Influence of Benzimidazole Fungicides on Phymatotrichum omnivorum and Phymatotrichum Root Rot of Cotton

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ABSTRACT

Two benzimidazole fungicides, Thiabendazole [2-(4- thiazolyl) benzimidazole] and Benlate [methyl 1-(butyl carbamoyl-)2-benzimidazole carbamate], applied as drenches around stems of 1-month-old cotton plants at rates of 2 lb. in 15 gal water/acre, proved effective in controlling *Phymatotrichum* root rot of cotton in greenhouse experiments. Control was based on inhibition of root lesions above the zone of lateral root formation. Benlate was better than Thiabendazole when applied at equivalent wt of active ingredient. Both chemicals were extremely toxic to growth of *Phymatotrichum omnivorum* when added to a synthetic culture medium; Benlate completely suppressed growth at 50 μM/liter, and Thiabendazole at the same concentration reduced growth 90% during a 50-day test. Neither chemical at fungitoxic levels influenced the respiration of germinating *Phymatotrichum* sclerotia. Phytopathology 60:726-728.

Phymatotrichum omnivorum (Shear) Duggar is indigenous to the calcareous soils of southwestern United States and northern Mexico, but it also has been found in over 80% of the counties in Texas (2). The disease is most severe throughout the Blackland, Coastal Bend, and lower Rio Grande regions of Texas.

Many different types of control measures for combating *Phymatotrichum* root rot have been tried, but most of them have proved ineffective or inconsistent. The use of chemicals has been disappointing because the distribution of the fungus within the soil profile where the sclerotia are produced at depths to 8 feet (4, 7) makes the fungus relatively impossible to control by conventional methods. Several attempts have been made to place chemicals deep in the soil to eradicate the pathogen. Rogers (3) placed crude oil 18 inches deep, using rates up to 15,000 gal/acre, and King & Hope (4) injected 1.5% formalin to depths of 6 ft. Recently, Lyda et al. (5) reported that deep placement of large quantities of a volatile dichloropropene-dichloropropane fumigant (Telone) effectively controlled the disease on cotton in Nevada. It was evident that the chemical must be placed deep in the soil to effectively control the pathogen. The large quantities of material required, however, made its use uneconomical for controlling the disease in low cash crops such as alfalfa and cotton. Because of the high cost of eradicative measures, it was decided that other methods should be investigated.

Under field conditions, *Phymatotrichum* root rot symptoms generally occur when the cotton plants begin squaring (early stage of floral initiation), or later. A careful examination of the tap root reveals that mycelial strands ascend the root peripherally, forming a mantle analogous to that of an ectotrophic mycorrhizal fungus. When the fungus reaches the region immediately below the soil surface and above the zone of lateral root formation, the epidermal cells of the root are invaded, followed by a collapse of the vascular tissues and death of the plants. If the fungus can be denied access to this region of the root, the plant should be able to survive in infested soils.

This paper presents the results of several tests using these two benzimidazole fungicides in laboratory and greenhouse studies on *P. omnivorum* and the disease it causes.

MATERIALS AND METHODS.—One isolate of *Phymatotrichum omnivorum,* obtained in 1967 from a diseased cotton plant at the Blackland Conservation Research Center, Temple, Texas, was used in all the tests. Its pathogenicity to cotton had been verified under greenhouse conditions.

Laboratory tests.—P. omnivorum was grown on a synthetic medium (40 g glucose, 1.18 g NH_4NO_3, 1.5 g KH_2PO_4, 1.55 g K_2HPO_4, 1.5 g $CaCO_3$, 750 mg $MgSO_4 \cdot 7\ H_2O$, 150 mg KCl, and 2.5 ppm each of Zn^{++}, Fe^{++}, Cu^{++}, Mo^{+++++}, and Mn^{++}, to one liter distilled water), to which varying concentrations of technical grade Thiabendazole (TBZ) and Benlate were added. The medium was dispensed into 250-ml Erlenmeyer flasks and autoclaved at 121 C for 30 min. Before cooling, 1 ml of fungicidal suspension was added to each medium to give concentrations of 500, 50, 5, or 0.5 μM/liter of each chemical. After a thorough mixing, approximately 20 ml of medium were added to each petri dish. A small disc of mycelium was placed in the center of each plate, and daily measurements were made of the culture diam until the growth on the controls extended across the medium. Periodic readings were continued on other plates up to 50 days, after which the test was discontinued. Eight replications were used for each treatment.

The influence of TBZ and Benlate were tested on the respiration rate of tissue from germinating sclerotia. Sclerotia were produced in sterile soil following a procedure outlined by Dunlap (1), recovered from the soil by wet sieving, and stored under refrigeration for 24 hr. The sclerotia were washed three times with sterile, distilled water and then incubated for 12 hr at 28 C before respiration measurements were made. Approximately 750 mg wet wt of sclerotia were placed in each Warburg vessel, which contained 2.5 ml 0.1 M phosphate buffer, pH 7.0, and 0.5 ml fungicidal solution in the sidearm. The center well contained 0.2 ml 20% KOH plus a 2-cm^2 fluted filter paper. All measurements were made with a Gilson differential respirometer over a 390-min period. The flasks were incubated in a water bath at 28 C. The tissue was permitted to respire in absence of the fungicide for 165 min, and the contents of the sidearm were introduced into the main compartment of the Warburg vessel. Readings were taken for another 225 min.

Three representative sclerotial samples were weighed and oven dried at 100 C for 24 hr to determine per cent moisture. All oxygen uptake data were calculated on 100-mg dry tissue basis.

Greenhouse studies.—Houston Black clay (HBC) soil was screened, and 4.4 kg were placed in each of 32 8-inch plastic pots. Laboratory-grown sclerotia (0.5 g) were placed in the center of the soil mass. Five Lankart 611 acid-delinted cottonseed were planted in each pot, and after emergence the plants were thinned to two/pot. At 30 to 40 days of age, TBZ and Benlate solutions were applied to the base of the cotton stem at a rate of 2 lb. active ingredient in 15 gal water/acre. Calculations were based on 39,640 plants/acre; therefore, 1.43 ml of solution were pipetted onto the stem of each plant at the ground line. Surfactant F (E. I. DuPont, Wilmington, Del.) was used with each chemical at the rate of 4 fluid oz/100 gal. Periodic recordings were made of disease development. This test was conducted twice.

The addition of Benlate to the covering soil at the time of planting was studied also. Rectangular, galvanized metal containers (11.4 × 12.7 × 17.8 cm) were filled with 1.53 kg air-dry HBC soil and gravimetrically adjusted to field capacity by adding 790 ml water. Five Lankart 611 cottonseed were placed on the moist soil surface and covered with the same soil to a depth of 5 cm. This layer of soil was wet with 240-ml Benlate solution containing 50, 5, or 0.5 ppm active ingredient. *Phymatotrichum* sclerotia (0.5 g) were placed in the center of the soil mass by dropping them into a hole made with a 16- × 150-mm test tube after the soil was adjusted to field capacity. There were 14 replications of each treatment, and all containers were completely randomized and maintained at 28 C in a controlled-temperature, circulating water tank. After emergence, the plants were thinned to leave two/container; however, a few plants were lost to causes other than *Phymatotrichum* root rot. Daily readings were taken when symptoms were noted. The test was terminated after 56 days.

RESULTS.—The two benzimidazole derivatives, TBZ and Benlate, inhibited the growth of *P. omnivorum* at very low concentrations (Table 1). Benlate was more inhibitory than TBZ at equal molar concentrations. Benlate completely suppressed the fungus when applied to the medium at 50 μM/liter, and at this dosage, TBZ reduced growth 91% over a 50-day test period. At 5 μM/liter Benlate reduced growth 75% over this same period, but the fungus growing on the medium containing TBZ at this concentration reached its maximum growth in 16 days. On an equal molar basis, TBZ did not appear to be as effective as Benlate in inhibiting the growth of *P. omnivorum*, but on a wt basis this could change since the molecular wt are quite different (e.g., 500 μM/liter of the two chemicals would be the same as 145 ppm Benlate and 100 ppm TBZ).

When tissue of germinating *Phymatotrichum* sclerotia was incubated in the presence of these chemicals, respiration was not affected significantly over a 125-min period (Table 2). No analysis was made to ascertain whether the chemical could be found internally. It was rather surprising to find a compound that was so inhibitory to growth and yet had virtually no influence on the respiration rate of the tissue.

Greenhouse tests.—A delayed, topical application of Benlate to the base of the cotton stem controlled

TABLE 1. The influence of various concentrations of Benlate and Thiabendazole on the growth rate of *Phymatotrichum omnivorum* on a solid, synthetic medium

Fungicide		Days, no.											
		1	2	3	4	7	8	9	16	23	29	39	50
μM/liter ppm		*Colony diam (mm)*[a]											
Benlate													
500	145	0	0	0	0	0	0	0	0	0	0	0	0
50	14.5	0	0	0	0	0	0	0	0	0	0	0	0
5	1.45	0	0	0	0	0	0	2	3	5	8	15	21
0.5	0.14	1	11	20	35	63	69	82					
Thiabendazole													
500	100	0	0	0	0	0	0	0	0	0	0	0	0
50	10	0	0	0	0	0	0	2	3	3	4	8	8
5	1	0	8	12	22	43	52	63	83				
0.5	0.1	4	13	27	41	67	75	83					
Check		2	11	20	30	55	67	83					

[a] Mean of eight replications.

TABLE 2. Respiration of germinating *Phymatotrichum* sclerotia incubated in various concentrations of Benlate and Thiabendazole

Fungicide[a]		Min 30	60	90	165	325	360	390
μM/*liter ppm*		*Cumulative O_2 uptake—μliter/100 mg dry tissue*[b]						
Thiabendazole								
500	100	13	27	41	76	127	146	159
50	10	13	26	39	71	123	144	157
Benlate								
500	145	10	22	34	65	113	135	148
50	14.5	10	21	32	61	104	124	136
5	1.45	12	23	35	63	111	131	143
Check		14	26	39	69	118	140	153

[a] The chemicals were introduced after 165 min.
[b] Mean of three replications.

Phymatotrichum root rot effectively (Table 3). In two separate tests, only 1 plant of 55 died in the Benlate treatment, but 18 out of 56 died in the TBZ treatment. This was still a very marked reduction in disease incidence when compared with the control, where 39 of 42 plants succumbed.

Benlate also proved to be effective when applied to the covering soil (Table 4). The high rate (68 mg/ml) caused noticeable phytotoxicity to the cotton plants; however, a tenfold dilution of this solution did not injure the plants, and prevented symptom development.

DISCUSSION.—The effectiveness of Benlate in controlling *Phymatotrichum* root rot under greenhouse conditions is most encouraging. The superficial nature of the mycelium has been noted since Pammel's second report of the disease in 1889. He stated, "If the roots of a dead cotton stalk or those which are wilting are examined, brown threads of a fungus, *Ozonium auricomum,* LK., are found to closely surround the taproot and some of the lateral roots" (6).

Apparently, a delayed, topical application of Benlate, made to the runoff stage, prevents further ascension of the fungus. In such cases, vascular tissues are not destroyed above the zone of secondary root formation, and the affected plants are capable of surviving by their lateral roots. Once the fungus girdles the taproot, the mycelium does not continue to move up the root. This type of host-parasite relationship appears to restrict further upward movement of the pathogen.

TABLE 3. The effect of topical application of Benlate and Thiabendazole to bases of cotton stems growing in soil artificially infested with *Phymatotrichum omnivorum*[a]

Fungicide[b]	Date applied, 1968	Plant age	Plants Total	Diseased
		days	*no.*	%
Benlate	April 4	31	12	0
Benlate	April 10	37	11	0
Thiabendazole	April 4	31	12	42
Thiabendazole	April 10	37	12	50
Control			12	100
Benlate	June 6	37	16	6
Benlate	June 11	42	16	0
Thiabendazole	June 6	37	16	19
Thiabendazole	June 11	42	16	25
Control			30	90

[a] Plants were grown in soil artificially infested with sclerotia of *P. omnivorum*.
[b] All chemicals were applied at the rate of 2 lb. active ingredient/15 gal water/acre.

TABLE 4. Influence of Benlate on incidence of *Phymatotrichum* root rot of cotton when added to upper 5 cm of soil covering the cottonseed[a]

Concn 50% Benlate	Active ingredient	Plants Total	Diseased
mg/ml[b]	*g*	*no.*	%
68	8.16	24	0
6.8	0.82	26	0
0.7	0.08	26	65
0.0		25	96

[a] The covering soil was adjusted to field capacity by addition of fungicidal suspension. *Phymatotrichum* sclerotia (0.5 g) were placed below the covering soil, which was adjusted to field capacity.
[b] 240 ml of each solution were added to 680 g dry covering soil in each container.

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