56.6, averaging 46.7% at Saskatoon, and between 37.6 and 55.9, averaging 44.6% at Scott. Thus, the fungicides gave no disease control. In general, this confirms the findings made in the preliminary test in 1973 in which plants were sprayed at an earlier growth stage.

DISCUSSION

Fungicides applied as sprays to the basal stems of wheat reduced sporulation of *C. sativus* on the basal stems, but not on the subcrown internodes. The lack of influence on subcrown internodes likely was due to the inability of the fungicides to reach this plant part which was usually 2-5 cm below the soil surface. Reduced sporulation on the basal stems was reflected by fewer conidia of *C. sativus* in the plant-row soil. Three of the fungicides (Duter, LFA 2043, and Panogen 15) were particularly effective; others were slightly less so or ineffective. Benlate increased numbers of conidia in the soil. Perhaps this fungicide was innocuous to *C. sativus* but was active against some of the microorganisms antagonistic to it on the basal stems, thus providing a more suitable condition for sporulation.

To control common root rot of wheat, a greater

TABLE 3. Average total population and viability of *Cochliobolus sativus* conidia in plant-row soil following spraying of the basal stems of Cypress wheat with fungicides at three dates at Saskatoon and Scott, Saskatchewan

Location	Fungicide	Total number of conidia per gram of plant/row soil in plot sprayed on				Average viability of conidia (%)
		15 July	29 July	6 August	Average[z]	
Saskatoon	Benlate	6,023	4,803	5,498	5,441 a	91.1
	Cyprex 65-W	4,539	3,238	3,175	3,651 b	90.8
	Duter	1,704	700	584	996 c	57.7
	LFA 2043	724	775	714	738 c	73.1
	Manzate D	2,241	2,571	1,434	2,077 c	80.2
	NC 5936	5,331	3,662	2,640	3,878 b	88.1
	Panogen 15	1,127	1,200	1,126	1,151 c	78.0
	Control	3,999	4,154	3,983	4,045 b	87.5
		17 July	21 July	5 August	Average[z]	
Scott	Benlate	598	327	311	412 a	96.1
	Cyprex 65-W	320	249	369	312 ab	93.6
	Duter	128	160	86	125 bc	82.7
	LFA 2043	58	78	29	55 c	81.7
	Manzate D	86	242	163	180 bc	88.4
	NC 5936	269	208	192	223 abc	94.9
	Panogen 15	43	92	70	68 c	82.6
	Control	255	249	264	256 abc	93.2

[z]Duncan's new multiple range test; values differing ($P = 0.05$) are followed by different letters.

reduction of *C. sativus* conidia in the soil than that observed in the present work is required since some studies (2, 11) have shown that a number as low as 27 conidia/g of soil can perpetuate the disease. To attain this objective, more must be known about sporulation of *C. sativus* on the basal stems and subcrown internodes of cereals under natural conditions and the factors that may enhance effectiveness of the fungicide sprays.

Sporulation began prior to mid-July which was earlier than had been anticipated since Spurr and Kiesling (18) did not find conidia in their study till the end of July in Michigan where cereals presumably mature earlier than on the Canadian prairie.

It is important to know why sporulation was more prolific on the basal stems than the subcrown internodes. Some of the differences may be due to the larger surface area of the basal stems which are longer and have larger diameters as compared to the shorter and smaller subcrown internodes. Furthermore, there are several stems and only one subcrown internode per plant. Differences in the nutritive constituents of the basal stems and subcrown internodes and in moisture, light, and aeration at these two sites also could contribute to sporulation. Sporulation, however, seems to be independent of the degree of infection. Although infection often was more pronounced on the subcrown internodes, sporulation was less evident there than on the basal stems. Estimates of counts at these two sites suggest that less than 10% of the *C. sativus* conidia in the plant-row soil came from the subcrown internodes with the remainder coming from the basal stems.

Sporulation, especially on the basal stems, and conidial populations were much higher at Saskatoon than at Scott. Environmental factors, such as soil types and plant nutrients, were suggested as possible causes for these differences in a previous study (3).

It is of interest to note the tremendous capacity of plants to support sporulation by *C. sativus*. At Saskatoon, 4,045 conidia were found in each gram of the 5 kg of plant-row soil from the control plot, thus indicating a total of about 20 million conidia. Assuming that 100 of the 125 seeds planted per row germinated and the plants matured, then each plant contributed about 200,000 conidia to the plant-row soil. When the basal stems of plants were examined on 5 September, many plants had a sporulation index of 5 indicating an estimated conidial number exceeding 1,000. None of the plants, however, appeared to possess more than 10 times this number. In fact, many had a sporulation index of 4 indicating conidial numbers under 1,000. This suggests that conidial production was prolific between 5 September and early October, when the soil was taken for analysis.

More must be known about the mechanism and factors influencing sporulation before an effective treatment can be developed for the control of common root rot. It is likely that more effective fungicides will be found since only 14 have been tested to date. Effectiveness may be improved by equipment that can give better coverage. Duter, in this study, not only influenced total number of *C. sativus* conidia but it also reduced the viability of the conidia, an important consideration.

From a practical standpoint, spraying should be done at times other than in mid-July and August. Aside from physical injuries to the crops if mechanized ground units were used, it is unlikely that sprays would reach the basal stems because the plant canopy at these dates of spraying is quite dense. Spraying experiments are now being made on plants at the four- to five-leaf stage and on stubble following harvest. Effect of spraying on common root rot of wheat also is under investigation.

LITERATURE CITED

1. CHINN, S. H. F. 1971. Biological effect of Panogen PX in soil on common root rot and growth response of wheat seedlings. Phytopathology 61:98-101.
2. CHINN, S. H. F. 1976. Influence of rape in rotation on prevalence of Cochliobolus sativus conidia and common root rot of wheat. Can. J. Plant Sci. 56:199-201.
3. CHINN, S. H. F. 1976. Cochliobolus sativus conidia populations in soils following various cereal crops. Phytopathology 66:1082-1084.
4. CHINN, S. H. F., R. J. LEDINGHAM, and B. J. SALLANS. 1960. Population and viability studies of Helminthosporium sativum in field soils. Can. J. Bot. 38:533-539.
5. FIENBERG, S. E. 1970. The analysis of multidimensional contingency tables. Ecology 51:419-433.
6. GARRAWAY, M. O. 1973. Sporulation in Helminthosporium maydis: inhibition by thiamine. Phytopathology 63:900-902.
7. GAVORA, J. S., E. S. MERRITT, A. A. GRUNDER, and R. S. GOWE. 1975. Effects of strain of chickens and vaccination with turkey herpesvirus on Marek's disease and lymphoid leukosis in breeding stocks. Br. Poult. Sci. 4:375-388.
8. GOODMAN, L. A. 1971. The analysis of multidimensional contingency tables: stepwise procedures and direct estimation methods for building models for multiple classification. Technometrics 13:33-61.
9. HORSFALL, J. G., and S. RICH. 1959. Antisporulant action of 2-(trichloropropyl)-benzothiazole. Phytopathology 49:541 (Abstr.).
10. HORSFALL, J. G., and S. RICH. 1960. Antisporulant action of hexachloro-2-propenol. Phytopathology 50:640 (Abstr.).
11. LEDINGHAM, R. J. 1961. Crop rotations and common root rot in wheat. Can. J. Plant Sci. 41:479-486.
12. LEDINGHAM, R. J., B. J. SALLANS, and A. WENHARDT. 1960. Influence of cultural practices on incidence of common root rot in wheat in Saskatchewan. Can. J. Plant Sci. 49:632-634.
13. MAY and BAKER, LIMITED. 1974. Technical data sheet, Report No. AR.864, Dagenham, Essex, England. 11 p.
14. MC INTOSH, D. L. 1969. A low-volume postharvest spray of benomyl prevents ascospore production in apple leaves infected by Venturia inaequalis. Plant Dis. Rep. 53:816-817.
15. MILLS, J. T., and H. A. H. WALLACE. 1968. Determination of selective action of fungicides on the microflora of barley seed. Can. J. Plant Sci. 48:587-594.
16. MILLS, J. T., and H. A. H. WALLACE. 1970. Differential action of fungicides upon fungi occurring on barley seed. Can. J. Plant Sci. 50:129-136.
17. RICHARDSON, L. T. 1972. Effectiveness of systemic fungicide seed dressing as protectants of barley seedlings against Cochliobolus sativus. Can. J. Plant Sci. 52:949-953.
18. SPURR, H. W., and R. L. KIESLING. 1961. Field and host studies of parasitism by Helminthosporium sorokinianum. Plant Dis. Rep. 43:941-943.

19. SZKOLNIK, M., J. R. NEVILL, and L. M. HENECKE. 1973. Fungicidal inhibition of production of new conidia from established foliar apple scab lesions. Phytopathology 63:208 (Abstr.).