The Residual Activity of TBZ or Benomyl Against *Cercospora Beticola* in Sugarbeets[1]

J. H. RIESSELMAN and J. L. WEIHING[2]

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Sugarbeet leaf spot, incited by *Cercospora beticola* Sacc., can be a limiting factor in sugarbeet production in certain areas of the world. Two fungicides which have been used to combat this disease are TBZ [2-(4'-thiazolyl)-benzimidazole] and benomyl [methyl 1-(butylcarbamoyl)-2-benzimidazolecarbamate] (2, 3, 5, 6).[3]

Previous research (4, 7) has shown that these systemic fungicides possess a long residual life. Hine *et al.* (4) in greenhouse tests observed that benomyl applied to cotton plants at a 50 ppm soil drench at 0, 4, and 6 weeks was detectable in roots, stems, leaves, and the soil up to 22 weeks following treatment. In field studies benomyl could be detected at 18 weeks but TBZ at similar rates could not. However, Solel (7) working with sugarbeets found that TBZ persisted in leaves and stems for 45 days without the loss of fungitoxic activity. In 1970 Biehn and Dimond (1) suggested that the ability of these fungicides to control certain diseases is dependent upon maintaining an effective level of the fungicide in the potting medium. The study reported herein was initiated to determine the residual effectiveness of benomyl and TBZ against *Cercospora beticola* and the longevity of each in the soil and leaf tissue when varying rates of each were added to the potting medium.

Methods and Materials

Sufficient fungicide was added to 1,000 g of air dried soil to bring the desired concentration of TBZ or benomyl to 10, 20, 40, 80, 160 or 320 ppm (w/w). After thorough incorporation with the soil, eight sugarbeet seeds (variety GW869-65R) were planted per clay pot. Following germination, each pot was thinned to one plant.

Beginning four months after the initial treatment and again at six and eight and one-half months, the leaves and soil from each treatment were sampled randomly for possible residual activity. The petioles and leaves were then separated, and bioassays were conducted on each of these components. The respective foliage samples were homogenized at 4,000 rpm for 30 seconds at a ratio of one gram of tissue per 10 ml of distilled water. Immediately after grinding, the macerated plant

[1]Published with the approval of the Director as Paper No. 3603 Journal Series, Nebraska Agricultural Experiment Station.

[2]Extension Assistant, Cooperative Extension Service, Lincoln, Nebraska and District Director & Superintendent, University of Nebraska Scotts Bluff Station, Mitchell, Nebraska.

[3]Numbers in parentheses refer to literature cited.

material was strained through cheese cloth and centrifuged for 15 min at 4,500 rpm. The supernatant was decanted and 0.1 ml was placed on a bioassay disc. Twenty-four hours later these plates were inoculated with a spore suspension of *C. beticola*. Using an Ott planimeter the zones of inhibition were recorded 72 hr after inoculation.

Prior to conducting bioassays of the soil each respective sample was autoclaved to remove contaminant organisms. We had previously determined that these fungicides were heat stable. Following autoclaving, 2 g of soil were added to 20 ml of distilled water, shaken for 24 hr at 27° and placed on the bioassay disc as previously described. At four and six months inoculation trials were conducted in an effort to detect residual activity. The plants in each treatment were placed in an inoculation chamber containing sugarbeets infected with *C. beticola* whose lesions were profusely sporulating.

Results

Phytotoxicity

When TBZ was applied as a pre-plant soil amendment, a noticeable phytotoxicity occurred. Within 21 days after planting, sugarbeets grown in soil that had been amended with TBZ at rates greater than 20 ppm showed a marked decline in the number of surviving seedlings. After six weeks severe stunting or death had occurred in all plants growing in soil amended with rates of TBZ greater than 20 ppm. The severity of the phytotoxicity limited the residual tests with TBZ to 10 and 20 ppm.

Plants grown in soil amended with benomyl did not display visible symptoms of phytotoxicity, other than a possible slight stunting early in the growing period at the 320 ppm level.

Fungitoxic activity

The initial bioassays, which began four months after treatment, indicated that through the 20 ppm level no fungitoxic material could be detected in the plants growing in the TBZ-treated soil or in the soil itself. Bioassays of both leaf and soil extracts obtained from the benomyl treatments revealed that fungitoxic activity determined by zones of inhibition was present at the 80 to 320 ppm level (Table 1). However, only trace amounts of fungitoxic activity were detected at the 80 ppm rate. In no tests could activity be detected in plants growing in soil or from soil treated with less than 80 ppm benomyl. Furthermore, bioassays of leaf petiole tissue at four months did not reveal the presence of any fungitoxicant.

At the end of six months there was a noticeable decline in the area of zones of inhibition obtained from soil bioassays when compared with those at four months, whereas the activity in the leaf extract

Table 1.—The residual activity of benomyl[A] in leaf and soil extracts as determined by bioassays. Readings taken at 4, 6 and 8.5 months. The test organism was *Cercospora beticola*.

Rate	Average Zone of Inhibition In In²	
	Leaf Extract	Soil Extract
	4 Months	
80 ppm	Tr[B]	Tr
160 ppm	1.13	2.84
320 ppm	1.59	5.49
	6 Months	
80 ppm	Tr	0
160 ppm	1.51	Tr
320 ppm	1.58	1.11
	8.5 Months	
80 ppm	0	0
160 ppm	0	0
320 ppm	0.04	0

[A]Benomyl applied as a pre-plant soil amendment.
[B]Tr indicates that the bioassay disc was not overgrown with the test organism.

bioassays remained relatively constant. By the end of the 8.5 month period only trace amounts of activity could be detected and then only in the 320 ppm leaf extract bioassays.

In order to determine if the fungitoxic principle present in bioassays would prevent infection, inoculations were conducted with *C. beticola* at the end of the four and six month period. These inoculations resulted in no infection, or in some instances, extremely limited infections at the 80, 160 and 320 ppm level when compared with untreated checks.

Discussion

The present data show that when high rates of benomyl (160 and 320 ppm) are applied to the soil as a pre-plant amendment, fungicidal activity could be detected in soil up to six months and in the leaf tissue up to eight and one-half months. Residual tests with TBZ at lower rates did not display fungicidal activity at four months. However, no residual tests with TBZ at higher rates were conducted because of the phytotoxic factors.

The data further show that when benomyl is applied to the soil at 320 ppm prior to planting, sufficient fungitoxicant is continually available to the leaves and can prevent infection by *C. beticola* at 6 months and possibly longer. It is interesting to note that leaf extract bioassays of plants grown in the 320 ppm amended soil had equal zones of inhibitions at both four and six months, whereas the soil bioassays from the same treatments showed that the available fungitoxicant in the soil dropped several-fold in the period between four and six months. Therefore, it appears that the sugarbeet plant continually

draws from the available benomyl reserves in the soil without accumulating unnecessarily large amounts in leaf tissue, thus preventing infections for as long as the fungitoxicant is available in the soil.

Even though further research is necessary, these data suggest the possibility that a single, strategically placed, in-furrow treatment at planting time with high rates of benomyl could provide the sugarbeets with a source of fungitoxicant throughout the growing season.

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