Seed Treatment for Control of Pyrenophora Leaf Stripe of Barley

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ABSTRACT

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The occurrence in 1978 of *Pyrenophora graminea* in a commercial field of Summit barley in Montana necessitated screening numerous registered and experimental seed treatment fungicides for efficacy in controlling barley leaf stripe. Tests were conducted over 2 yr and involved applying materials at varying rates. Seed treatment increased emergence, decreased infection, and increased yield. Seven experimental fungicides completely controlled the disease. Delayed seeding increased emergence and decreased both infection percentage and yield. The fungicides had no residual effect on emergence or infection when seed harvested from the field was grown in the greenhouse.

Additional key words: fungicides, Helminthosporium stripe, *Hordeum vulgare*

Barley leaf stripe caused by *Pyrenophora graminea* Ito & Kurib. (conidial state: *Drechslera graminea* (Rab. ex Schlecht.) Shoem., syn. *Helminthosporium gramineum* Rab. ex Schlecht.) is a seedborne disease in which barley (*Hordeum vulgare* L.) seedlings are systemically infected coincidentally with germination (10). Infection and symptom development are favored by low temperature (<15 C) during the first 4 wk of development (6,11,17). The initial faint chlorotic leaf striping that appears soon after emergence develops into widespread necrosis and decimates a susceptible plant before maturity. Infected plants produce lightweight seed, reducing yield 75–80% (7,15). However, during commercial harvest this lightweight seed is usually lost, resulting in a 100% yield loss per infected plant (13,16).

Spores produced on leaves infect ovaries near flowering. At maturity, resting mycelium of *P. graminea* is found in the hull and pericarp (10). These locations provide excellent protection for the fungus, making control by chemical means difficult. The volatile nature of the organomercurial fungicides made these compounds effective against barley stripe (6,12). However, because of their deleterious effects in the environment, mercurial fungicides were banned from interstate transport in the early 1970s, and supplies are now depleted.

P. graminea was first identified in Montana in 1977. The pathogen was found throughout the state in experimental plots containing barley lines of European origin (9). In 1979, one commercial field of Summit barley contained 25–30% stripe-infected plants.

Summit is a two-row spring barley developed in England. Regional testing in the United States began in 1972. When Summit was grown in 1978 as breeders seed, the plants were known to carry a trace of barley stripe. In 1979, after its release as a variety, an irrigated field in Montana was found that contained 30% infected plants. Laboratory assay of the seed harvested from this field determined that the infection level in the seed was >90%. In two growing seasons, the infection level increased from a trace to 30% to >90%.

Although much of Montana's barley is produced in an arid environment, a condition that would reduce the possibility of a barley stripe epidemic (9), 23% is currently produced under irrigation, and the proportion is likely to increase. The introduction of *P. graminea* into irrigated seed-producing areas could have serious consequences for barley production in Montana and other western states.

Before 1980, no research on the effectiveness of the newer systemic fungicides for controlling barley stripe under Montana's environmental conditions had been reported. At present, only 2-(thiocyanomethylthio)benzothiazole and carboxin + thiram formulations are registered for use against this disease. There have been conflicting reports on the efficacy of carboxin for controlling barley stripe (3,11). Kline and Roane have reported that twice the recommended rate of carboxin + thiram provides control (4).

This paper reports on the efficacy of numerous systemic seed treatments against *P. graminea*. Fungicide application rate and planting date are also considered.

MATERIALS AND METHODS

The research was conducted at the Montana State University Arthur Post Experimental Farm during two harvest years. Consequently, the materials and methods as well as the results are divided chronologically.

1980. Seed of Summit barley produced in 1979 with >90% infection was used. The infection percentage of this seed was determined by plating 100 surface-sterilized (3 min in 0.5% sodium hypochlorite) half-seeds onto water agar. From those seeds with fungal growth, mycelia were transferred to potato-dextrose agar. Because this fungus does not sporulate readily in culture, identification was based on culture pigmentation and morphology. In addition, this seed lot was planted in the greenhouse in the manner described below. The infection percentage observed was found to be >90%, thus substantiating the laboratory assay.

Five systemic fungicides were applied to this seed lot at one and two times the label recommended rate, in the manner described below. The materials tested were carboxin (17%) + thiram (17%) (Uniroyal); carboxin (5%) + thiram (10%) (Evershield RTU 1050, Cargill); fenapronil (24%; Rohm & Haas); methfuroxam (8.4%; Uniroyal); and 2-(thiocyanomethylthio)benzothiazole (30%; TCMTB, Wilbur Ellis). In addition, ethylmercury *p*-toluene sulfonamide (DuPont) and an untreated check were utilized as controls.

The liquid formulations were diluted to 5 ml with distilled water and sprayed onto 82 g of seed. Ethylmercury *p*-toluene sulfonamide was dusted over the seed surface. Each lot of treated seed was mechanically tumbled at 35 rpm for 3 min to ensure uniform coverage. The seed was planted on 29 April and 20 May 1980 in single-row, 3.6-m plots (150 seeds per row) in a split plot design with four replicates. Planting date was considered to be the main plot effect, with seed treatments handled as subplots.

Emergence and infection counts from

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each seeding date were determined at Feekes' scale one and three, respectively (5). The entire row was read. At maturity, the center 2.4 m was harvested from each plot for yield and thousand kernel weight measurements. Airflow through the Vogel plot thresher was minimal, to reduce the loss of light seed.

A sample of 125 seeds from each plot harvested in August 1980 was planted in the greenhouse to determine the incidence of pathogen transmission from one year's crop to the next. The seed was planted in flats 30.5 × 20.3 × 6.4 cm, filled with sand, watered sparingly, and maintained at a 5/21 C (night/day) temperature regime, with 4 hr supplemental fluorescent lighting daily. Emergence and infection readings were made at the one- and three-leaf stage, respectively.

1981. The seed used had 20% *P. graminea* infection as determined by the greenhouse seedling assay described above. Seven experimental systemic fungicides were each applied at five rates, covering a 16-fold increase from lowest to highest rate. The materials tested were 1[2-(2,4-dichlorophenyl)-4-ethyl-1,3-dioxolan-2-ylmethyl]-1 *H*-1,2,4-triazole (13.5%; CGA 64251, Ciba-Geigy); 2,5-dimethyl-*N*-cyclohexyl-*N*-methoxy-3-furancarboximide (50%; Gus 215, Gustafson); fenapronil (22.2%; Rohm & Haas); imazalil (5%; Wilbur Ellis); nuarimol (97.9%; EL-228, Eli Lilly); prochloraz (40%; Boots Chemical); and triadimenol (14%; Mobay). Carboxin (10%) + thiram (10%) (RTU 1010, Cargill); carboxin (17%) + thiram (17%) (Uniroyal); and ethylmercury *p*-toluene sulfonamide (1.93%; DuPont) were used for treated controls.

The infected seed was treated in 50-g lots by dusting the dry formulations (mercury and nuarimol) over the seed surface or by spraying the liquid formulations onto the seed. Triadimenol and the carboxin + thiram formulations were diluted to 3 ml with distilled water before application. The other liquid formulations are not miscible with water and were diluted to 3 ml in 95% ethanol before application. Each lot of treated seed was tumbled as described earlier. Seed treated with mercury was kept in a sealed container for 24 hr. A noninfected control was also used to help determine the actual yield reduction due to infection by *P. graminea*.

The seed was planted on 16 April 1981 in single-row (150 seeds per row), 3.6-m plots in a randomized block design replicated four times. Infection counts and observations on phytotoxicity were taken when the plants had reached growth stages one and two (Feekes' scale). At maturity, 2.4 m was harvested from the center of each row for yield determinations.

RESULTS

1980. Statistical analysis indicated that the fungicides tested responded in a similar manner on both planting dates. Data presented in tabular form are from the 29 April planting date only. When applied at the recommended rate, all of the fungicides tested increased emergence (Table 1). Fenapronil when applied at twice the recommended rate was phytotoxic. Emergence from treated seed increased 169% compared with the nontreated check. A 3-wk delay in seeding date increased emergence of nontreated seed by 62% and treated seed by 5.7%. Doubling the application rates did not significantly increase emergence.

The incidence of barley stripe infection was reduced in all treatments (Table 1). In general, doubling the application rates resulted in an average of 35% less infection than occurred at the recommended rates. Delayed seeding reduced infection in both treated and nontreated rows by 43% compared with the earlier seeding date.

Yield was also significantly ($P = 0.05$) increased by the use of seed treatment (Table 1). Infection was highly correlated with yield ($r = -0.79$). The reduction in percentage of infection due to increased application rate resulted in a 12% increase in yield. A 3-wk delay in seeding reduced yield by 30% across all treatments.

Delayed seeding reduced thousand kernel weight an average of 5.5%. Although application rate had no effect on thousand kernel weight, the individual treatments increased seed weight by 4–12%. Data from the greenhouse trials indicated that seed harvested from the early seeding date had 11% higher emergence than seed harvested from the latter seeding date. Neither application rate nor individual chemicals had a residual effect on emergence. The percentage of seed infected was 26% lower in seed harvested from the early date of seeding compared with the later date. Infection levels were not significantly affected by either the rate of application or the chemical formulation used.

1981. Regardless of the application rate, the seven experimental fungicides significantly ($P = 0.05$) reduced the incidence of barley stripe when compared with the infected, nontreated check (Table 2). With the exception of Gus 215 applied at 0.122 and 0.244 ml a.i./kg and CGA 64251 applied at 0.004 ml a.i./kg, nearly 100% disease control was obtained. Mercury-treated seed produced 100% healthy plants.

Plots with barley stripe infection yielded 22% less than plots with 100% healthy plants. Five of seven treatments were phytotoxic at the highest application (Table 2). Plots planted with seed treated with Gus 215 applied at 0.122 ml a.i./kg and the formulation with 10% each of carboxin + thiram did not yield significantly ($P = 0.05$) better than those plots planted with nontreated seed. Several of the plots treated with nuarimol and imazalil yielded as much as 15% more than the healthy control (Table 2).

DISCUSSION

Our results indicate that seed treatment can be important in preventing barley leaf stripe. The newer systemic materials now under evaluation are particularly promising. Materials currently registered significantly reduced the level of stripe infection, but none completely controlled it. Our data are evidence that organomercurial compounds control barley stripe if the volatile nature of these compounds is utilized. Although the mercury data in Tables 1 and 2 appear contradictory, the complete control obtained in the second year can be attributed to the mercury-treated seed in 1981 being kept in a sealed container for 24 hr before planting. In 1980, the treated seed was planted immediately.

Although all materials reduced the impact of the seedling blight phase of this disease, the low correlation ($r = -0.42$) between increased emergence and decreased infection could imply that disease control is dependent on how

Table 1. Effect of fungicide seed treatment and application rate on emergence, infection, and yield of Summit barley seed infected with >90% *Pyrenophora graminae*

Fungicide	Rate: ml or (g) a.i. per kg of seed	Emergency (%)	Infection (%)	Yield (hl/ha)
Methfuroxam (8.4%)	0.33	64.7 b[y]	28.3 cd	83.4 cd
	0.65	61.7 b	18.0 bc	81.4 cd
Carboxin (17%) + thiram (17%)	0.88	67.5 bc	30.3 cd	68.6 bcd
	1.76	70.7 bc	11.5 ab	90.1 d
Carboxin (5%) + thiram (10%)	0.42	65.3 b	47.5 e	47.0 ab
	0.84	64.0 b	49.8 e	52.5 abc
TCMTB[z] (30%)	0.30	69.7 bc	36.5 de	76.5 cd
	0.61	60.2 b	20.3 bc	81.3 cd
Fenapronil (24%)	0.65	56.3 b	0.5 a	96.8 d
	1.30	30.9 a	0.0 a	72.9 bcd
Mercury (1.93%) check	(0.50)	81.5 c	30.8 cd	82.8 cd
Nontreated check	···	39.0 a	72.5 f	29.1 a

[y] Column means with a letter in common are not significantly different at $P = 0.05$ according to Newman-Keuls multiple comparison test.

[z] 2-(Thiocyanomethylthio)benzothiazole.

Table 2. Effect of six experimental fungicides applied at decreasing rates on infection and yield of *Pyrenophora graminae* infected (20%) Summit barley seed

Fungicide	Rate ml or (g) a.i. per kg of seed	Infection[a] (no.)	Yield (hl/ha)
Nuarimol (97.9%)	(0.019)	0.5	95.8
	(0.038)	0.0	85.0
	(0.075)	0.0	89.7
	(0.150)	0.0	83.4
	(0.300)	0.3	87.8
Prochloraz (40%)	0.050	0.0	90.0
	0.100	0.0	90.9
	0.200	0.3	94.0
	0.350	0.5	87.3
	0.500	0.0	63.6[b]
Fenapronil (22.2%)	0.041	0.3	93.9
	0.081	0.0	91.7
	0.163	0.3	87.8
	0.325	0.0	92.9
	0.650	0.0	79.0[b]
Imazalil (5%)	0.006	0.5	95.3
	0.013	0.3	94.8
	0.025	0.3	83.4
	0.050	0.0	86.6
	0.100	0.3	88.7
CGA 64251 (13.5%)	0.004	7.5	81.1
	0.007	1.3	89.9
	0.015	0.0	83.4
	0.029	0.0	83.4
	0.059	0.0	71.6[b]
Triadimenol (14%)	0.020	1.0	89.2
	0.041	0.3	91.4
	0.081	0.0	87.4
	0.162	0.0	88.1
	0.325	0.0	74.5[b]
Gus 215 (50%)	0.122	15.5	77.2
	0.244	14.8	86.7
	0.488	0.0	87.5
	0.975	1.8	84.6
	1.951	0.0	81.3[b]
Carboxin (10%) + thiram (10%)	0.572	10.0	79.1
Carboxin (17%) + thiram (17%)	0.663	6.0	85.3
Mercury (1.93%) check	(0.050)	0.0	84.8
Infected nontreated check	...	28.0	66.5
Healthy nontreated check	...	0.0	81.4
LSD ($P = 0.05$)		2.2	12.7

[a] Number of infected plants per 3.6 m of row at Feekes' scale one to two.
[b] These treatments had observable phytoxic symptoms in at least 50% of the replicates.

effectively each chemical reaches mycelium deep within the pericarp.

The reduced infection observed between planting dates in 1980 may be attributed to soil temperatures at seeding. On 29 April, the temperature was 10 C, whereas on 20 May it was 16 C. Lower soil temperatures are conducive to maximum disease development (6,11,17). The harvested seed from the 29 April planting had the lowest level of infection. Although we do not have data to explain this, several variables may be important: a) moisture at flowering and b) more diseased tissue for inoculum production later in the growing season.

An interesting comparison can be made between the three materials that are formulations of carboxin + thiram. In both test years, the formulation containing 17% each carboxin and thiram was observed to be more effective than either of the other two materials, probably because of the greater quantity of each active ingredient when applied at the label recommended rate (Tables 1 and 2).

The detrimental effect of delayed seeding on yield is well known (2) and is not a viable means of reducing the effects of *P. graminea*. This reinforces the importance of seed treatment for disease control, allowing for the earlier seeding that usually results in increased yield.

In 1981, the experimental plot was seeded in an area known to contain *Cochliobolus sativus* (Ito & Kurib.). Imazalil and nuarimol have both been reported to be effective against this pathogen (1,8). The plots that were planted with seed treated with these two materials yielded better than the healthy control. Apparently, both nuarimol and imazalil are effective against *P. graminea* and *C. sativus*.

Five of the six experimental fungicides (ie, nuarimol, prochloraz, fenapronil, imazalil, CGA 64251, and triadimenol) are known inhibitors of ergosterol biosynthesis (14). These five materials were effective in controlling *P. graminea* at low rates. In fact, 95% disease control was obtained with rates of <50 mg a.i./kg of seed. Both imazalil and CGA 64251 were active at <10 mg a.i./kg of seed. In contrast, carboxin, which has been recommended for controlling *P. graminea*, was only 50% effective at 440 mg a.i./kg of seed. The effectiveness and spectrum of activity of these new systemic fungicides is impressive.

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