Tolerance in Cercospora arachidicola to Benomyl and Related Fungicides

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ABSTRACT

Isolates of *Cercospora arachidicola* tolerant to 5 μg/ml benomyl in vitro were found in six locations in four Georgia counties. Sensitive isolates did not grow on potato-dextrose agar with 0.5 μg/ml, while tolerant isolates grew with 160 μg/ml benomyl. Approximately one-third of the lesions collected from experimental plots that received six applications of Benlate, yielded tolerant isolates. No reduction in peanut yields was evident, however, when compared to other fungicides. Cross-tolerance was also found to methyl thiophanate (Topsin M) and 2-(methoxycarbamoyl)-benzimidazole (BAS 3460).

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Additional key words: methyl thiophanate, peanut, *Arachis hypogaea*.

Cercospora leaf spot caused by *Cercospora arachidicola* Hori is a serious disease of peanuts in the United States and other countries of the world (7). Severe economic losses have been drastically curtailed with modern fungicides (1, 6, 11). Recent reports document tolerance of plant pathogenic fungi to benzimidazole compounds (2, 4, 5, 10, 13). Benlate, a benzimidazole compound has been widely used since 1970 for the control of Cercospora leaf spot of peanuts, and has resulted in dramatic disease control and increased yield (9, 11). Because of recent reports on tolerance among species of *Cercospora*, research was initiated to: (i) determine if benomyl-tolerant strains of *C. arachidicola* were present in Georgia during the 1973 season, and (ii) investigate the influence of benomyl concn in vitro on growth of tolerant and sensitive isolates. A preliminary report has been published (8).

MATERIALS AND METHODS.—Infected peanut leaves were collected during July-August, 1973, from thirteen locations in six Georgia counties where Benlate was used in the spray program. Specimens were also collected 9 October from replicated fungicide spray plots at the Southwest Branch Experiment Station, Plains, Ga. Fungicides were applied every 14 days beginning 19 June and ending 28 August. Disease severity ratings were made, and the peanuts were lifted 9 October. Benomyl-tolerant *Cercospora* isolates were determined by visible growth on potato-dextrose agar (Difco, 39 g/liter, PDA) amended with 5 μg/ml (active ingredient) of benomyl. Desired concns of benomyl were obtained by suspending 50 mg of Benlate (benomyl) [methyl-1-(butylcarbamoyl)-2-benzimidazolecarbamate] in 10 ml of acetone and adding appropriate amounts to 500 ml of PDA. To obtain freshly produced conidia, infected leaflets were washed in running tap water, soaked 1.0 min in Clorox (diluted 1:9, v/v with H_2O), blotted dry, and placed in moist chambers maintained at 25 C. Conidia were removed from individual lesions with a glass rod, transferred directly to the test medium, and incubated at 26 C for 7 days.

Three benomyl-tolerant isolates (C-27, C-32-2, C-17-11A) and one sensitive isolate (C-2) were chosen to determine the effect of various concns of benomyl on growth and conidial size. Concentrations employed in

TABLE 1. Influence of fungicide sprays on percent lesions yielding benomyl-tolerant isolates of *Cercospora arachidicola*, percent defoliation, and yield of Florunner peanut[a]

Fungicide	Dosage/application (per hectare)	Lesions[b] (%)	Defoliation[c] (%)	Yield (Kg/hectare)
Benlate	0.42 kg	31.8	51.5 A[d]	4532 A
Bravo 6F	1.8 liters	0	39.0 A	4440 A
Fungi-Sperse	9.4 liters	0	43.5 A	4486 A
Duter	0.42 kg	0	71.1 B	4302 A
Control	...	0	95.2 C	1922 B

[a]Data obtained from counts of five stems in each of four replicate plots.
[b]Percent lesions collected 10/9 yielding isolates tolerant to benomyl.
[c]Percent defoliation obtained by dividing number of leaflets abscinded by total number per central stem × 100.
[d]Data followed by the same letter are not significantly different, $P = 0.05$, according to Duncan's multiple range test.

TABLE 2. Influence of fungicide combinations on percent lesions yielding benomyl-tolerant strains of *Cercospora arachidicola* from Tifspan peanut leaves

Fungicide	Dosage/application (per hectare)	Lesions[a] (%)
Benlate + Manzate + oil	0.28 kg + 1.4 kg + 2.4 liters	80.0
Benlate + oil	0.28 kg + 2.4 liters	76.9
Benlate + Manzate	0.28 kg + 1.4 kg	68.2
Benlate	0.28 kg	56.3
Benlate + Bravo 6F	0.28 kg + 1.17 liters	4.8
Control	...	0

[a]Percent lesions collected 9 October 1973 yielding isolates tolerant to benomyl.

PDA were 0.5, 5, 10, 20, 80, and 160 μg/ml. Each plate was seeded with approximately 50,000 conidia suspended in 1.0 ml of water and incubated 8 days at 28 C.

Benomyl-tolerant isolates were seeded on PDA amended with 1 and 5 μg/ml of methyl thiophanate; 2-(methoxycarbamoyl)-benzimidazole (BAS 3460), or chlorothalonil (Bravo 6F). Plates were allowed to incubate for 8 days at 26 C.

RESULTS AND DISCUSSION.—A total of six locations representing Webster, Sumter, Lee, and Colquitt Counties, yielded isolates of *C. arachidicola* tolerant to benomyl. Approximately 32% of the lesions from leaves of plants in fungicide spray plots which had received six applications of benomyl, yielded tolerant isolates. No tolerant isolates were obtained from unsprayed control plants (Table 1). Plants sprayed with Benlate alone or in combination with Manzate 200 after Cercospora leaf spot had reached epidemic proportions, yielded tolerant isolates from 56 to 80% of the lesions tested (Table 2). Less than 5% tolerant isolates were found when Benlate was used in combination with Bravo.

Tolerant isolates grew at 160 μg/ml, but the number of colonies per plate was significantly reduced as compared to nonamended controls. Sensitive isolates did not grow at 0.5 μg/ml while all tolerant isolates grew at 160 μg/ml. Tolerant isolates varied in the number of colonies per plate at concns above 0.5 μg/ml. The most tolerant isolate tested (C-17-11A) was restricted only slightly at 80 μg/ml, while isolate C-32-2 was severely inhibited at this concn. Concentrations above 5 μg/ml reduced amount of growth of all isolates.

Conidial measurements and microscopic observations showed no evidence of reduction in size or change in morphology of conidia when the fungus was grown on benomyl-amended media. This is not in agreement with observations by Griffee with *Colletotrichum musae* (5) in which conidial size was reduced by culturing on a benomyl-amended medium.

Based on 1 year's data in test plots at Plains, Ga., the presence of benomyl tolerance in the population of *C. arachidicola* was not associated with detectable increases in disease severity or significant decreases in yield. Percent defoliation was not significantly higher on Benlate-sprayed plants than plants receiving other fungicides in which tolerant isolates were detected (Table 1). Berger (2) reported *C. apii* progressed as rapidly in benomyl-sprayed celery plots as in unsprayed controls. It appears that benomyl-tolerant strains of *C. arachidicola* are not as virulent as *C. apii* under field conditions.

In agreement with other research, isolates tolerant to benomyl were also tolerant to methyl thiophanate and experimental fungicide BAS 3460 [2-(methoxycarbamoyl)-benzimidazole] (2, 3, 4, 5). A characteristic lag period in appearance of colonies after seeding on PDA amended with benomyl or other systemic fungicides, was evident in all in vitro studies when compared to PDA controls. Bravo 6F (chlorothalonil) prevented growth of all benomyl-tolerant isolates at 1.0 μg/ml (active ingredient).

Detection of benomyl tolerance in *C. arachidicola* before widespread losses occur and establishment of a range in tolerance is an advantage in the formulation of a disease control program (13). It is not known whether tolerant isolates will survive or if such isolates are as competitive or virulent as sensitive strains; however, precautions should be employed to prevent widespread increases of benomyl-tolerant strains in the peanut-growing belt (13). Additional research is underway to find answers to these questions.

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