Movement of Methyl 2-Benzimidazolecarbamate into Soybean Seeds After Foliar Application of Benomyl

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ABSTRACT

Methyl 2-benzimidazolecarbamate (MBC) was detected in the seedcoat and cotyledons of soybean (*Glycine max*) seeds from plants sprayed with benomyl. MBC moved through the pod walls and into seeds and was absorbed through stem tissues and translocated upward into seeds.

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Benomyl is rapidly hydrolyzed into methyl 2-benzimidazolecarbamate (MBC) in solution (1, 4, 5) and in plants (4, 5). Benomyl (MBC) is absorbed by germinating soybean seeds and is translocated to aboveground parts of soybean seedlings from roots treated with the fungicide. The systemic movement and fungitoxic properties of benomyl in soybean are due to MBC (9). Foliar applications of benomyl to soybeans reduced the occurrence of *Diaporthe phaseolorum* var. *sojae* (*Phomopsis* sp.) and other fungi in seed from sprayed plants (3, 6). We report the detection of MBC in soybean seeds from plants sprayed with benomyl in the greenhouse and field.

MATERIALS AND METHODS.—Soybean [*Glycine max* (L.) Merr. 'Beeson'] seeds were planted in the field and 53 days later, whole plants were dug up with a shovel to include the rhizosphere soil and two plants were transplanted into each of several 11-liter plastic greenhouse pots. After 48 hours, suspensions of either 100 or 500 μg/ml benomyl were applied to: (i) two entire plants each by spraying to just before runoff, (ii) 10 pods of each of two plants by swabbing with a cotton swab, and (iii) the main stem of the bottom halves of two plants each, using a cotton swab. After 24 and 48 hours, three pods were collected from each plant per treatment. For treatment iii, pods were collected from above the swabbed stems.

Masking tape was wrapped around the stems of 12 plants each about half way up the stems. The lower half of six plants each was sprayed with either 100 or 500 μg/ml benomyl. Two pods were collected from each plant above and below the tape at 24, 48, and 72 hours after treatment.

The pods and the seeds from the pods collected from the above experiments and all other experiments in this study were bioassayed using Gibco potato-dextrose agar (PDA) seeded with spores of *Penicillium expansum* (2) unless otherwise stated. All pods were aseptically opened along the sutures and the seeds removed. Pods and seeds were washed for 1 minute in distilled water. One-half of each pod was cut into approximately five equal sections and plated in sequential order from the petiole to the tip. Seeds were also plated in sequential order from pods onto bioassay plates. Zones of inhibition were measured after 18 hours at 4 C followed by 48 hours at 30 C. All plant material was frozen for at least 24 hours before assay. Pods from nontreated plants served as controls.

A field plot of Beeson soybeans was planted on June 11. Randomized blocks with three 6.1-meter rows on 76-cm centers containing approximately 90 plants per row were sprayed with suspensions of benomyl. A row of nonsprayed plants separated each sprayed row. Treated rows were sprayed with 2 liters of either 50, 100, 500, 1,000 or 5,000 μg/ml (a.i.) Benlate four times at 2-week intervals beginning 55 days after planting. Sprays were applied with a hand sprayer.

Bioassays of seeds from 50 randomally-selected pods per treatment were made at 48 hours after each of the first three sprays, and again at 2 weeks after the third spray. Seeds from 50 pods per treatment harvested at 48 hours after the second and third sprays and at 14 days after the third spray were bioassayed on PDA seeded with the mycelium of *Diaporthe phaseolorum* var. *sojae* (*Phomopsis* sp.) (*Dps*).

Dps was grown on soybean broth for 7 days. Soybean broth was prepared by boiling 100 g of soybean seeds in 1 liter of distilled water for 30 minutes. The broth was filtered through cheesecloth and autoclaved [121 C and 1 atmosphere (15 psi)] for 20 minutes. The mycelium mat was separated from the culture medium by filtering through Whatman #1 filter paper. Mycelium (3 g wet weight) was homogenized 3 minutes in a sterile Waring Blendor in 100 ml of sterile, distilled water, then added to 1,000 ml of liquid PDA (50 C). The seeds were bioassayed as previously described.

Pods were collected again from each treatment at 7, 14, 21, and 31 days after the fourth spray. At 31 days after the fourth spray, one-half of each treated and nontreated row was harvested and seeds stored at 4 C. The remaining plants were harvested at 30 days after the first harvest. After the first harvest, but before the second harvest, pods were collected from each treatment at 41, 53, and 61 days after the fourth spray. Seeds from 25 pods per treatment for each collection date were bioassayed.

Fifty seeds per treatment were removed from cold storage at 10, 30, and 43 days after the first harvest and at 13 days after the second harvest and bioassayed for fungicide activity.

Up to 14 days after the third spray, all seeds were green and succulent and entire seeds were bioassayed. At 14 days after the third spray, seeds began to mature. All

seeds collected after 15 days after the third spray, were cut in half and plated with the cut surface downward on the bioassay agar.

MBC activity was located in soybean seeds by collecting 25 pods from each treatment 48 hours after the second and third sprays and bioassaying with *P. expansum* as follows: seeds were removed aseptically from pods, then the seedcoats removed and each placed separately into beakers of distilled water for 1 minute. The beakers were numbered to maintain the order in which the seeds occurred within the pod. The seedcoat and embryo of the same seeds were plated opposite each other on seeded PDA and zones of inhibition were measured.

The presence of MBC was confirmed in seeds from benomyl-treated plants by harvesting 30 g of seed from plants sprayed with 5,000 μg/ml benomyl 48 hours after the third spray. The seeds were homogenized 15 minutes in a Waring Blendor in 150 ml of chloroform, the homogenate poured out, the Blendor was washed with an additional 150 ml of chloroform, and combined with the homogenate. This mixture was heated to 80 C and held there for 15 minutes, allowed to cool to room temperature (24 C), and centrifuged for 15 minutes at 3,500 *g*. The supernatant was filtered through two layers of Whatman No. 1 filter paper three times, and reduced in volume to 10 ml with a rotating flash evaporator under vacuum. Thirty grams of seed from nontreated plants were processed identically and the extract used as a control.

Twenty microliters of each of the following solutions were spotted separately on Eastman 6061 silica gel or 13255 cellulose (6064) chromatography (TLC) plates without fluorescent indicator; (i) extracts from nontreated and treated seeds, (ii) technical grade benomyl, (iii) technical grade MBC, and (iv) chloroform. Solvent fronts were run from 12 to 14 cm in three systems using either 6061 plates and acetone or ethyl acetate plus chloroform (60/40, v/v) or 6064 plates with ethyl acetate, chloroform and acetic acid (1:1:0.04). All plates were developed using the bioautography technique of Peterson and Edgington (4) and *P. expansum*. Each treatment was chromatographed three times.

RESULTS.—All extracts of seeds from benomyl-treated plants of all TLC plates developed in all solvent systems had R_f values equal to that of MBC. Therefore, the fungicidal activity in seeds tested was due to MBC (4, 5). No zones were observed from extracts from seeds from control plants. A different R_f value was obtained for benomyl than for either extract from treated plants or MBC.

All the pods and 66 and 100 percent, respectively, of the seeds bioassayed from greenhouse plants sprayed with either 100 or 500 μg/ml benomyl produced zones of inhibition. All pods and 63 and 100 percent, respectively, of the seeds from pods swabbed with either 100 or 500 μg/ml benomyl produced zones of inhibition. All pods and 63 and 100 percent of the seeds from pods swabbed with either 100 or 500 μg/ml benomyl produced zones of inhibition. All pods and 29 and 67 percent of the seeds from plants whose lower stems were swabbed with either 100 or 500 μg/ml benomyl, respectively, produced zones of inhibition. All pods from above and below the tape markers from plants whose lower half had been sprayed with benomyl showed zones of inhibition. The percentage number of seeds which produced a zone of inhibition after 24, 48, and 72 hours from plants sprayed with 100 μg/ml benomyl taken from below the tape marker was 66, 78, and 89, respectively, and for seeds taken from above the tape marker, 39, 33, and 61, respectively. The percentage number of seeds producing a zone of inhbition taken from below the tape marker of plants sprayed with 500 μg/ml benomyl was 67, 100, and 61, respectively, and for above the sprayed zone, 55, 83, and 55, respectively.

Zones of inhibition were produced around seeds harvested 48 hours after the third spray at all levels of benomyl. Zones were produced about seeds from plants sprayed with either 500, 1,000, or 5,000 μg/ml benomyl, but not those sprayed with either 50 or 100 μg/ml at 14 days after the third spray.

Zones of inhibition were produced around seeds from all treatments of field-sprayed plants at 7, 14, 21, 31, 41, and 53 days after the last spray except those sprayed with 50 μg/ml and from seeds harvested at 21, 31, 41, and 53 days from plants sprayed with 100 μg/ml. Generally, the average size of inhibition zones about seeds decreased the further from the petiole the seeds were located in the pod and with increase in time of the field. No zones of inhibition were produced on any seeds from control plants.

Inhibition zones were not produced from stored seeds of the first harvest from plants sprayed with either 50 or 100 μg/ml benomyl, but zones were produced about seeds from the other three treatment levels for the three assays. The only seeds of the last harvest that produced zones of inhibition were from plants sprayed with either 1,000 or 5,000 μg/ml benomyl.

Seedcoats of seeds from treated plants always produced larger zones of inhibition than those produced by the seeds from which they were removed. In a few cases, zones of inhibition were produced around seedcoats and not around cotyledons from which they were removed. Seeds from all treatments up to 14 days after the third harvest produced zones of inhibition on *Dps*-seeded PDA. At 14 days after the third spray, seeds from all treatments except plants sprayed with either 50 or 100 μg/ml produced zones of inhibition.

DISCUSSION.—MBC was absorbed through the wall of developing pods and entered into the seeds within 24 hours after application of benomyl under greenhouse conditions. MBC was also absorbed through stem tissues and moved apoplastically (7) into the pod tissues and seeds above the point of application. When MBC enters soybean seeds, it moves into the seedcoat and cotyledons, with the greatest concentration occurring in the seedcoat. This is the first report of the detection of fungicide activity (presumably due to MBC) in soybean seeds from plants sprayed with benomyl. Peterson and Edgington (5) reported that MBC moved into pod tissues of bean *(Phaseolus* sp.) after uptake by roots.

Field studies using similar rates of benomyl showed a decrease in seed-borne *Dps* and other fungi (3, 6), but fungicide activity in the seeds was not studied. Our data show that MBC may be present in seeds from benomyl-treated plants prior to harvest in concentrations sufficient to inhibit the growth of *Dps* mycelium and the germination of *P. expansum* spores. Thus, seeds from treated plants are given protection against seed-infecting fungi, but the fungicide may not be detectable in the seeds

after harvest and storage when low concentrations of benomyl are used. This may, in part explain the failure of other workers to detect MBC in soybean seeds after foliar application of benomyl (6).

Fungicide activity in seeds decreased with an increase in time after treatment. MBC was reported to be broken down in plant parts (8). The decrease in activity appeared to occur slowly in seeds which were dried and stored, but rapidly when seeds were allowed to stay on dead plants in the field under moist conditions.

Illinois growers of certified seeds often harvest late because of wet weather. This was the case in 1972 and 1974. This results in reduced seed quality and germination, and an increase in seed-borne fungi (10). Seed infection may be partially prevented with the use of benomyl about mid-season and when the crop is maturing.

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