# Effect of Benomyl on Soybean Endomycorrhizae

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ABSTRACT

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Soil drenches of benomyl (methyl-1-[butylcarbamoyl]-2-benzimidazole carbamate) at 2.5, 25, 125, and 250 μg/g of soil were added to pots newly planted to soybeans and containing 120 chlamydospores of *Glomus fasciculatus* per 100 g of soil. Mycorrhizal infection (recorded as percent of root length colonized) was decreased from 70–80% to approximately 45% by 25 μg benomyl per gram of dry soil in one experiment and by 2.5 μg/g in another. Concentrations as high as 250 μg/g did not decrease infection further. Benomyl prevented increased plant growth due to the vesicular-arbuscular mycorrhizae even with fungal colonization of the root system as high as 48%.

*Additional key words:* Benlate, pesticide.

It is generally accepted that vesicular-arbuscular (VA) mycorrhizae improve the growth of agricultural crops (8,10,12,15,18,19,21). The increased growth of mycorrhizal plants is due largely to increased efficiency of nutrient uptake. The mycorrhizal fungal network, for example, translocates phosphorus as far as 7 cm through soil to roots (17). Understanding the effects of pesticides on the development and efficiency of VA mycorrhizal associations is critical if these associations are to be used advantageously in agricultural production.

Several nematicides (1,3,13), one insecticide (14), and several nonsystemic (10,16,17) and systemic fungicides (2,11,20) adversely affect mycorrhizal development. Among these the toxicity of benomyl to endomycorrhizal fungi (20) was unexpected because previous testing showed it to be ineffective against several phycomycetous fungi (4,6,7). This study was conducted to reevaluate the effects of benomyl on the development of VA mycorrhizae using soybean (*Glycine max* [L.] Merr., 'Hark') and *Glomus fasciculatus* (Thaxt.) Gerd. and Trappe.

## MATERIALS AND METHODS

**Preparation of inocula.**—*Glomus fasciculatus* (9) was maintained on soybean in a growth chamber. The plants were grown to senescence, then discarded, retaining only the soil. The retained soil was mixed and sampled for chlamydospores of *G. fasciculatus* using a modified wet sieve-centrifugation technique (5). After the number of spores per gram of soil was determined, the soil was stored at 4 C as inoculum for all experiments.

**Planting and inoculation.**—Soybean seeds were inoculated with a suspension of *Rhizobium japonicum* and planted in 1 kg of autoclaved soil in 1-L plastic cups. Each cup had a drainage hole in the bottom covered by a nylon screen. The soil had a pH of 7.7, contained 5% organic matter, and had the following nutrient status: nitrate ($NO_3$), 51 ppm: phosphorus, 8 ppm; potassium, 4 ppm; calcium, 143 ppm; and magnesium, 29 ppm. To each cup was added 1,200 chlamydospores of *G. fasciculatus;* one-half of the spores were placed 5 cm from the soil surface and one-half approximately 9 cm from the soil surface. Three soybean seeds were added to each cup and after emergence the seedlings were thinned to one per cup. Nonmycorrhizal plants were prepared as above except that the inoculum (soil containing *G. fasciculatus* chlamydospores) was autoclaved for 30 min at 120 C. To insure that organisms other than *G. fasciculatus* also were present in the pots with nonmycorrhizal plants, nonautoclaved soil-chlamydospore washings was passed through a 45 μm sieve three times and equal portions of the filtrate were added to each pot.

**Benomyl application.**—A fresh suspension of benomyl (50% wettable powder in water) was added as a drench after the seeds and spores were planted in each cup. Four concentrations of benomyl were used (2.5, 25.0, 125.0, and 250.0 μg/g of soil) in each of five to six mycorrhizal and nonmycorrhizal plants in separate cups. Four to six mycorrhizal and nonmycorrhizal plants not treated with benomyl served as controls. Each pot was drenched with enough water with benomyl to saturate (but not to drip from) the soil and give the desired concentration. Benomyl concentrations are reported as micrograms of benomyl (active ingredients) per gram of dry soil. Plants were grown 70 days in a growth chamber (20 C-night [10 hr] and 26 C-day [14 hr]) at a light intensity of $18.3 \times 10^3$ lumens/$m^{-2}$ (fluorescent) and 1.1 lumens/$m^{-2}$ (incandescent). Plants were watered daily with distilled water.

**Infection rating.**—Roots were cleared by autoclaving in 10% KOH (weight basis) at 10.34 Newtons/$cm^2$ (15 lb) pressure and 120 C for 3 min, acidified by decanting the

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KOH and rinsing with 0.01 N HCl, and stained by autoclaving as above with acid fuchsin stain in lactophenol. The roots were rinsed several times in fresh lactophenol and placed in petri dishes that had been marked in 1-mm parallel increments. The roots were separated and aligned perpendicularly to the markings; 200 mm of the roots was randomly examined at ×112 for hyphae and vesicles of the fungus. Infection was reported as a percentage of root length infected.

**Leaf area measurements.**—Leaf areas were measured by cutting each leaf (that had unrolled) at the base of the blade and then inserting the leaves in a (LI-COR, Model LI-3000) leaf area measurer.

## RESULTS

When no benomyl was applied, 70% of the root length was infected by *G. fasciculatus*. Percent root infection decreased with increasing concentrations of benomyl to 25 μg/g. At concentrations ≥ 25 μg/g no further decreases below 39% infection were found (Fig. 1). Infection was significantly less in mycorrhizal plants with benomyl than in mycorrhizal plants without benomyl when 25, 125, and 250 μg/g applications were used (*t*-test, $P < 0.10$ for 25 μg/g and $< 0.02$ for 125 and 250 μg/g). Nonmycorrhizal plants showed no infection at any level of benomyl application. When repeated, this experiment gave similar results except that the lowest level of infection (approximately 40%) was reached at 2.5 μg/g concentration.

Leaf area of mycorrhizal plants was significantly ($P < 0.01$) larger than that of nonmycorrhizal plants when 0, 2.5, and 25 μg/g concentrations were added to the soil (Fig. 2). These differences were not present, however, with benomyl concentrations of 125 and 250 μg/g of soil, at which concentrations the leaf areas of mycorrhizal and nonmycorrhizal plants were similar. The increase in leaf area of mycorrhizal plants at 2.5 μg/g, compared with that at 0 μg/g, was not repeatable.

## DISCUSSION

These experiments demonstrated that *G. fasciculatus* spores can germinate and infect soybean plants when benomyl concentrations as great as 250 μg/g are present. The infection process was hindered, however, by all concentrations of benomyl tested. In this study reduced mycorrhizal infection could have been due to reduced germination of chlamydospores, reduced hyphal growth, or reduced host-fungus compatibility.

Increases in mycorrhizal plant growth at 0, 2.5, and 25.0 μg/g are attributed to the mycorrhizal association, and the lack of increases at 125.0 and 250.0 μg/g is attributed to the effects of benomyl on the fungus or the host-fungus relationship or both. The failure of high concentrations of benomyl to decrease infection below approximately 40% is not understood at this time. No significant change in growth occurred when benomyl was applied to nonmycorrhizal plants; thus, phytotoxicity cannot account for these growth responses.

Because 2.5 μg of benomyl per gram of soil (a rate higher than that anticipated under field conditions) did not affect growth of mycorrhizal plants, field usage should not present a hazard to this particular host/symbiont combination. An important implication of this work is that a decrease in infection may not necessarily negate growth increases normally thought to result from a mycorrhizal infection. This point is illustrated by the dramatic decreases in infection when 2.5–25.0 μg/g concentrations were applied without a concomitant decrease in host growth (ie, leaf area). In addition, the amount of infection may be substantial with no apparent benefit to the plant, since when 125.0 and 250.0 μg of benomyl were applied, 35 and 45% of roots were infected without concurrent mycorrhizal growth increases. Therefore, benomyl affects both fungal colonization of the root and the efficiency of the symbiosis. This indicates that "percentage type" measurements of mycorrhizal infection may not be

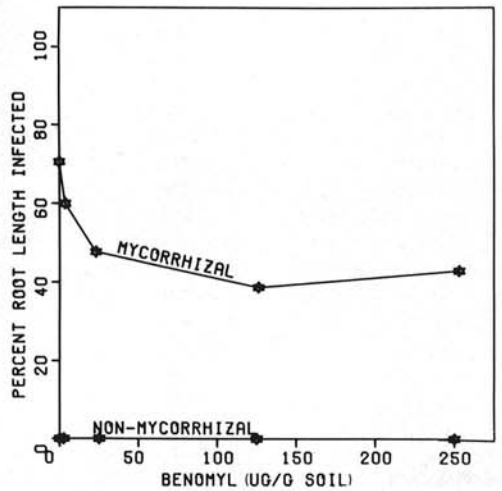


**Fig. 1.** Effects of various concentrations of benomyl applied to soil on *Glomus fasciculatus* endomycorrhizal infection of soybean. Each point represents the average root length infected of four to six plants after 70 days of growth.

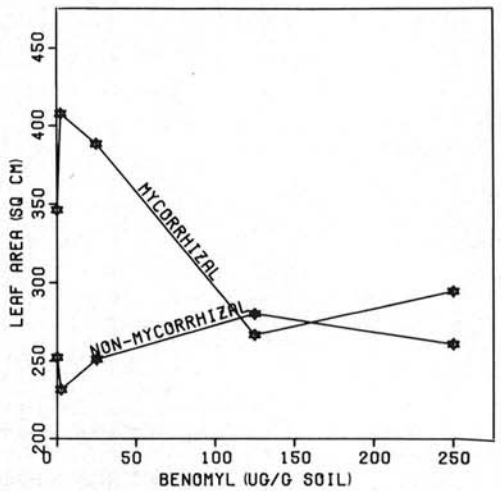


**Fig. 2.** Effects of various concentrations of benomyl applied to soil on the leaf area of mycorrhizal and nonmycorrhizal soybean plants. Each point represents the average leaf area ($cm^2$) of four to six plants.

sufficient to indicate the presence of functional mycorrhizal associations. Hence, studies of potentially hazardous chemicals to mycorrhizal associations should include growth response measurements (ie, yield, vegetative growth, etc.) as well as indications of the extent of root colonization.

## LITERATURE CITED


1. BACKMAN, P. A., and E. M. CLARK. 1977. Effect of carbofuram and other pesticides on vesicular-arbuscular mycorrhizae in peanut. Nematropica 7:12-17.
2. BAILEY, J. E., and G. R. SAFIR. 1977. Uptake and effect of benomyl in mycorrhizal soybean plants. Proc. Am. Phytopathol. Soc. 4:93.
3. BIRD, G. W., J. R. RICH, and S. U. GLOVER. 1974. Increased endomycorrhizae of cotton roots in soil treated with nematicides. Phytopathology 64:48-51.
4. BOLLEN, G. J., and A. FUCHS. 1970. On the specificity of the in vitro and vivo antifungal activity of benomyl. Neth. J. Plant Pathol. 76:299-312.
5. CAVENESS, F. E., and H. J. JENSEN. 1955. Modification of the centrifugal-flotation technique for the isolation and concentration of nematodes and their eggs from soil and plant tissue. Proc. Helminthol. Soc. Wash. 22:87-89.
6. DELP, C. J., and H. L. KLOPPING. 1968. Performance attributes of a new fungicide and mite ovicide candidate. Plant Dis. Rep. 52:95-99.
7. EDGINGTON, L. V., K. L. KHEW, and G. L. BARRON. 1971. Fungitoxic spectrum of benzimidazole compounds. Phytopathology 61:42-44.
8. GERDEMANN, J. W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. Annu. Rev. Phytopathol. 6:397-418.
9. GERDEMANN, J. W. 1974. The Endogonaceae in the pacific northwest. Mycol. Mem. 5. 76 p.
10. GERDEMANN, J. W. 1975. Vesicular-arbuscular mycorrhizae. Pages 575-591 *in* J. G. Torrey and D. T. Clarkson, eds., The Development and Function of Roots. Academic Press, London.
11. JALALI, B. L., and K. H. DOMSCH. 1975. Effect of systemic fungi-toxicants on the development of endotrophic mycorrhiza. Pages 619-626 *in* F. E. Sanders, B. Mosse, and P. B. Tinker, eds., Endomycorrhizas, Academic Press, New York. 626 p.
12. KAHN, A. G. 1972. Mycorrhizae and their significance in plant nutrients. Biologia special supplement. April, 1972. p. 42-78.
13. KLEINSCHMIDT, G. D., and J. W. GERDEMANN. 1972. Stunting of citrus seedlings in fumigated nursery soils related to the absence of endomycorrhizae. Phytopathology 62:1447-1453.
14. KRUCKELMANN, H. W. 1973. Die vesikulär-arbuskuläre mykorrhiza und ihre beeinflüssung in landwirtschaftlichen Kulturin. Ph.D. Thesis, Diss. Naturwiss. Fakultät Tech. Universität. Carolo-Wilhelminas, Brauschweig. 56 p.
15. MOSSE, B. 1973. Advances in the study of vesicular-arbuscular mycorrhiza. Annu. Rev. Phytopathol. 11:171-196.
16. NESHEIM, N. O., and M. B. LINN. 1969. Deleterious effects of certain fungitoxicants on the formation of mycorrhizae on corn by Endogone fasciculatus and corn root development. Phytopathology 59:297-300.
17. RHODES, L. H., and J. W. GERDEMANN. 1975. Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol. 75:555-561.
18. SAFIR, G. R. 1978. Vesicular-arbuscular mycorrhizae and crop productivity. In P. Carlson, ed., Biology of Crop Productivity, Academic Press, New York. (in press).
19. SUTTON, J. C. 1973. Development of vesicular-arbuscular mycorrhizae in crop plants. Can. J. Bot. 51:2487-2493.
20. SUTTON, J. C., and B. R. SHEPPARD. 1976. Aggregation of sand-dune soil by endomycorrhizal fungi. Can. J. Bot. 54:326-333.
21. TINKER, P. B. 1975. Soil chemistry of phosphorus and mycorrhizal effects on plant growth. Pages 353-371 *in* F. E. Sanders, B. Mosse, and P. B. Tinker, eds., Endomycorrhizas, Academic Press, London. 626 p.