Prevention of Apothecial Formation in Gloeotinia temulenta by Benzimidazole Compounds

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ABSTRACT

Methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl) at 2 mg/92 cm^2 (2 lb./acre) in a single application at the soil surface over overwintered, infected seeds of *Lolium perenne* prevented formation of apothecia of *Gloeotinia temulenta*. Most apothecia were also suppressed by 0.5 or 1 mg of benomyl; 2-(4-thiazolyl) benzimidazole (thiabendazole or TBZ) prevented apothecial formation at 2 mg in one test, but failed in a second test; 2-(2-furyl)-benzimidazole (Bayer 33172) and 2,6-dichloro-4-nitroaniline (Botran or DCNA) suppressed apothecia at 10 and 8 mg, respectively. Fairly good control but incomplete suppression of apothecia was obtained with pentachloronitrobenzene (PCNB) at 20 mg and with a mixture of 20 mg PCNB plus 10 mg 5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (Terrazole). Benomyl eliminated apothecia during April-May from a single application made the previous November, December, or January in tests during 2 separate years. Seed infection was prevented after soil application and root uptake in *L. perenne* by benomyl at 60 mg but not by TBZ at 20 through 160 mg/600 ml of soil. Spraying the inflorescences before anthesis with benomyl at 10^{-2}, 10^{-3}, and 10^{-4} did not prevent seed infection. Prevention of apothecial formation by low rates of benzimidazole compounds can eliminate ascosporic inoculum in grass fields, and may provide the first feasible chemical control of blind seed disease in grass seed crops. Phytopathology 60:1259-1261.

Additional key words: systemic-fungicides, apothecial-suppression, blind-seed disease, *Lolium perenne, Gloeotinia temulenta,* benomyl.

Burning straw and stubble in fields after harvest in Oregon has controlled blind seed disease by killing the causal fungus, *Gloeotinia temulenta* (Prill. & Del.) Wilson, Noble, & Gray, in infected seeds of perennial ryegrass, *Lolium perenne* L., at the soil surface (2). Annual field-burning since 1949 has progressively reduced the incidence of *G. temulenta,* and during 1968, 99% of *L. perenne* fields had no disease and 1% had only trace infestations based on spore recovery tests on cleaned seed (1).

Because smoke from field-burning contributes to air pollution in Western Oregon, severe restriction of field-burning is anticipated. Control of blind seed disease in Oregon is critically dependent on field-burning (3), and restricting this practice would necessitate development of substitute control methods for several susceptible grasses (1). Because breeding for resistance, crop rotation, and seed treatment are either impractical or inadequate for blind seed disease control, development of chemical control is imperative.

No satisfactory chemical control of blind seed disease has been reported. Chemotherapeutic prevention of seed infection in *L. perenne* after soil application and root uptake of methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate (benomyl) was reported recently (4); however, heavy dosages were required. A more attractive approach to blind seed disease control would be the elimination of ascosporic inoculum. Promising results on prevention of apothecial formation with benzimidazole compounds in comparison with a few other chemicals are reported here.

MATERIALS AND METHODS.—*Flower inoculations.*—Field plants were dug in midwinter. Sections of crowns were transplanted to 600 ml of a sandy loam soil 8.5 cm deep in 10-cm square plastic pots with four drainage holes and were returned outdoors. After winter conditioning, plants were brought into the greenhouse. Just prior to chemical treatment, the plants and soil were transferred to pots with no drainage holes to prevent loss of chemicals. For study of systemic activity after uptake by roots, chemicals were added to the soil in water suspensions. For evaluation of chemicals in sprays, plants were sprayed to runoff and inverted to drain and dry. Water was added only to soil as needed. Plants were inoculated during anthesis by spraying flowers with a water suspension of macroconidia produced on potato-dextrose agar enriched with peptone and malt extract (1).

Apothecial suppression tests.—Infected seeds (pseudosclerotia) of *L. perenne* were planted on the surface (92 cm^2) of soil 8.5 cm deep in square plastic pots (about 500 seeds/pot) with 4 drainage holes, frozen to kill seedlings, and incubated outdoors over winter. Chemicals were applied once with sufficient water to aid distribution over the soil surface. Pots were brought into the greenhouse in early spring to induce apothecial development. The soil surface was kept continuously moist by holding pots in saucers that were constantly supplied with water. Results from four replications were measured by counting and removing mature apothecia with attached seeds at weekly intervals.

In addition to benomyl, the following chemicals were studied in wettable powders: 2-(4-thiazolyl)-benzimidazole (thiabendazole or TBZ); 2-(2-furyl)-benzimidazole (Bayer 33172); 2,6-dichloro-4-nitroani-

TABLE 1. Suppression of *Gloeotinia temulenta* apothecia by benomyl applied 28 March 1969 over infected *Lolium perenne* seeds at the soil surface

Benomyl/92 cm² (mg)	No. apothecia/infected seeds					
	Dates seeds with apothecia removed during 1969					
	29 April	5 May	12 May	26 May	18 June	Total
0.5	0	0	3/1	1/1	0	4/2
1	0	0	4/3	1/1	0	5/4
2	0	0	0	0	0	0/0
4	0	0	0	0	0	0/0
8	0	0	0	0	0	0/0
None	243/125	266/127	74/40	18/8	4/3	605/303

line (Botran or DCNA); and pentachloronitrobenzene (Terraclor or PCNB). Terraclor Super-X, a mixture containing 10% PCNB and 5% 5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (Terrazole) was tested as a granular formulation.

RESULTS.—*Foliar and inflorescence sprays.*—During 1968, benomyl in single sprays at 10^{-2}, 10^{-3}, and 10^{-4} was applied to *L. perenne* plants in the greenhouse after inflorescences had emerged, but 2 weeks before anthesis. No control of seed infection was apparent after flowers were inoculated during anthesis with a water suspension of macroconidia.

Chemicals applied to soil before flower inoculation.—Benomyl applied to soil around test plants of *L. perenne* during the spring of 1967 prevented seed infection at 80 and 160 mg but gave no apparent control at 20 and 40 mg/600 ml soil. During 1968, benomyl applied to soil again prevented seed infection in *L. perenne* at 60, 80, and 100 mg. TBZ gave no control of seed infection by soil application at 20, 40, 80, and 160 mg/600 ml soil.

Suppression of apothecia.—Benomyl prevented apothecial formation by a single application over seeds overwintered at the soil surface at 4 mg/92 cm² (4 lb./acre) applied 20 December 1967 or 8 January 1968, or at 2 mg applied 22 April 1968, when seeds in pots were moved into the greenhouse. In a separate test, benomyl at 1 mg applied 26 November 1968 prevented

TABLE 2. Prevention of apothecial formation in *Gloeotinia temulenta* by chemicals applied over infected seeds at the soil surface

Chemical/92 cm² (mg)	No. apothecia/infected seeds removed					
	Weeks after chemical applied					
	4	5	6	7	8	Total
Benomyl						
1/32	99/52	111/59	33/19	8/8	11/8	262/146
1/16	52/22	154/79	15/13	11/8	11/7	243/129
1/8		33/22	13/9	4/4	2/1	52/36
1/4		5/2	3/3	0/0	2/1	10/6
1/2	0/0	0/0	3/1	0/0	0/0	3/1
1	0/0	0/0	0/0	0/0	0/0	0/0
TBZ						
2	0/0	0/0	0/0	0/0	0/0	0/0
4	0/0	0/0	0/0	0/0	0/0	0/0
10	0/0	0/0	0/0	0/0	0/0	0/0
20	0/0	0/0	0/0	0/0	0/0	0/0
BAY-33172						
2	30/14	109/56	75/46	14/10	27/20	255/146
4	0/0	20/9	22/16	0/0	11/8	53/33
10	0/0	0/0	0/0	0/0	1/1	1/1
20	0/0	0/0	0/0	0/0	0/0	0/0
DCNA						
0.5	225/92	174/89	12/10	0/0	0/0	411/191
1	103/44	210/108	27/17	0/0	12/7	352/176
2	55/18	167/70	32/19	0/0	10/5	264/112
4	0/0	64/29	35/20	0/0	24/19	123/68
8	0/0	0/0	0/0	0/0	4/1	4/1
PCNB						
2	169/65	174/95	21/14	0/0	1/1	365/175
5	78/30	161/75	56/30	0/0	0/0	295/135
10	12/5	114/45	22/14	0/0	9/4	157/68
20	0/0	10/3	0/0	4/2	0/0	14/5
None	110/68	219/118	32/21	10/8	5/5	376/220
None	187/110	160/90	32/20	3/3	5/4	387/227

formation of apothecia and at 0.5 and 1 mg applied 28 March 1969 suppressed nearly all apothecia (Table 1).

In another test, pots were brought into the greenhouse early in April. Soil and seeds were allowed to dry to forestall apothecial development. Chemicals listed in Table 2 were applied 1 May 1969 in a water suspension to the soil surface, and water was added to saucers that held the test pots to permit apothecial development to resume. Mature apothecia were counted and removed with attached seeds weekly for 5 weeks, beginning 4 weeks after chemicals were applied. Benomyl at 1 mg completely suppressed apothecia, and most apothecia were suppressed at 0.5 and 0.25 mg/92 cm^2. TBZ prevented apothecial development at 2 mg; however, in a more recent test, TBZ failed to prevent apothecial formation at 2 mg/92 cm^2. The other benzimidazole compound, Bayer 33172, and DCNA suppressed apothecia at 10 and 8 mg, respectively, for 4 weeks. Fairly good control but incomplete suppression of apothecia was obtained by 20 mg PCNB. A mixture of 20 mg PCNB and 10 mg Terrazole in a granular formulation was slightly less effective than 20 mg PCNB alone applied as a wettable powder.

DISCUSSION.—Both benomyl and TBZ apparently become systemic in grasses by root uptake after soil application, but only benomyl prevented infection of seeds following inoculation of flowers with macroconidia of *G. temulenta*. Although benomyl provided the first chemotherapeutic control, the heavy rates required will exclude its use in grass crops.

Chemotherapeutic control of *G. temulenta* by systemic fungicides may be possible eventually, but with the pressing need for control methods to substitute for field burning, elimination of ascosporic inoculum through prevention of apothecial formation by chemicals is more likely to provide a feasible chemical control in the near future. Of the three benzimidazole compounds evaluated for apothecial inhibition, benomyl was highly promising; TBZ may be promising; but Bayer 33172 was definitely inferior. Benomyl was also much superior to DCNA, PCNB, and a mixture of PCNB and Terrazole. Attractiveness of benzimidazole compounds is enhanced by the control from benomyl applied either in the fall or spring.

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