Absorption and Movement of Benomyl into Cotton Bolls and Control of Boll Rot

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ABSTRACT

In laboratory studies, peduncles of detached, immature cotton bolls did not absorb and translocate sufficient concentrations of benomyl from aqueous suspensions to protect locules from infection by *Aspergillus flavus*. The chemical was not detectable by direct assay or chloroform extracts in carpel tissue, lint, seed coat, or embryo tissue. When detached bolls were shaken in benomyl suspensions, some absorption occurred, primarily through bract tissue. Benomyl formulated in glycerine, Volck oil, Puregrow Supreme 90 oil, or DuPont Surfactant F or Colloidal Products R-552 and sprayed on plants in the greenhouse and in the field was not detectable in lint, seed coat, or embryo tissue. Benomyl did not control boll rot caused by *A. flavus* and other fungi in field plots at El Centro and Brawley, Calif. Benomyl has not been detected in bolls from plants growing in soil containing as high as 500 µg/g (active) air-dry, soil weight basis. Phytopathology 61:1134-1136.

Additional key words: systemic fungicides.

Results of greenhouse and field experiments with cotton (*Gossypium hirsutum* L.) conducted during 1968 and 1969 indicated that benomyl or its breakdown product (3) was absorbed from soil by roots and moved systemically (4, 5, 7, 8). Also, movement occurred from sprayed cotton foliage into unsprayed new foliage. In neither case, however, was benomyl detected in carpel tissues, lint, or seed of bolls (8). Results of these tests appear to be contradictory to other studies which showed that benomyl, at a dosage rate of 500 µg/ml (active), protected detached, immature bolls from infection by boll-rotting microorganisms, presumably by absorbing the toxicant from suspension through the peduncle of the boll (2). Because of the high degree of activity of benomyl against a number of important boll-rotting fungi, and the practical significance of possible translocation of the chemical into cotton bolls, further studies in the greenhouse and field were initiated on absorption and movement of benomyl into cotton bolls. These results are reported here. An abstract has been published (6).

MATERIALS AND METHODS, RESULTS.—*In vitro studies.*—Standard in vitro agar tests at chemical concentrations ranging from 1 to 50 µg/g (active) were used to determine the effect of benomyl on growth of *A. flavus* Lk. ex Fr. (isolate 606) and other boll-rotting fungi, including *Rhizopus arrhizus* Fisch., *Aspergillus niger* v. Tiegh., *Fusarium roseum* Lk., *Penicillium* sp., and *Trichothecium* sp.

Incorporation of 5 µg/g (active) of benomyl into potato-dextrose agar (PDA) prevented mycelial growth of *A. flavus,* although spore germination took place at concentrations of 50 µg/g. Mycelial growth of *R. arrhizus* was not affected by concentrations of benomyl at 50 µg/g. Minimum concentrations preventing mycelial growth were 1 µg/g for *Trichothecium* and *Penicillium,* and 5 µg/g for *F. roseum.* Mycelial growth of *A. niger* was inhibited approximately 80% at 50 µg/g.

Movement of benomyl into in situ cotton bolls from foliage sprays and soil applications.—Foliage and nearly mature but green bolls were sprayed in the field to run off with concentrations of benomyl as high as 2,000 µg/ml (active) formulated in Volck oil, glycerine, Puregrow Supreme 90 oil (5% v/v), or with DuPont Surfactant F or Colloidal Products R-552 (4 oz/100 gal). Assays for benomyl were made 1 and 3 weeks after spraying. Bolls were separated into carpel tissue, lint, seed coats, and embryo tissue. The materials were ground to pass a 20-mesh screen. Five-g samples of each were shaken 1 hr in 200 ml of chloroform in 250-ml Erlenmyer flasks to extract benomyl. Chloroform extract residues were evaporated to ca. 2 ml, and 0.2-ml samples were pipetted into a well in PDA. After the chloroform evaporated, the wells were filled with warm agar and the plates seeded with a spore suspension of *Penicillium expansum* Lk. ex Thom. adjusted to ca. 80% light transmittance at 550 nm. Zones of inhibition were read after 48 hr at 25 C. Boll tissues were also frozen and placed directly on plates seeded with *P. expansum.*

Plants with squares but no flowers or bolls were also sprayed in the greenhouse with 3,000 µg/ml (active) benomyl formulated in the above surfactants. After bolls were formed, they were detached and were injected at two sites with a water suspension of conidia of *Aspergillus flavus.* The inoculated bolls were held at 30 C in plastic bags for 5 days, removed, and dried at 30 C. Locules that fluoresced bright greenish yellow under ultraviolet light were considered to be infected by *A. flavus* (1).

A peat-perlite-soil mixture in the greenhouse, in which approximately 1-year-old cotton plants were growing, was treated by applying benomyl in water suspension as a drench. The fungicide drench (50 µg/g active wt:soil-wt basis) was applied 3 times at 0, 4, and 6 weeks. Assays were made from soil, roots, stems, leaves, and boll tissue up to 22 weeks after the

last application. Concentrations of benomyl from 100 to 500 μg/g (active) were also incorporated into soil in pots in greenhouse studies. Assays were made periodically on boll tissues.

Benomyl was not detected in interior boll tissues when foliage and nearly mature green bolls were sprayed in the field to run off with concentrations of benomyl as high as 2,000 μg/ml (active) formulated in Volck oil, glycerine, Puregrow Supreme 90 oil (5% v/v), or with DuPont Surfactant F or Colloidal Products R-552 (4 oz/100 gal). Assays (chloroform extracts and *P. expansum*) made of lint, seed coat, and embryo tissue 1 and 3 weeks after field spraying were negative, although benomyl was readily detected on the exterior surface of the boll wall and on bract tissue (Fig. 1). Bolls from plants that were sprayed in the greenhouse with 3,000 μg/ml (active) benomyl and inoculated with conidia of *A. flavus* were rotted readily.

Benomyl was not detected in boll tissue from plants drenched with the fungicide or from plants growing in soil containing as high as 500 μg/g (active) air-dry, soil wt basis. Bolls on plants growing in treated soil also were not protected from infection by *A. flavus*.

Absorption of benomyl by detached, immature cotton bolls through the peduncle.—Near-mature but green bolls (Deltapine 16 and Acala 4-42), free from injury with peduncles attached but bracts removed were supported on a metal screen over water or benomyl at 500 μg/ml (active). The peduncles of the bolls were immersed in the liquids contained in 0.5-pint sealed or unsealed jars. This technique is similar to a previously described method (2). After 10 days at 30 C, each boll was injected at two sites with a water suspension of conidia of *A. flavus*. The inoculated bolls were held at 30 C in plastic bags for 5 days, removed, and dried at 30 C. When dry, locules were removed and examined in ultraviolet light for infection. In another similar study, possible benomyl movement was determined by exposing peduncles of detached cotton bolls to benomyl suspensions for 13 days. The peduncles were then removed, the bolls frozen, and lint, seed, locular wall, and carpel wall material were assayed for presence of benomyl by use of a *P. expansum* bioassay using a described technique (4). A total of 124 bolls were treated in the above experiments.

In a number of tests, both in an open (aqueous suspensions of benomyl exposed to air) and closed system (aqueous suspension in sealed jars), benomyl was not absorbed and translocated through peduncles of detached bolls in sufficient amount to protect locules from infection by *A. flavus*. Peduncles of detached cotton bolls placed in 500-μg/ml (active) suspensions of benomyl for 10 days at 30 C and inoculated with spore suspensions of *A. flavus* were rotted readily. In one experiment, 94% of 149 locules from bolls whose peduncles were immersed in benomyl suspensions were rotted. Benomyl also was not detected in the carpel wall, lint, or seed by direct bioassay of these parts or by assay of chloroform extracts of these parts.

Field studies with benomyl for control of boll rot.—Replicated field plots were established during sum-

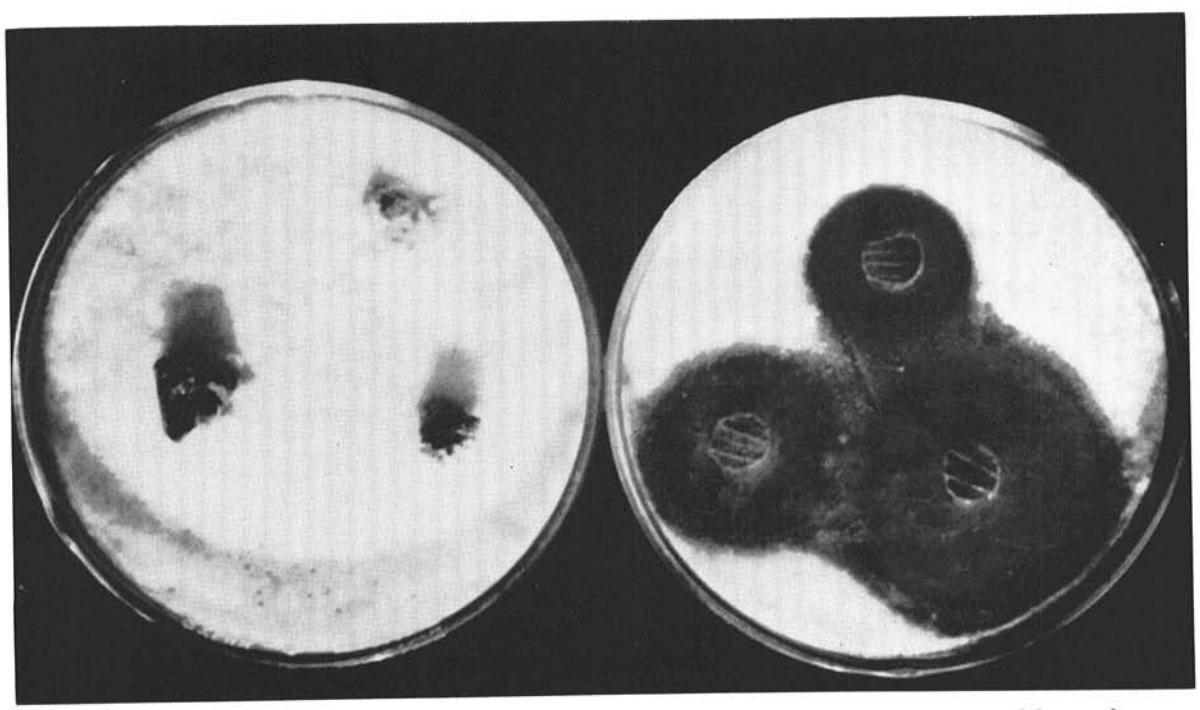

Fig. 1. Zones of inhibition (right) with *Penicillium expansum* bioassay test indicating presence of benomyl on exterior of cotton boll wall 3 weeks after spray application in the field. Interior tissues (left) are negative for presence of the chemical.

mer, 1970, to determine if foliage sprays of benomyl would control boll rots caused by *A. flavus* and other fungi. Sprays were applied on 27 July in plots at the University of California Meloland Field Station (El Centro) and at the USDA Southwest Irrigation Field Station (Brawley). At El Centro, benomyl sprays were applied at 2 lb. and 0.5 lb. (active)/acre in 100 gal of water/acre and the plot sprinkled-irrigated once a week starting 1 week after spraying until 15 August, when sprinkling was stopped. At Brawley, benomyl was applied with a high-clearance 4-row sprayer at rates of 1.5 and 0.25 lb. (active)/acre in 30 gal of water/acre and the plot furrow-irrigated. No surfactants were used. Assays, as previously described, were made for benomyl from lint, seed coat, and embryo tissue from 75 bolls from the 1.5-lb. active rate at Brawley 8 weeks after spraying. Boll rot incidence was determined on 19 October 1970. Locules were removed and examined in ultraviolet light. Locules that fluoresced bright greenish yellow were considered to be infected by *A. flavus*.

Boll rot determinations made at the El Centro and Brawley plots indicated that benomyl sprays did not significantly reduce rot caused by *A. flavus* or other fungi (Table 1). Assays from seed coat and embryo tissue from 75 bolls from the 1.5-lb. active rate at Brawley, made 8 weeks after spraying, were negative for benomyl. Assays for benomyl in lint from bolls in the same test showed benomyl presence in 6 of 300 locules. Disease incidence at El Centro, where the plots were sprinkle-irrigated, was ca. 3 times higher than at Brawley (Table 1).

DISCUSSION.—Our data agree with reports (4, 5, 8) which show that benomyl or its breakdown product (3) is absorbed by roots and leaves and is translocated to untreated foliage. But while benomyl was readily detected in foliage, it was never detected in bolls, in either greenhouse or field experiments, regardless of whether plants were grown in treated soil or whether they were sprayed with the substance. Benomyl was detected in 6 of 300 locules from a field experiment, but the possibility that the bolls were partially opened, when sprayed, could not be excluded.

Results of our bioassay and infection experiments show that benomyl is not absorbed into detached bolls via the peduncle. Thus, our results seem to differ with results reported by Bagga (2), who reported control of boll rot in a similar system. Although it is possible that the fungi he studied were more sensitive to benomyl than *A. flavus*, it should be noted that he did not report which fungi were involved in the boll rot disease complex he was studying. Also, no effort was made to detect benomyl in peduncles or boll tissue. Our information appears to be reasonable in light of other data (9) which indicate that benomyl is translocated in the xylem stream to regions of the plant having evaporating surfaces. In this regard, neither our tests or tests of Bagga, made in sealed jars, favored evaporation from the surface of bolls. But since our results were the same whether bolls were in sealed jars or whether they were exposed to air, it appears that water usage by nearly mature bolls is largely for growth, not transpiration. Thus, movement by diffusion appears to be the only logical mechanism for movement of the material in detached bolls. Results of other experiments (data to be reported separately) show that benomyl can be absorbed by detached bolls when shaken in benomyl suspensions. The process is quite slow, however, and appears to be due to diffusion, with benomyl detected only in carpel tissue after 2 days and in lint after 10 days. Lastly, the fungicide did not control boll rot in the field caused by *A. flavus* and other fungi when applied as a single foliage spray to immature, closed bolls. These negative results appear to be explained by results of the laboratory and greenhouse experiments.

TABLE 1. Effect of a single foliage spray of benomyl on cotton boll rot

Location[b]	Treatment, benomyl (Benlate 50% W.P.)	% Boll rot[a]		
		Aspergillus flavus	Other fungi	Total
El Centro	4 lb./acre	12.5[c]	24.1	36.6
	1 lb./acre	12.8	25.1	37.9
	Check	14.1	23.7	37.8
Brawley	3 lb./acre	3.5	5.2	8.7
	0.5 lb./acre	3.9	5.2	9.1
	Check	5.0	5.2	10.2

[a] Percentages are based on total number of infected locules of 500 locules examined.

[b] The foliage sprays were applied in 100 gal and 30 gal water/acre at El Centro and Brawley, respectively. The El Centro plot was sprinkle-irrigated.

[c] The data were not statistically different from the control at either location at the 5 and 1% level.

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