

Cercospora and Cercosporidium Tolerance to Benomyl and Related Fungicides in Alabama Peanut Fields

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ABSTRACT

Cercospora arachidicola and *Cercosporidium personatum* spores, isolated from commercial peanut fields in Alabama and cultured on fungicide-containing media, were found to tolerate levels of benomyl and related fungicides at rates 10 times higher than those previously recorded. No tolerance was found in a field that had not previously been treated with benomyl or related fungicides.

Phytopathology 64:1476-1477

Additional key words: *Arachis hypogaea*; peanut leafspot.

Two related fungi, *Cercospora arachidicola* Hori and *Cercosporidium personatum* (Berk. and Curt.) Deighton cause early and late leafspot respectively, of peanuts, *Arachis hypogaea* L. Both fungi have been controlled during the past 3 yr with benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] = duPont's Benlate 50WP. Tolerance to benomyl has been reported in *Cercospora apii* Fres. (1, 2) and *C. beticola* Sacc. (5). In 1973, inadequate leafspot control was observed in several fields in south Alabama while under the recommended benomyl program of 170 g (6 oz)/acre applied at 14-day intervals. Tests were conducted to determine: (i) if *C. arachidicola* and *C. personatum* had developed tolerance to benomyl; (ii) if the proportion of benomyl-tolerant biotypes of *Cercospora* in problem areas differed from that in other areas; and (iii) if the tolerance detected also applied to fungicides chemically related to benomyl.

MATERIALS AND METHODS.—Leaves infected

with *Cercospora* and *Cercosporidium* spp. were obtained from fields with three different leafspot control histories. Field I was an only peanut field in an area where peanuts are not grown and had never received benomyl; field II received benomyl and other fungicides in previous years and benomyl exclusively in 1973 with good results. Benomyl was used in the area around field III in 1971 and 1972 with good results, but its use in 1973 resulted in inadequate control. Leaves were washed, placed on moistened filter paper in petri dishes, and incubated at 28 C for 4-6 days under constant illumination by white fluorescent tubes. Sporulating lesions were either sampled immediately or were air-dried for subsequent use. Single spores removed from the lesions were placed on PDA-tetracycline-streptomycin agar (PDATS) (6) and subsequently examined at weekly intervals for six wk. Transfers of mycelial fragments were made from colonies which developed on the original fungicide-amended media to media containing different fungicides and rated to observe the effect on colony growth. Fungicides and rates tested were benomyl at 5 and 50 µg/ml, thiophanate methyl, [1,2-bis(3-methoxycarbonyl-2-thioureido) benzene = Pennwalt's Topsin M 70WP] at 5 and 50 µg/ml, and methyl 1-((5-cyanopentyl)amino) carbonyl-1H-benzimidazol-2-yl) carbamate = Chemagro's Bay Dam 18654 at 5 µg/ml. All fungicides for these tests and also for the spore germination tests were added to the PDATS after autoclaving.

RESULTS AND DISCUSSION.—Spores of *C. arachidicola* and *C. personatum* germinated on PDATS plates containing 5 µg/ml benomyl. However, marked differences in development after germination were noted. Some germ tubes ceased development early and were assumed to be susceptible, while others showed tolerance by continuing to grow and producing viable colonies in the presence of the fungicide. An intermediate response was also noted, in which the spores germinated and showed some growth, but failed to develop into viable colonies. Growth on PDATS amended with 5 µg/ml benomyl was considered indicative of tolerance since 0.5 µg/ml was adequate to inhibit *C. arachidicola* development when benomyl first became available (authors, unpublished). Also before tolerance to benomyl had developed in *C. apii*, less than 1.0 µg/ml was sufficient to completely inhibit that species (1). *C. arachidicola* spores from field I (Table 1) showed very little tolerance with only 1.0% of the spores giving short-

TABLE 1. Germination and growth of *Cercospora arachidicola* spores on control and benomyl-amended media

Field ^a	Medium ^b	Total spores (No.)	Germination (%)	Short-term growth (%)	Continued ^c growth (%)
I	PDA	40	78	0	100
	PDA + 5 µg/ml	105	89	1	0
II	PDA	112	95	0	100
	PDA + 5 µg/ml	107	96	82	1
III	PDA	28	79	0	100
	PDA + 5 µg/ml	41	78	56	44

^aField I: no present or past leafspot control with Benlate. Field II: good leafspot control with Benlate in 1973. Field III: poor leafspot control with Benlate in 1973.

^bConcentration expressed as µg/ml of active ingredient of formulated product: Benomyl 50% W.P.

^cCalculated on basis of spores that germinated.

term growth. An intermediate level of tolerance probably existed in field II from which 82% of the isolated spores showed short-term growth and 1.0% produced viable cultures on the PDATS amended with 5 $\mu\text{g}/\text{ml}$ benomyl. Forty four percent of spores from field III developed into good colonies on the fungicide-amended medium, indicating a level of tolerance sufficiently high so that there was inadequate control by benomyl of leafspot in the field. *C. personatum* occurred at a very low frequency in all fields; however, development of germ tubes from spores isolated from the three fields showed a similar trend. Cultures tolerant to 5 $\mu\text{g}/\text{ml}$ benomyl were obtained. Transfer of cultures of *C. arachidicola* resistant to 5 $\mu\text{g}/\text{ml}$ benomyl indicated that these cultures could tolerate 50 $\mu\text{g}/\text{ml}$ benomyl, 50 $\mu\text{g}/\text{ml}$ thiophanate methyl and 5 $\mu\text{g}/\text{ml}$ Bay Dam 18654. Growth at 50 $\mu\text{g}/\text{ml}$ benomyl and thiophanate methyl was much slower than at the lower concn.

Considering that benomyl and thiophanate methyl have a common fungitoxic breakdown product, MBC (methyl 2-benzimidazolecarbamate) (3, 4), it is likely that the tolerance observed in these biotypes is to MBC.

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