Prevention of Apothecial Formation in Gloeotinia temulenta by Systemic and Protectant Fungicides

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ABSTRACT

Prevention of apothecial formation in *Gloeotinia temulenta* (blind seed disease) was studied in a greenhouse by application of 28 systemic and 26 protectant fungicides over infected seeds of *Lolium perenne*. For complete or a high degree of apothecial suppression, effective dosages per 92 cm² of soil surface for the most promising systemic compounds were: 1,2-bis(3-ethoxycarbonyl-2-thioureido)benzene, 1,2-bis(3-methoxycarbonyl-2-thioureido)benzene, parinol, and benomyl at 1 to 2 mg, and triarimol at 0.2 to 0.5 mg. Of the protectant-type fungicides tested, only four show promising activity. Cadmium succinate at 1 mg, cadmium chloride at 0.5 to 1 mg, phenyl-5,6-dichloro-2-trifluoromethyl-l-benzimidazolecarboxylate (Lovozal) at 1 mg, and triphenyltin acetate at 2 mg gave either complete or nearly complete suppression of apothecia compared with a very high degree of control from benomyl at 1 mg and triarimol at 0.1 to 0.5 mg/92 cm² of soil surface. These nine compounds were vastly superior to the other chemicals tested, and justify field testing for elimination of ascosporic inoculum of *G. temulenta* to obtain field control of blind seed disease in grasses.

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The burning of straw and stubble in fields of perennial ryegrass, *Lolium perenne* L., has provided outstanding control of blind seed disease in Oregon by killing the causal fungus, *Gloeotinia temulenta* (Prill. & Del.) Wilson, Noble, & Gray, in infected seeds at the soil surface (2). Since 1949, annual field burning has progressively reduced the incidence of blind seed disease, and since 1968 more than 99% of *L. perenne* fields had no disease based on spore recovery tests on cleaned seed (6).

Air pollution by smoke from field burning has resulted in strict regulation, and outlawing of field burning in Oregon is anticipated. Control of blind seed disease is now dependent on field burning (3, 6), and loss of this practice will necessitate development of alternative control methods for several susceptible perennial grasses (1). Breeding for resistance, crop rotation, and seed treatment have been either impractical or inadequate for blind seed disease control. Prevention of seed infection by chemotherapy does not yet appear practical. Only one chemical, benomyl, has shown activity by root uptake after soil application of heavy dosages (4, 5). Elimination of ascosporic inoculum would be an attractive approach to control. Prevention of apothecial formation was obtained with benzimidazole compounds (5); however, some systemics probably will be too expensive for low-income crops such as perennial ryegrass and tall fescue, *Festuca arundinacea* Schreb. In a continuing search for effective chemicals to replace field burning, protectant and systemic fungicides were evaluated for apothecial suppression.

MATERIALS AND METHODS.—Seeds of *L. perenne*, many of which were infected with *G. temulenta*, were placed on the surface of a sandy loam, pH 5.8, soil 8.5 cm deep in 10-cm square plastic pots with four bottom drainage holes (about 500 seeds/pot). The soil and seeds were moistened, and after germination of healthy seeds, the pots were frozen to kill seedlings. The pots were held outdoors overwinter, or at 5 C in a constant temperature chamber for 30 to 90 days to condition the pseudosclerotia for apothecial production.

Just before chemical treatment, the pots were brought into a greenhouse to force apothecial development. The soil was pressed firmly to provide a flat surface area of 92 cm^2 and to prevent the chemicals from running down the inner walls of the pot. The chemicals were applied once in a suspension or solution with sufficient water to aid distribution in a uniform layer of chemical over the surface after the water was absorbed by the soil. Dosages are all expressed as actual ingredient.

The soil surface was maintained continuously moist by holding the pots in plastic saucers constantly supplied with water. Results from three pots treated with each chemical dosage were measured by the counting and removing of mature apothecia with attached seeds at weekly intervals starting 3 or 4 weeks after chemical application for a period of 8 to 10 weeks.

RESULTS.—*Systemic fungicides.*—Most systemic fungicides applied at 2, 4, and 10 mg/92 cm^2 of soil surface failed to suppress apothecial formation, including: symmetrical dichlorotetrafluoroacetone (DCTFA); 1,1,1-trichloro-3-nitro-2-propanol (TCNP); N-tridecyl-2,6-dimethylmorpholine (NIA9211 = BAS2203-F); carboxin; 5,6-dihydro-2-methyl-N-(2-biphenylyl)-1,4-oxathiin-3-carboxamide (F427); 5,6-dihydro-2,2′,3′-trimethyl-1,4-oxathiin-3-carboxanilide (F827); oxycarboxin; 2,4-dimethyl-5-carboxanilidothiazole (G696); 2,4-dimethyl-5-N-(2-methylphenyl) carboxamidothiazole (H115); 1-methyl-3-(6-methoxy-3-pyridyl)-urea (LCS761); 2-methyl benzanilide (BAS3050F); 2-methyl-5,6-dihydro-4-H-pyrane-3-carbonic acid anilide (HOE2989); 4-*n*-butyl-1,2,4-triazole (RH124); chloroneb; P-(2-ethyl-imidazol-2-yl)-P-imidazol-1-yl-N,N-dipropyl (PTA); 1-imidazolylphenylpiperidine (PS); 3-(2-methylpiperidino)propyl 3,4-dichlorobenzoate (EL211); 5-n-butyl-2-ethylamino-4-hydroxy-6-methylpyrimidine (PP149); 5-n-butyl-2-dimethylamine-4-hydroxy-6-methylpyrimidine (PP675); and 4-amino-6-chloro-2-(methylthio)pyrimidine (U8342).

The oxathiin derivative, 5,6-dihydro-2,2′,3′-trimethyl-1,4-oxathiin-3-carboxanilide-4,4-dioxide (F872) prevented apothecia for 5 weeks and reduced the total apothecial production by 80% at 10 mg, but F872 was unsatisfactory at 4 mg. Complete suppression of apothecia was obtained by 10 mg of piperazin-1,4-diyl-*bis*[1-(2,2,2-trichloroethyl)formamide] (W524), and it gave complete suppression for 5 weeks at 2 and 4 mg, although abundant apothecia were produced 6 to 10 weeks after the chemical was applied.

Nearly complete suppression of apothecial formation was obtained by several systemic fungicides at 1 to 4 mg/92 cm^2 of soil surface including: 1,2-bis(3-ethoxycarbonyl-2-thioureido)-benzene (thiophanate) at 4 mg; 1,2-bis(3-methoxycarbonyl-2-thioureido)benzene (thiophanate-methyl) at 2 mg; benomyl at 1 mg; triarimol at 0.25 and 0.5 mg; and parinol at 1 mg.

The compounds selected for further testing for confirmation of activity in comparison with the active systemics previously reported, benomyl and 2-(4-thiazolyl)-benzimidazole (Thiabendazole) (5), were: thiophanate, thiophanate-methyl, triarimol, and parinol. In these tests at reduced rates, benomyl again gave a high degree of control at 1 and 2 mg; whereas, Thiabendazole was unsatisfactory at these rates. The two closely related compounds, thiophanate and thiophanate-methyl, both gave a high degree of control at 2 mg. The greatest potency was shown by triarimol, which gave nearly complete control at 0.5 mg and a high degree of control at 0.1 and 0.2 mg. The promising pyridine compound, parinol, gave nearly perfect control at 1, 2, and 4 mg.

The most promising chemicals were evaluated at reduced dosages in an additional test in which large numbers of apothecia were recovered from untreated seeds. In this test, benomyl gave incomplete control at 0.5, 1, and 1.5 mg. At 0.1, 0.2, and 0.5 mg, parinol was very inferior to triarimol, which gave complete

TABLE 1. Prevention of apothecial formation in *Gloeotinia temulenta* by fungicides applied to infected seeds at the soil surface

Chemical[a]	mg/ 92 cm^2	No. apothecia/attached seeds removed from three pots Weeks after chemical application					
		4	5	6	7	8	9-12
Benomyl	0.5	0/0	24/19	38/27	49/36	9/8	15/15
	1.0	0/0	0/0	3/3	5/5	3/3	15/15
	1.5	0/0	0/0	0/0	19/13	0/0	4/4
Triarimol	0.1	6/2	4/4	3/3	74/34	5/5	48/33
	0.2	0/0	0/0	0/0	17/6	1/1	8/8
	0.5	0/0	0/0	0/0	0/0	0/0	0/0
Parinol[b]	1.0	0/0	0/0	0/0	0/0	0/0	1/1
	2.0	0/0	0/0	0/0	0/0	0/0	4/4
	4.0	0/0	0/0	0/0	0/0	0/0	0/0
Thiophanate-methyl	1.0	0/0	6/5	12/11	35/23	25/19	23/23
	2.0	0/0	0/0	0/0	0/0	3/2	8/7
	4.0	0/0	0/0	0/0	0/0	0/0	1/1
Thiophanate	1.0	40/26	23/18	12/11	10/8	3/2	19/18
	2.0	0/0	0/0	1/1	3/3	1/1	8/8
	4.0	0/0	0/0	0/0	0/0	0/0	2/2
Cadmium succinate	0.5	0/0	10/8	0/0	0/0	0/0	1/1
	1.0	0/0	0/0	0/0	0/0	0/0	0/0
	2.0	0/0	0/0	0/0	0/0	0/0	0/0
Cadmium chloride	0.1	28/13	20/12	12/8	13/7	2/1	7/7
	0.2	45/33	10/5	6/6	0/0	0/0	2/2
	0.5	0/0	0/0	0/0	0/0	0/0	0/0
TPTA[c]	0.5	3/3	60/18	34/30	32/25	19/18	16/15
	1.0	10/5	22/19	5/4	13/8	10/10	9/9
	2.0	3/3	0/0	0/0	1/1	1/1	8/8
Lovozal	0.5	124/78	66/45	17/16	13/7	1/1	5/3
	1.0	0/0	4/4	5/3	0/0	0/0	0/0
	2.0	0/0	0/0	0/0	0/0	0/0	0/0
None		213/186	84/74	36/29	17/12	6/5	19/13

[a] Thiophanate-methyl = 1,2-bis(3-methoxycarbonyl-2-thioureido)benzene. Thiophanate = 1,2-bis(3-ethoxycarbonyl-2-thioureido)benzene. TPTA = triphenyltin acetate. Lovozal = phenyl-5,6-dichloro-2-trifluoromethyl-1-benzimidazolecarboxylate.
[b] Results taken from another test in which 280 apothecia attached to 222 seeds were harvested from three untreated pots.
[c] Results taken from separate test in which 150 apothecia were harvested from 111 seeds in three untreated pots.

control at 0.5 and a high degree of control at 0.2 mg. The two thiophanate compounds again gave good control at 2 mg (Table 1).

Protectant fungicides.—In a first test at 4, 10, and 20 mg/92 cm^2 of soil surface (about 4, 10, and 20 lb./acre), most of the protectant fungicides failed to suppress apothecial formation, including: captan; chloranil; chlorothalonil; sodium-*p*-(dimethylamino)benzenediazosulfonate (Dexon); dichlone; *N*-[(1,1,2,2-tetrachloroethyl)sulfenyl]-*cis*-4-cyclohexene-1,2-dicarboximide (Difolatan); dodine; ferbam; folpet; 2,3-dicyano-1,4-dithiaanthraquinone (Thynon); and zineb.

Some of the protectant fungicides produced good control after application at 20 mg but not at 4 or 10 mg, including: maneb; maneb plus zinc ion (Dithane M-45), thiocyanomethylbutyl sulfone (TCMBS); thiram; and ziram.

A few protectant fungicides significantly reduced apothecial production after application at 10 mg but not at 4 mg including: N-(dichlorofluoromethylthio)-N′,N′-dimethyl-N-phenylsulfamide (BAY47531); mixture of ammoniates of [ethylenebis(dithiocarbamato)] zinc with ethylenebis [dithiocarbamic acid] bimolecular and trimolecular cyclic anhydrosulfides and disulfides (Polyram); and 2-(thiocyanomethylthio)benzothiazole (TCMTB). In tests at 1, 2, and 4 mg, monosodium salts of 2,2′-methylenebis(2,4,6-trichlorophenol) (Isobac 20) proved unsatisfactory.

Several protectant fungicides inhibited apothecial production for 7 weeks after application of 4 mg/92 cm^2 soil surface, including: cadmium succinate; 2-(thiocyanomethylsulfinyl)benzothioazole (TCMTOB), and triphenyltin acetate (TPTA). Nearly complete control was obtained at 4 mg by triphenyltin hydroxide (TPTH). The experimental miticide, phenyl-5,6-dichloro-2-trifluoromethyl-1-benzimidazolecarboxylate (Lovozal), suppressed all apothecia at 4 mg.

The most effective chemicals selected for testing for confirmation of activity and control at lower

dosages included: cadmium succinate, TCMTOB, Lovozal, TPTA, and TPTH. Cadmium chloride was included after activity of cadmium succinate was discovered. Apothecial production was prevented by cadmium succinate and cadmium chloride at 0.5, 1, and 2 mg, by Lovozal at 1 and 2 mg, and by TPTH at 4 mg/92 cm^2. Fair control but incomplete suppression of apothecia was obtained by TPTA at 2 mg, Polyram at 8 mg, TCMTB at 6 and 8 mg, and by TPTH at 4, 6, and 8 mg, but these chemicals are decidedly inferior to the most promising chemicals.

In an additional test, the most promising chemicals were tested at reduced rates to determine minimum effective dosages in comparison with benomyl and triarimol. Both cadmium succinate and cadmium chloride suppressed all apothecia at 1 and 0.5 mg, respectively, comparable to that for triarimol at 0.5 mg. Lovozal was unsatisfactory at 0.5, but gave nearly complete control at 1 mg and complete control at 2 mg (Table 1).

DISCUSSION.—No promising activity was shown by representatives of several groups of systemic chemicals in the present tests, although most of the chemicals have merit for control of various other diseases. Promising activity of benomyl, triarimol, parinol, and the thiophanates suggests that other derivatives of benzimidazoles, pyrimidines, pyridines, and thiophanates should be evaluated for possible activity in suppressing apothecial formation in *G. temulenta.*

Similarly, no promising activity in suppressing apothecial development at low dosages was shown by most representatives of the several groups of protectant and eradicant fungicides tested. Pentachloronitrobenzene (PCNB), PCNB plus 5-ethoxy-3-(trichloromethyl)-2,4-thiadiazole, and 2,6-dichloro-4-nitroaniline were also found ineffective in earlier tests (5).

Activity of cadmium compounds is interesting, because cadmium chloride at the rates shown to be effective could be cheaper than most other fungicides (S. Frederiksen, *personal communication*, Mallinckrodt Chemical Works). Results in present tests warrant tests of cadmium compounds under field conditions.

Although it is better known as an experimental miticide, Lovozal was added to the tests because it is a derivative of benzimidazole, one analog of which (benomyl) has shown promising activity. The good activity of Lovozal indicates that other benzimidazole derivatives should be tested.

Many grass seed crops, particularly Lolium ryegrasses and tall fescue, are low-value crops, and little latitude exists for additional production expense. It is unfortunate, therefore, that the widely used protectants are not more active in suppressing apothecial development, because these fungicides are readily available and would be less expensive than the new systemics.

Certain systemic fungicides, while not used as chemotherapeutants, have shown stronger activity than protectant fungicides generally. The pyrimidine compound, triarimol, consistently has completely suppressed apothecial formation at dosages lower than all other systemic and protectant chemicals tested.

Suppression of apothecia for 4 to 7 weeks after application may be adequate for elimination of ascosporic inoculum by chemicals that are applied in late spring, perhaps within a month before anthesis. In this respect, a portion of the dosage of a multiple-use fungicide applied for foliar disease control would perhaps reach the soil surface in sufficient quantity to contact the infected seeds and coincidentally suppress apothecial formation. Thus, several applications, especially during April and May, for control of foliar diseases of grasses perhaps could provide a cumulative dosage to the soil surface effective for apothecial suppression. The cost of blind seed control by at least some broad-spectrum chemicals hopefully could be offset by simultaneous control of leaf and stem diseases. Results from the present tests indicate, however, that very few systemic or protectant fungicides are sufficiently active to provide apothecial suppression at reasonable dosages.

The practicality of the various chemicals that show activity in suppressing apothecial formation will be determined by the dosage required and cost, because most grass seed crops are low in acre value. Although benomyl is effective, the projected price may limit its use in the near future. Triarimol and certain other pyrimidines and the pyridines are also expected to be expensive (I. F. Brown, *personal communication*, Eli Lilly & Co.). The thiophanates may represent economically feasible chemicals for blind seed disease control.

Control of *G. temulenta* may become possible through prevention of apothecial formation by application of one of several chemicals, but for successful grass seed production, ergot (*Claviceps purpurea*) must also be controlled. The principal grass hosts for *G. temulenta*; viz., *L. perenne* and tall fescue, *Festuca arundinacea*, are susceptible to *C. purpurea* and subject to severe infections in the same areas where blind seed disease is troublesome. Therefore, a chemical to be feasible for blind seed disease should also control ergot, because it is unlikely that more than one chemical can be used economically for control of the two diseases in perennial ryegrass and tall fescue. Tests are, therefore, in progress to determine the effectiveness of these chemicals in suppression of ascocarp formation in *C. purpurea.*

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