EFFECT ON BENOMYL ON SOYBEAN ENDO - MYCORRHIZAE AND UPTAKE OF BENOMYL BY MYCORRHIZAL ROOTS

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ABSTRACT

EFFECT OF BENOMYL ON SOYBEAN ENDO-MYCORRHIZAE
AND UPTAKE OF BENOMYL BY MYCORRHIZAL ROOTS

By

Jack Eugene Bailey

Soil drenches of benomyl (methyl-1-(butylcarbamoyl)-2-benzimidazole-carbamate) at 2.5, 25, 125, and 250 µg/g soil were added to pots newly planted to soybeans (Glycine max (L.) Merrill) and containing 120 chlamydospores of the vesicular-arbuscular (VA) mycorrhiza-forming fungus Glomus fasciculatus per 100 g soil. Mycorrhizal infection, recorded as percent of root length colonized, was decreased from 70 - 80% to approximately 45% by concentrations of 25 µg/g in one experiment and 2.5 µg/g in another. Concentrations as high as 250 µg/g did not further decrease infection. Benomyl prevented increased growth due to the VA mycorrhizae even with colonization as high as 48%. Benomyl uptake was measured in a bioassay using the benomyl-sensitive fungus Penicillium digitatum and extracts of plant parts. No consistent uptake differences between mycorrhizal and non-mycorrhizal plants were found.

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By

Jack Eugene Bailey

A THESIS

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To my wife and parents

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I would like to thank Dr. Gene Safir for his advice and encouragement in helping me to prepare this manuscirpt, and in his guidance in all matters for the last two and one half years.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

INTRODUCTION AND LITERATURE REVIEW

Vesicular arbuscular (VA) mycorrhizal fungi are common in soil and in plant roots. They occur on wild herbaceous plants, shrubs, trees and agricultural crops (30). The first descriptions of these organisms appeared in the late 1800's, however, their investigation was limited until the 1950's (30). From 1955 to 1968 most research dealt with distribution and morphology. More recently emphasis has been placed on their symbiotic and other effects on plant growth and development (56).

Fungi which form VA mycorrhizae with plant roots are members of the family Endogonaceae (29). The large amount of diversity within this family may eventually lead to the displacement of some species to other families (29), such as the Mortierelaceae (47,29,1,83). There are seven genera in the Endogonaceae as defined by Gerdemann (29): Endogone, Gigaspora, Acaulospora, Glomus, Sclerocystis, Glaziella and Modicella. All but Modicella and Glaziella are known to form VA mycorrhizae (29).

The beneficial effect of VA mycorrhizal infection on plant growth is well documented (6,7,26,27,14,30,56,31,41,82). Increases in nutrient uptake can occur via the fungal hyphae (37) which increase

the growth of mycorrhizal plants (37,67). Recently, Rhodes and Gerdemann (67) showed that phosphorus (P) absorption, via hyphae, can take place up to 7 cm from the root surface. Greater P uptake is associated with increased plant growth (6,26,49,50,71), making P fertilization less beneficial to mycorrhizal than non-mycorrhizal plants. In some cases P reduces mycorrhizal-related growth stimulation (7,15,49,50,55,58,70), and mycorrhizal infection (55,57). Safir et al. (72) showed decreased resistance to water transport in mycorrhizal compared to non-mycorrhizal soybean roots. This phenomenon may result from increased P concentration of the plant (73). Other nutrients such as manganese, magnesium, copper, zinc, sulphur, and strontium also can be taken up more readily by mycorrhizal plants (30,32,36,45). Their uptake, like accelerated water recovery (72,73), may not only be due to direct hyphal translocation. For example, sulphur uptake may not be as greatly dependent on an intact hyphal network when sulphur is present near the root, indicating a general increase in root permeability to this compound (68).

Since mycorrhizal plants generally grow and yield better than NM plants the effect of chemicals on mycorrhizal fungi is an important consideration when dealing with agricultural systems. Soil fumigants, for example, have sometimes been detrimental to mycorrhizal fungi (11, 52,71). Nesheim and Linn (59) tested eight different soil fungitoxicants (arasan, botran, captan, lanstan, mylone, terraclor, vapam, and vorlex). Captan was the least and terraclor the most harmful to the mycorrhizal formation. Kleinschmidt and Gerdemann found chemical toxicity detrimental to VA mycorrhizae. Stunting of citrus seedlings

after fumigation in nurseries could be partially overcome by fertilization. Inoculation of fumigated soils with VA mycorrhizal fungi restored growth and reduced demand for fertilizer. The workers suggested that mycorrhizal inoculum could be produced en masse for agricultural purposes.

From 1966 to 1968, oxathiins, pyrimidines, and benzimidazoles were introduced as the basis for systemic fungicides (22). In 1968 Delp (16) demonstrated that benomyl showed promise as a soil and seed treatment in the greenhouse and as a foliar spray and seed treatment in both the greenhouse and field against a wide variety of diseases (16,18,19,33,80). Benomyl apparently acts on cell spindle fibers (personal communication, Charles Delp, DuPont de Nemours and Co.) as an antimetabolite (2) competing with purine-like compounds which prevents mitotic division (35,36).

Benomyl is toxic to a wide spectrum of fungi and in many cases its effectiveness appears to follow taxonomic lines (17). In 1971, Edgington et al. (17) did a comprehensive in vitro study to identify taxonomic/fungitoxicity relationships for the three benzimidazoles: benomyl, thiabendazole and furidazol. He found a striking similarity between the activity spectrum of the three compounds and concluded that the benzimidazole moeity was responsible for these similarities. Five phycomycetes had a ED_{50} greater than 100 ppm, and were considered to be insensitive. This supported the observation made by Delp (16) that phycomycete caused diseases were not controlled by benomyl.

Jalali and Domsch (46) showed that systemic compounds such as benomyl and trifoline had ill effects on mycorrhizal development when

used as seed treatments or foliar sprays. Foliar sprays were thought to induce a physiological incompatibility since systemic fungicides are generally not translocated downward to any appreciable extent (22, 60,63,54). Inhibition from the seed treatments may be due to passive translocation of the fungicide during root growth as well as a physiological incompatibility.

In 1976, Sutton and Shepard (79) showed that benomyl and thiabendazol were toxic to an unidentified Glomus species. Concentrations as low as 15 ppm of benomyl (dry soil) prevented mycorrhizal development. Forty spores of the fungus served as inoculum in the planting medium consisting of sand dune soil and silica sand (3:1), a mixture virtually void of organic matter. The fact that benomyl is toxic to a fungus in the class Zygomycetes (Phycomycetes) is not consistent with previous views (17,16). However, in 1970, Bollen and Fuchs (10) observed a great deal of variation in Mortierella sensitivity to benomyl in vitro. This variability may have been an indication that strict class adherence to benomyl resistance was on overgeneralization, and that possibly the Morierellaceae-Endogonaceae line of evolution (1) may represent an exception to the rule. In light of Sutton and Shepard's report (79), it is of interest to see what effects benomyl has on other soil microorganisms, including other VA mycorrhizal fungi. Siegel (75) found that benomyl was metabolized by soil microorganisms in nutrient-amended soil. In addition, he found a 2- to 3-fold reduction in the number of soil fungi and actinomycetes but not bacteria. Berg and Bollen (8) also found quantitative decreases in the mycoflora when 10 ppm benomyl was added to potting soil.

Benomyl stimulates the growth of some fungi both *in vivo* and *in vitro*. Robinson *et al*. (34,69) found that subinhibitive concentrations increased the growth of *Ustilago striiformis in vitro*. Smith *et al*. (77) noted that a turf disease incited by an unidentified Basidiomycete became more prevalent following benomyl treatments and that the fungus grew on potato dextros agar ammended with 25 ppm benomyl. Jackson (44) reported that benomyl enhanced *Helminthosporium* leaf spot on Kentucky bluegrass. Peeple's work (62) showed no alteration in microbial populations in field soil amended up to 89.6 kg benomyl/hectar. He also found only a small initial rise in respiration rates of soil in the laboratory when 10 ppm benomyl was added. Benomyl-sensitive fungi such as *Penicillium*, *Asperigillus*, and *Trichoderma* were consistently isolated from benomyl treated soils. Ponchet and Tramier (66) found no change in the number of species after benomyl application. In addition, Kaastra and Gams showed (48) no qualitative or quantitative changes in soil microflora amended with 10 ppm benomyl. Among the fungi most commonly isolated from amended soils were *Trichoderma pseudokoningii*, *T. viride*, *Monilia pruinosa*, *Penicillium janthinellum*, *Volutella ciliata*, and *Chrysosporium meridarium* which according to Bollen and Fuchs (10), are very sensitive to benomyl. Kaastra and Gams indicated that the low level of metabolic activity in the soil may have accounted for the lack of sensitivity in their experiment. Weeds and Hedrick (84) found that an undefined microorganism population in greenhouse potting soil had an increased respiration rate when 100 ppm benomyl was added. However, they made no attempt to qualitatively or quantitatively measure their population.

These works show benomyl to have a low level of toxicity to soil microorganisms even those known to be highly sensitive *in vitro*. Two factors probably explain why sensitive fungi can persist after application of benomyl to soil: benomyl's fungistatic nature (10,48), and its affinity to organic matter (5,42). Baude (4) found that the majority of benomyl and its breakdown products, methyl-2-benzimidazole-carbamate (MBC) and 2-aminobezimidazolecarbamate (AB), did not penetrate three different soil types more than 10 cm after a soil drench application. Consequently, there is an inverse relationship between organic matter and uptake of benomyl by plants (43,73). Peat moss, for example, added to sand, reduced uptake of benomyl by cotton plants (21). Leach *et al*. (53) suggested that the inability of benomyl to control Verticillium wilt of cotton in the field was due to lack of contact with the compound. They mixed the same amount of benomyl into 100, 50, 25, 12, 6, or 3% of the soil before potting. After 119 days, the greatest uptake was achieved from the most thorough mixing (100%), and no uptake occurred in the mixture involving only 3% of the soil. Benomyl movement in soil was increased by acidification, increasing soil moisture, or adding a surfactant such as Tween 20 (61).

Benomyl in its MBC form is rapidly translocated apoplasticly (24, 65) from the roots to existing leaves. Thus, new leaves are unprotected because the fungicidal reservoir is quickly depleted (24). However, benomyl itself tends to concentrate in the roots and is slowly released to the leaves as it degrades to MBC (24). As the compound reaches the leaves it moves with the transpiration stream and becomes concentrated at leaf margins (65).

Much of benomyl's persistence in soil is due to its retention and slow release from the soil. Erwin (20) showed that cotton plants grown in 50% peat and 50% sandy loam were protected against Verticillium wilt four months after treatment of 50 ppm. Rates of 100 and 500 ppm gave even longer protection.

Soil applications have proven to be much more successful in the greenhouse than in the field (22), where uneconomically high rates are needed. Even distribution in the soil is important so that a large portion of the root system can come into contact with the fungicide.

The possibility that mycorrhizal plants might be more permeable to benomyl could be valuable in field situations. The possible ill effects of benomyl on mycorrhizal fungi would not necessarily negate an increase in absorption rates. Pentachloronitrobenzene (PCNB), a fungicide that has been used to render external hyphae non-functional (31), does not decrease water uptake in mycorrhizal plants (72,73) after short term applications. Since benomyl uptake by soybean plants is proportional to water uptake (personal communication, Dr. L. V. Edgington) indirect effects on general root permeability may allow for increase absorption.

To date the only two works (48,79) which have dealt with the effect of benomyl on endomycorrhizae have had sand as their planting medium. In an agricultural situation, organic matter is present, at least to some degree, and would be expected to reduce benomyl toxicity. In addition, in neither study was benomyl used as a soil drench, which is the usual way mycorrhizae come in contact with the compound. Thus far, seed treatments, foliar sprays (46), and thorough mixing in the

soil (43) have been tested, the latter which would be found only in a greenhouse situation. Both studies used unidentified species of a VA mycorrhizal fungus and the numbers of chlamydospores were not adjusted to levels commonly found in the field (39). Furthermore, the influence of mycorrhizae on benomyl uptake by plants was not tested. Finally, there have been no studies of the relationship between mycorrhizal-stimulated growth and benomyl application (i.e., effect on the symbiosis). Sutton et al. (79) and Jalali et al. (46) dealt with infection per se, but not with mycorrhizal growth responses. Hayman and Mosse (40) found no correlation between plant growth stimulation and rates of infection in some cases, therefore, if growth data along with infection data are not collected, as was the case with Sutton et al. and Jalali et al., the effects of a given chemical on mycorrhizal symbioses cannot be estimated.

This study sought to: 1) evaluate the effects of benomyl on the development of a functional VA mycorrhizal relationship involving soybean and the mycorrhizal fungus Glomus fasciculatus and 2) evaluate the relationship between VA mycorrhizal infection and the uptake of benomyl by soybean plants.

MATERIALS AND METHODS

Preparation of Inocula

Glomus fasciculatus (29) was maintained on soybean, *Glycine max* (L.) Merril, (cv. Hark) plants in a growth chamber and in the greenhouse. The plants were grown to senescence then discarded and the soil retained. The soil was combined, mixed and sampled for chlamydospores of *G. fasciculatus* using a centrifugation technique. One hundred cc samples of soil were placed in a 5.5 l plastic bucket filled 3/4 full with water and swirled by hand to suspend all spores. The mixture was allowed to settle approximately 20 seconds to remove larger soil particles and decanted through a 45 μm sieve. The fraction retained on the sieve was transferred to centrifuge tubes containing .35 ml water and centrifuged at 450 g for four minutes. The supernatant fluid was decanted and the pellets resuspended in a sucrose solution of 1.14 specific gravity. The resultant suspensions were centrifuged again at 450 g for one minute, quickly decanted into a 45 μm sieve, washed with distilled water, rinsed into a petri dish and the chlamydospores counted at 30 X magnification. The pellets remaining in the test tubes were resuspended in sucrose and reextracted several times until all spores were recovered. The number of spores

per gram of soil was calculated. This "calibrated" soil was stored at 0°C and used as inocula for all experiments.

Planting and Inoculation

Soybean seeds were inoculated with a suspension of Rhizobium japonicum and planted into 1 Kg autoclaved soil in one liter plastic (Sweetheart) cups. Each cup had a drainage hole in the bottom covered by a nylon screen. The soil contained 5% organic patter and had a pH of 7.7. The soil had the following nutrient status: nitrates, 51 ppm; phosphorus, 8 ppm; potassium, 4 ppm; calcium, 143 ppm; and magnesium, 29 ppm. Three seeds were planted in each cup.

Controls (NM) were prepared as above but the mycorrhizal inoculum was autoclaved for 30 minutes at 120°C. To insure that organisms other than the mycorrhizal fungus present in the chlamydospore inoculum were also present in the NM pots, new soil-chlamydospore inoculum was passed three times through a 45 µm sieve and the screened liquid was added to each pot.

Benomyl Application

A stock solution of benomyl at 500 ppm (in water) was prepared. Benomyl suspensions were added to each pot in enough additional water to saturate the soil and give the appropriate soil concentration. Benomyl concentrations in soil are reported as µg/g soil.

Benlate is formulated as a 50% wettable powder. The active ingredient, benomyl (methyl-1(butylcarbamoyl)-2-benzimidazole-carbamate),

hydrolyses to MBC in water (12) and in plant tissue when applied as a root dip or soil drench (63,75).

Bioassay Procedures

In order to test for fungistatic activity in the roots and leaves of treated plants, a bioassay technique was utilized. Penicillium digitatum (Link) was used as the bioassay organism and maintained in stock culture on 3% potato-dextrose-agar (PDA) at 2°C until needed. To obtain spores, the plates were grown at ca. 22°C for approximately one week. Spores were washed from the plates with distilled water containing Tween 20. The spores were transferred to Klett spectrophotometer tubes and adjusted to 50 Klett units using a 420 nm filter.

One g samples of finely chopped leaf or root tissue were placed into 30 ml test tubes, frozen overnight at 0°C, and extracted in 10 ml of acetone. Extraction was done by placing the test tubes on a reciprocal-shaker at 100 cycles per minute for 24 hours. Aliquots (100 µl) of the extract were spotted on bioassay disks (12.7 mm; No. 740-E Schleicher & Schuell) with a micro-syringe. The disks were held by No. 325 insect pins which were placed through the middle of each disk and stuck into a flat sheet of styrofoam. Once the acetone had evaporated completely, each disk was placed in the petri dish (100 x 15 mm) containing 6 ml of 3% water agar. The disks were subsequently covered with 4 ml of PDA containing conidia of P. digitatum (PDA-P). This bilayer agar method is similar to that of Thornberry (81). The

PDA-P mixture consisted of one part spore suspension (as described above) to 100 parts (1.5%) PDA. The spore suspension was added to molten PDA held between 45-50^{o}C and stirred on a magnetic stirrer to insure a uniform mixture. The petri plates were then stored at room temperature and zones of inhibition were measured two to three days later (see also Appendix B).

Infection Rating

This procedure is similar to that of Sutton (78). Root samples were washed, blotted dry, weighed, and placed in a small glass petri dish. The roots were cleared by heating in 10% KOH (wt/wt) in an autoclave at 120^{o}C with 15 pounds pressure for five minutes. The KOH was decanted and the roots acidified using 0.01 N HCL. This was decanted and a solution of acid fuchsin stain in lactophenol was added and autoclaved as above. The roots were removed from the stain and placed in fresh lactophenol without acid fuchsin stain and allowed to destain overnight. Before observation, the roots were placed in petri dishes which had been marked off into 1 mm increments, and covered with fresh lactophenol. The roots were separated, aligned perpendicularly to the markings, and 200 mm of the roots were examined under 112X for any internal signs of the fungus (i.e., hyphae and vesicles). Infection was reported as a percentage of root length infected (see also Appendix A).

RESULTS

Fungal Tolerance to Benomyl

Preliminary testing showed that infections of soybean roots by Glomus fasciculatus was highly tolerant to benomyl. When benomyl was applied to heavily infested soil (600 G. fasciculatus chlamydospores/ 100 g soil) to which soybean seeds had been sown, high levels of infection resulted even when 250.0 µg/g soil was applied.

In another experiment, the effect of different benomyl concentrations on mycorrhizal development was evaluated. Four concentrations of benomyl were used (2.5, 25.0, 125.0 and 250.0 µg/g soil) with five to six M and NM plants per concentration. Four to six M and NM plants not treated with benomyl served as controls. Benomyl was added directly after the seeds and spores, and infection was rated 70 days later. The percent root infection decreased with increasing concentrations of benomyl applied as a soil drench up to 25.0 µg/g (Figure 1). At 25.0 µg/g and above no additional decreases below 39% infection were found. Infection in mycorrhizal plants was significantly less than that in non-treated mycorrhizal controls when 25.0, 125.0, and 250.0 µg/g benomyl was applied. Non-mycorrhizal plants showed no infection at any level of benomyl application. This experiment was repeated once with

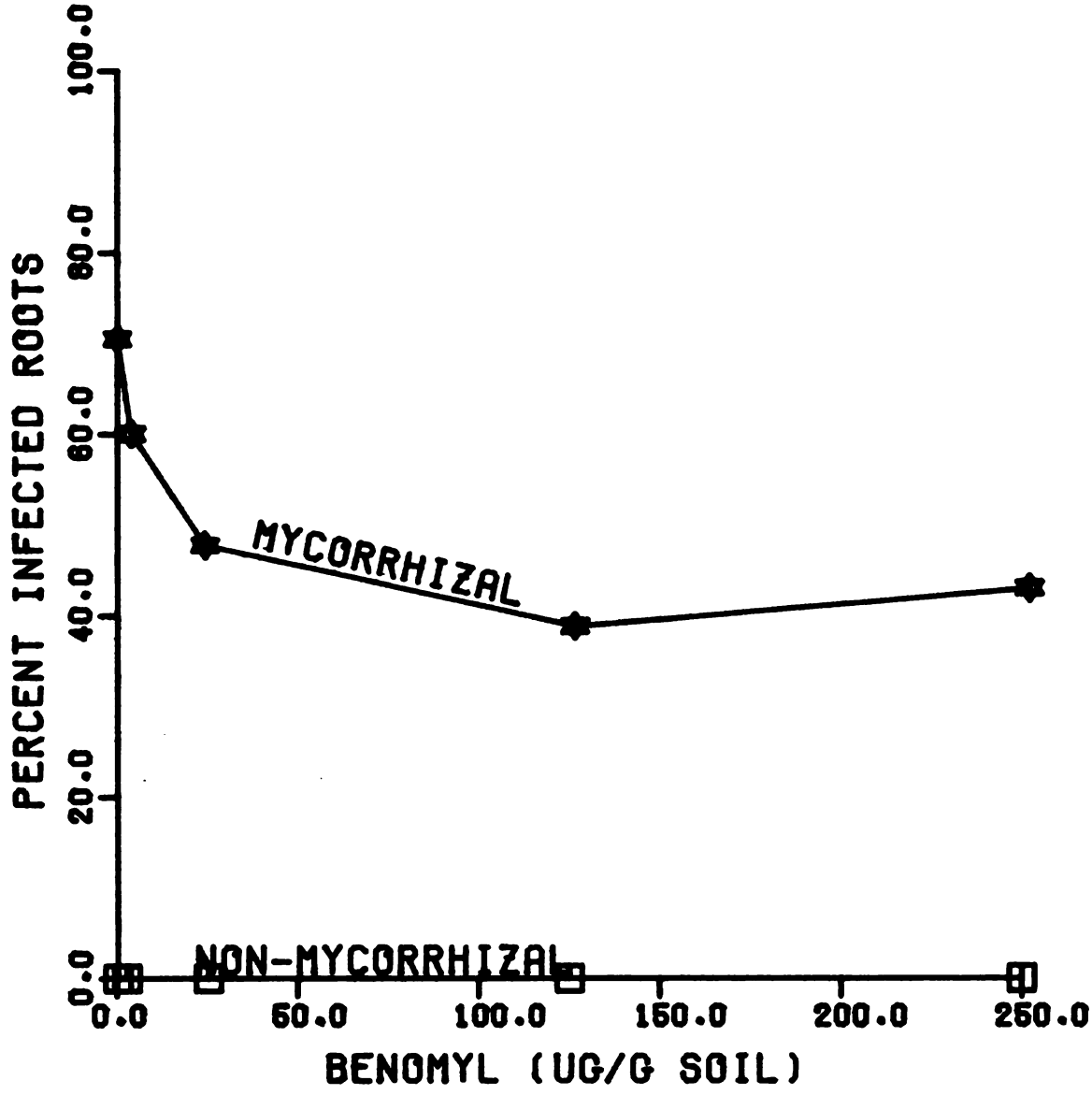
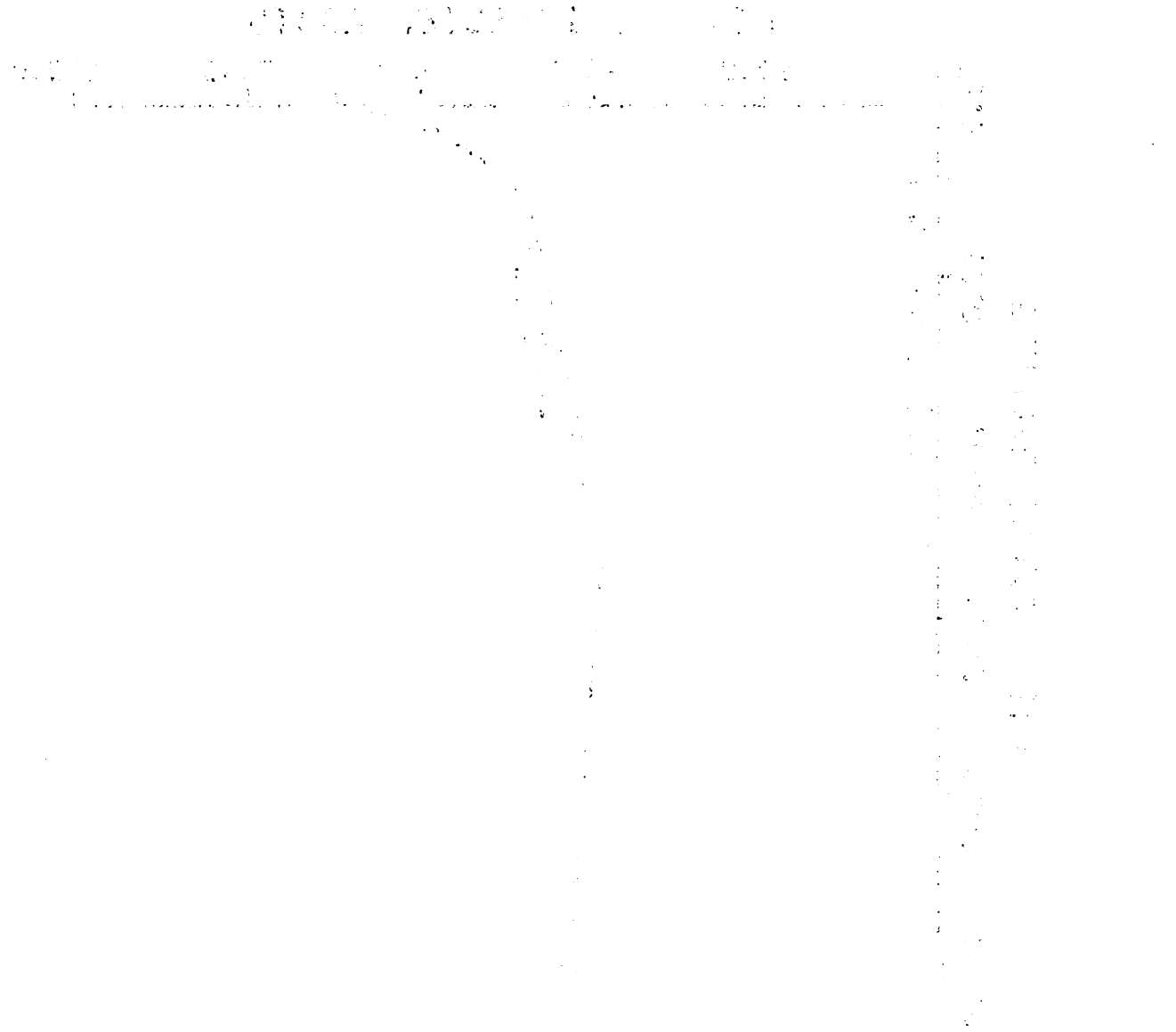


Figure 1. Effect of various concentrations of benomyl applied to soil on G. fasciculatus infection of soybean. Each point represents the average infection rating of 4-6 plants after 70 days growth.



essentially the same results. However the lowest level of infection in this instance (between 32% and 50%) was reached at 2.5 µg/g benomyl (Appendix D, Figure 14).

Using leaf area as a growth parameter of M and NM plants, the M plants were significantly ($P < 0.01$) larger than the NM plants for 0, 2.5, and 25.0 µg/g soil (Figure 2). These differences, however, were smaller with increasing benomyl concentrations. At 125.0 and 250.0 µg/g soil, total leaf area of M and NM plants were similar. The growth of NM plants remained unchanged at all elvels of benomyl. When this experiment was repeated similar results were obtained; however, like the percent infection data, the reduction in M infection and plant size occurred at lower concentrations (Appendix D, Figure 15).

Uptake of Benomyl by Soybean Plants

Since M plants have larger leaf areas than do NM plants the effect of leaf area on benomyl uptake was tested. Six to seven M and NM plants were grown for 50 days when all but the third, fourth, and fifth trifoliate leaves were removed from half of the M and NM plants. All plants were treated with 45 µg/g soil drench of benomyl. Two days later each plant was bioassayed. There was no apparent relationship between leaf area and amount of benomyl taken up (Figure 3). This experiment was repeated with similar results. Hence, leaf area was not considered as a major source of variability in subsequent experiments.

Preliminary testing indicated that M plants may have a greater ability than NM plants to take up benomyl. To further investigate this

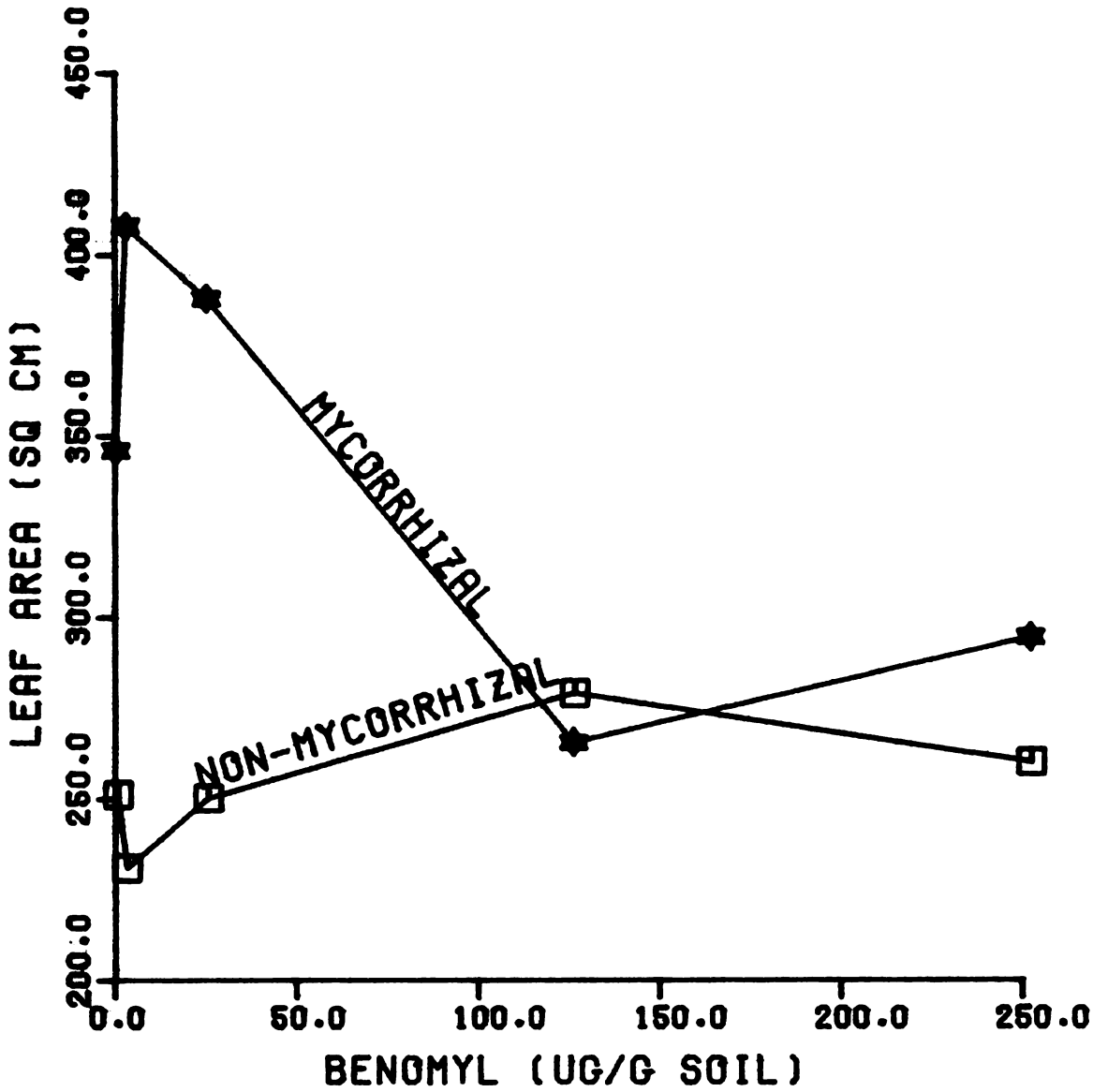
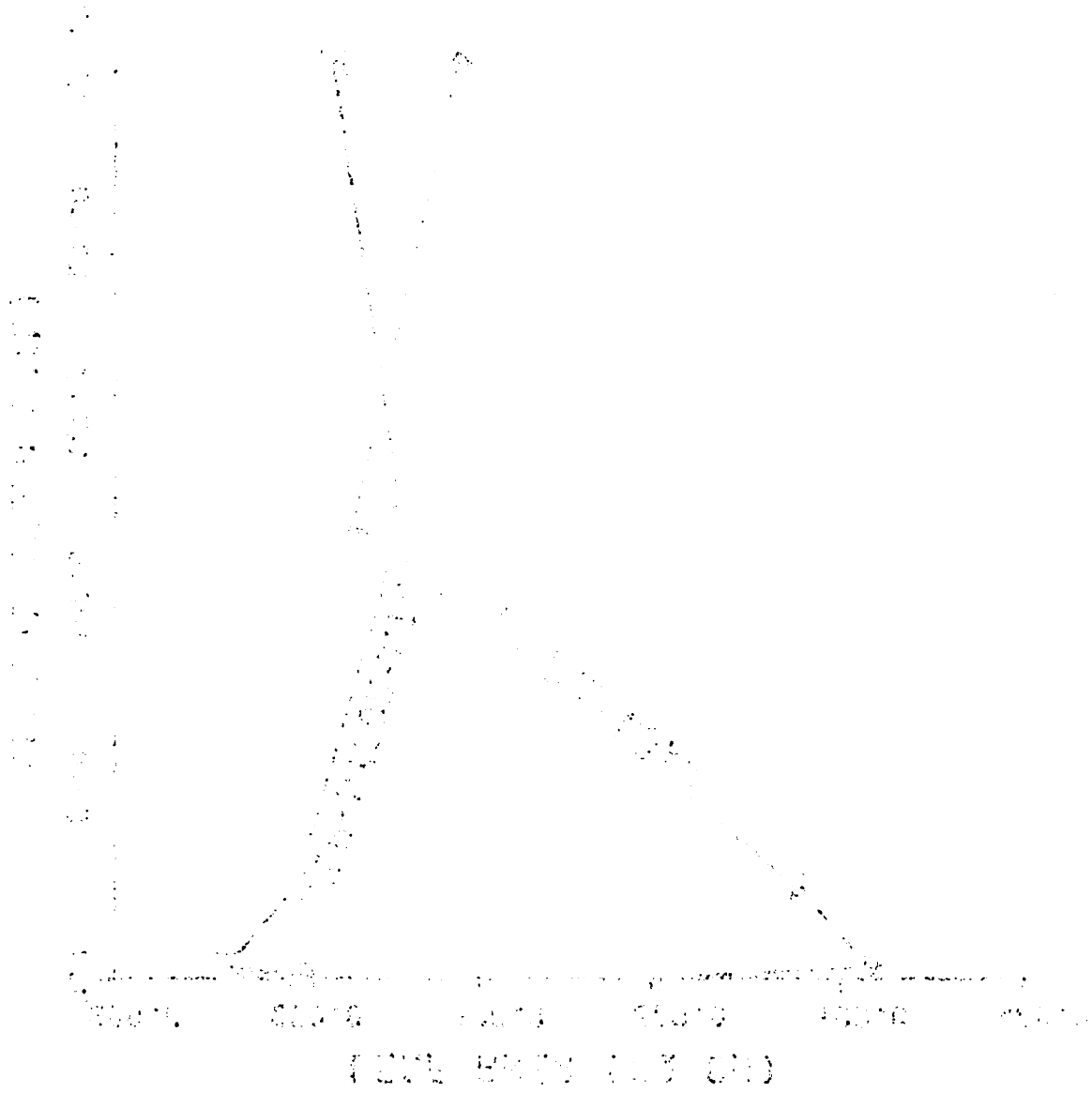


Figure 2. Effect of various concentrations of benomyl applied to soil on the leaf area of soybean plants with (mycorrhizal) or without (non-mycorrhizal) a G. fasciculatus infection. Each point represents the average leaf area (cm^2) of 4-6 plants.



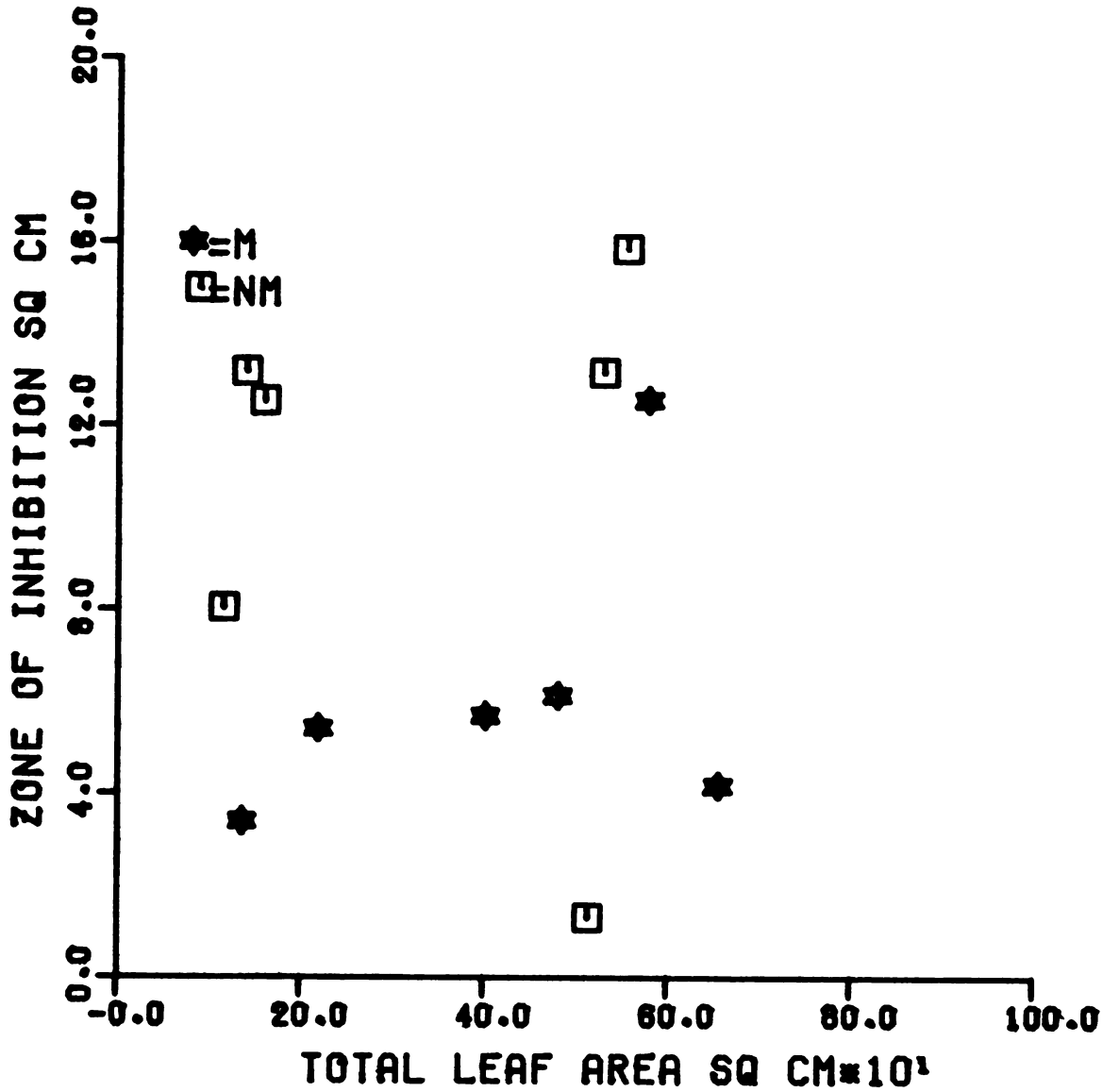


Figure 3. Benomyl uptake with relation to leaf area. Leaf bioassays were conducted two days after a soil drench of benomyl.

= NM, = M.

possibility benomyl was added as a soil drench (25 µg/g soil) to plants which were 45 days old. Plants were bioassayed five days later. Four M and NM plants were used per treatment, with one additional untreated M and NM plant used as controls. The experiment was done three times (Appendix D, Table 5).

Even though fungal colonization of the roots had taken place, no mycorrhizal growth stimulation occurred (Appendix D, Table 6). Figure 4 shows the results of one of these experiments. No differences in uptake could be demonstrated. All experiments had similar results except in one experiment where the leaves of the NM plants contained approximately 2X as much benomyl (Appendix D, Figures 22, 23). This was not a repeatable phenomenon.

In order to determine if varying the period of exposure to benomyl would allow detection of differing capabilities of M and NM plants to take up the fungitoxicant, benomyl was applied as a drench (25 µg/g soil) on four different dates. Benomyl was added on days 19, 26, 33, 40, and assayed on day 45, resulting in 26, 19, 12, and 5 days exposure, respectively. Five M and NM plants were drenched on each date. Four M and NM plants were left untreated as controls. The experiment was conducted two times. These two experiments showed similar concentrations of benomyl in roots and leaves of M and NM plants at all exposure times and it was concluded that under the conditions tested there were no differences in the ability of M and NM plants to take up benomyl. Figures 5 and 6 show the results of one of these experiments. The results of the other experiment were similar (Appendix D, Figures 19, 20)

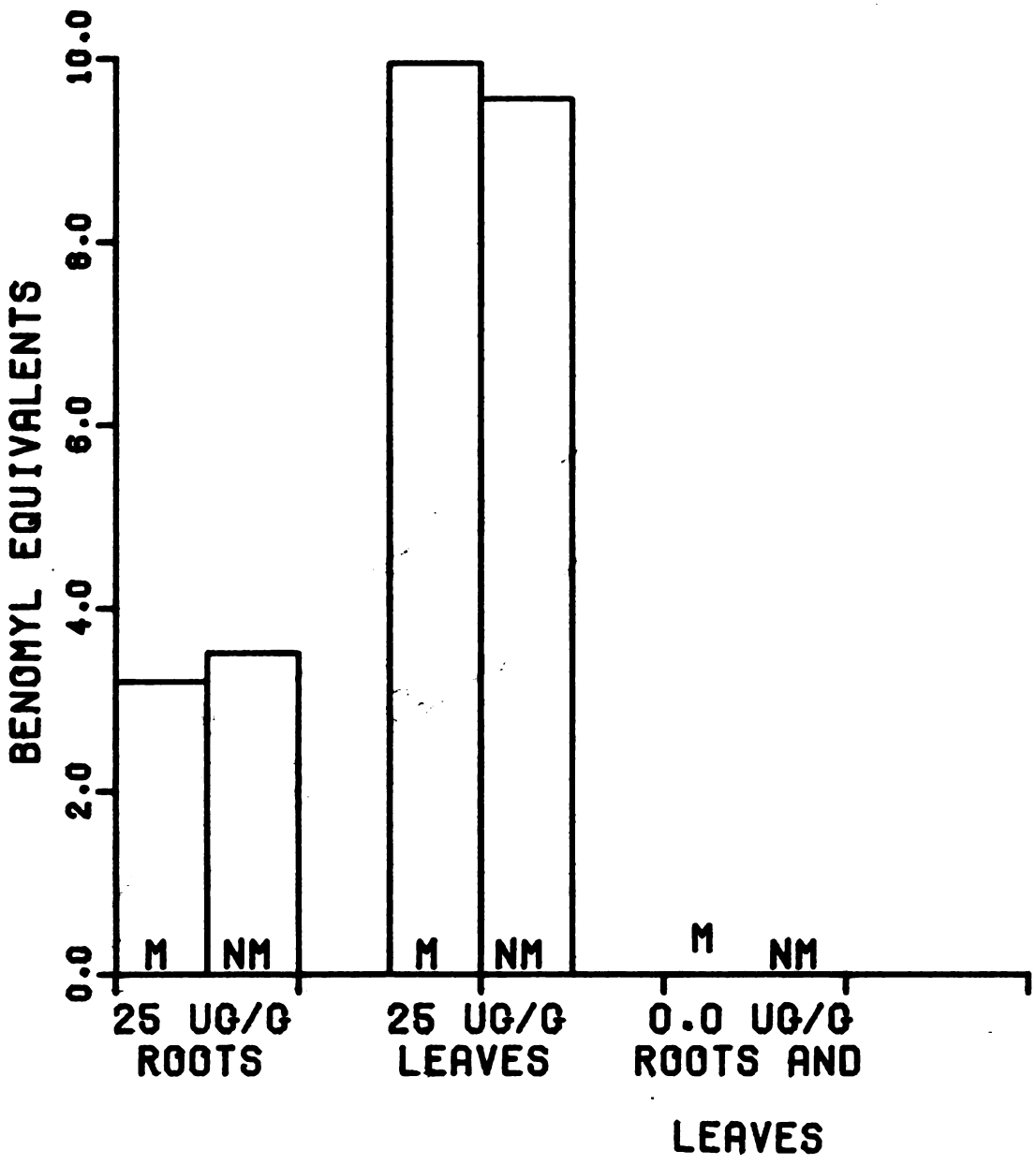
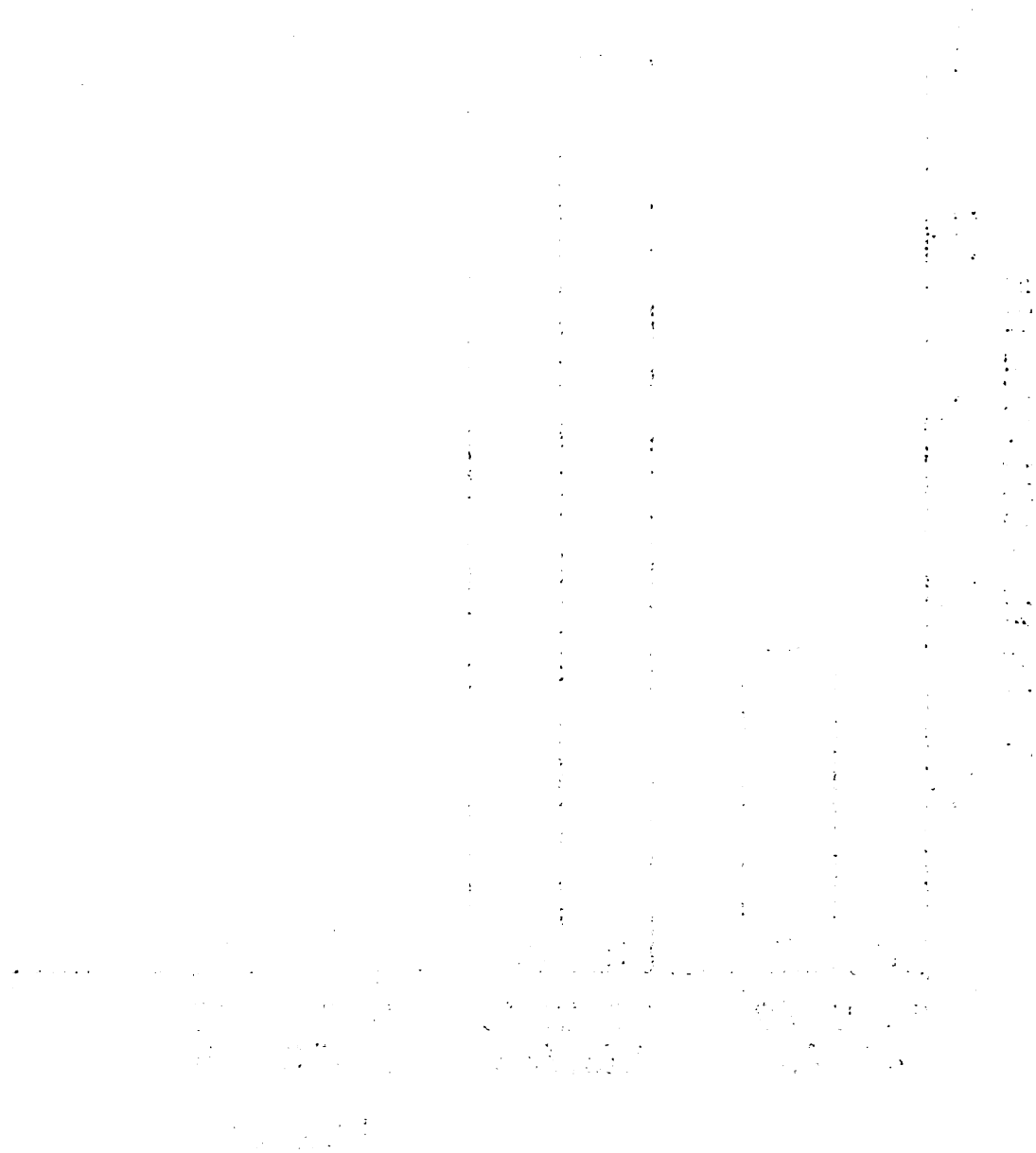


Figure 4. Benomyl distribution in roots vs. leaves of soybeans with (M) or without (NM) a G. fasciculatus infection. Each bar represents the mean of five plants except the 0.0 µg/g bar which represents one plant.



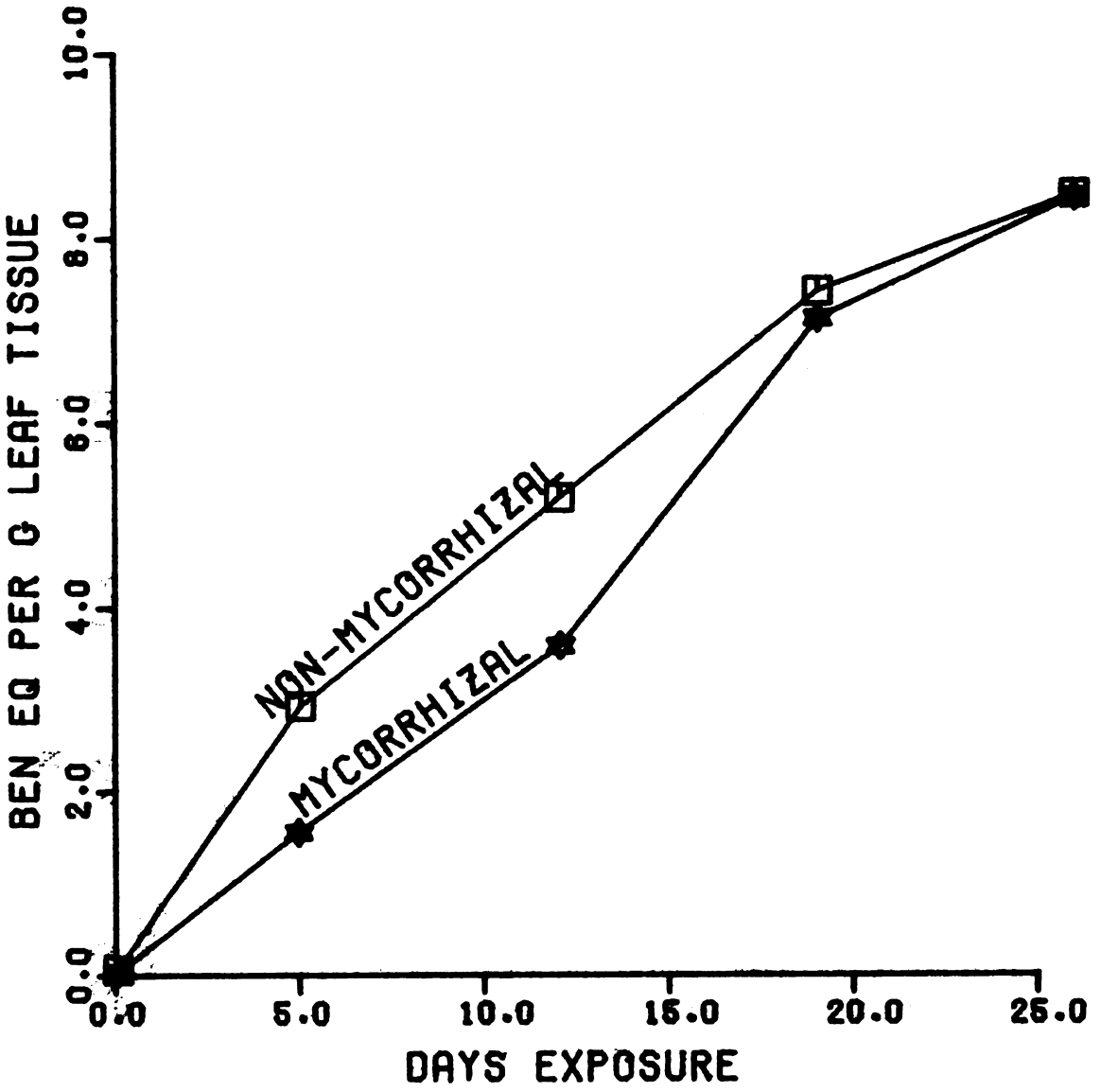


Figure 5. Benomyl concentration in leaves vs. days exposure to a 25.0 µg/g soil drench. Each point represents the average of five soybean plants either with (mycorrhizal) or without (non-mycorrhizal) a G. fasciculatus infection.

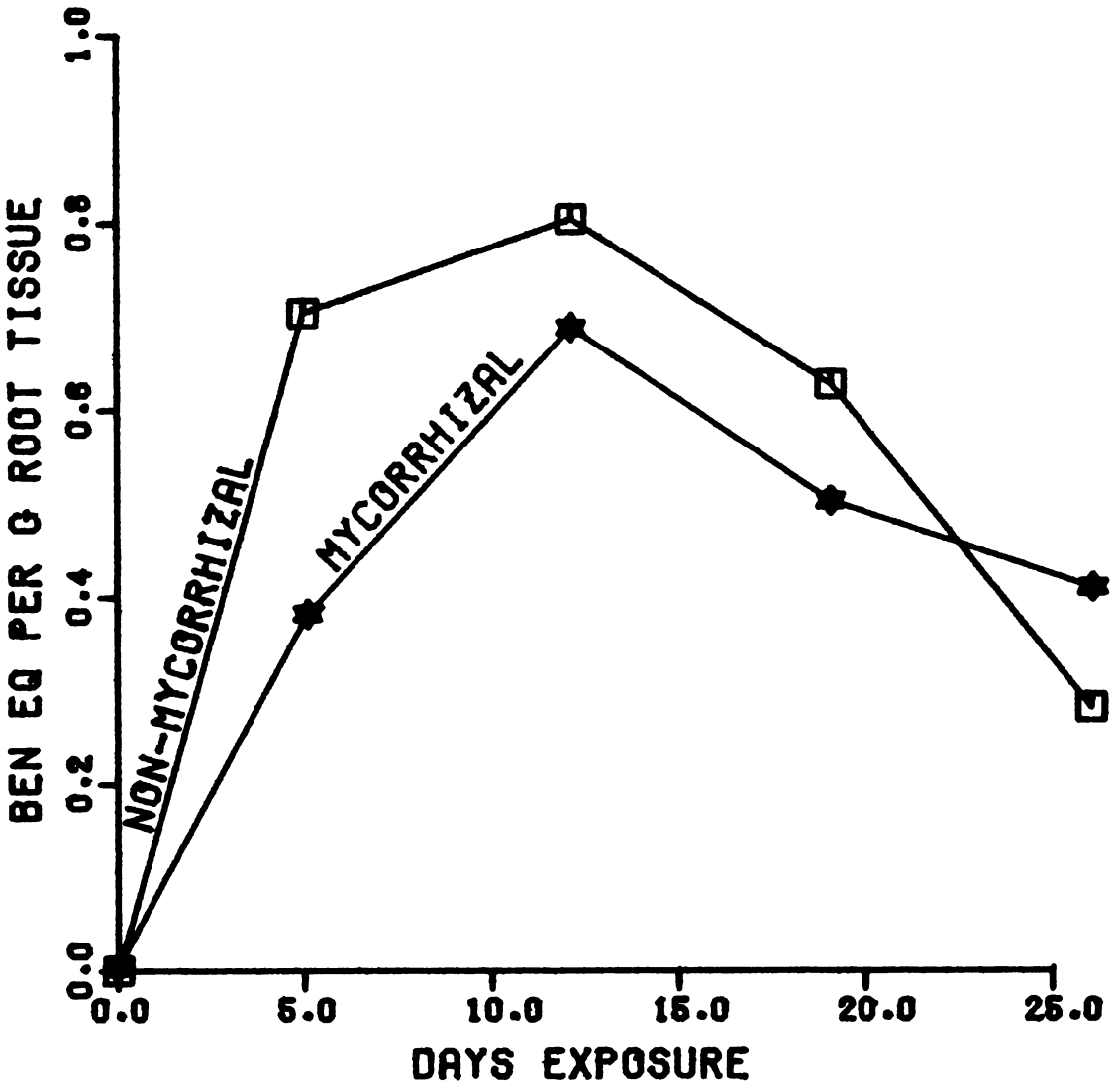


Figure 6. Benomyl concentration in roots vs. days exposure to a 25.0 µg/g soil drench. Each point represents the average of five soybean plants either with (mycorrhizal) or without (non-mycorrhizal) a G. fasciculatus infection.

0.0
0.2
0.4
0.6
0.8
1.0
0.0
0.05
0.10
0.15
0.2
0.25

There was an interesting trend for the plants to form less foliage with increasing exposure periods to benomyl (Figure 7). This was much more pronounced for the M plants than the NM plants and would appear to support the results in the tolerance studies (Figure 2) which also showed that M plants developed more poorly in the presence of benomyl then when no benomyl was present.

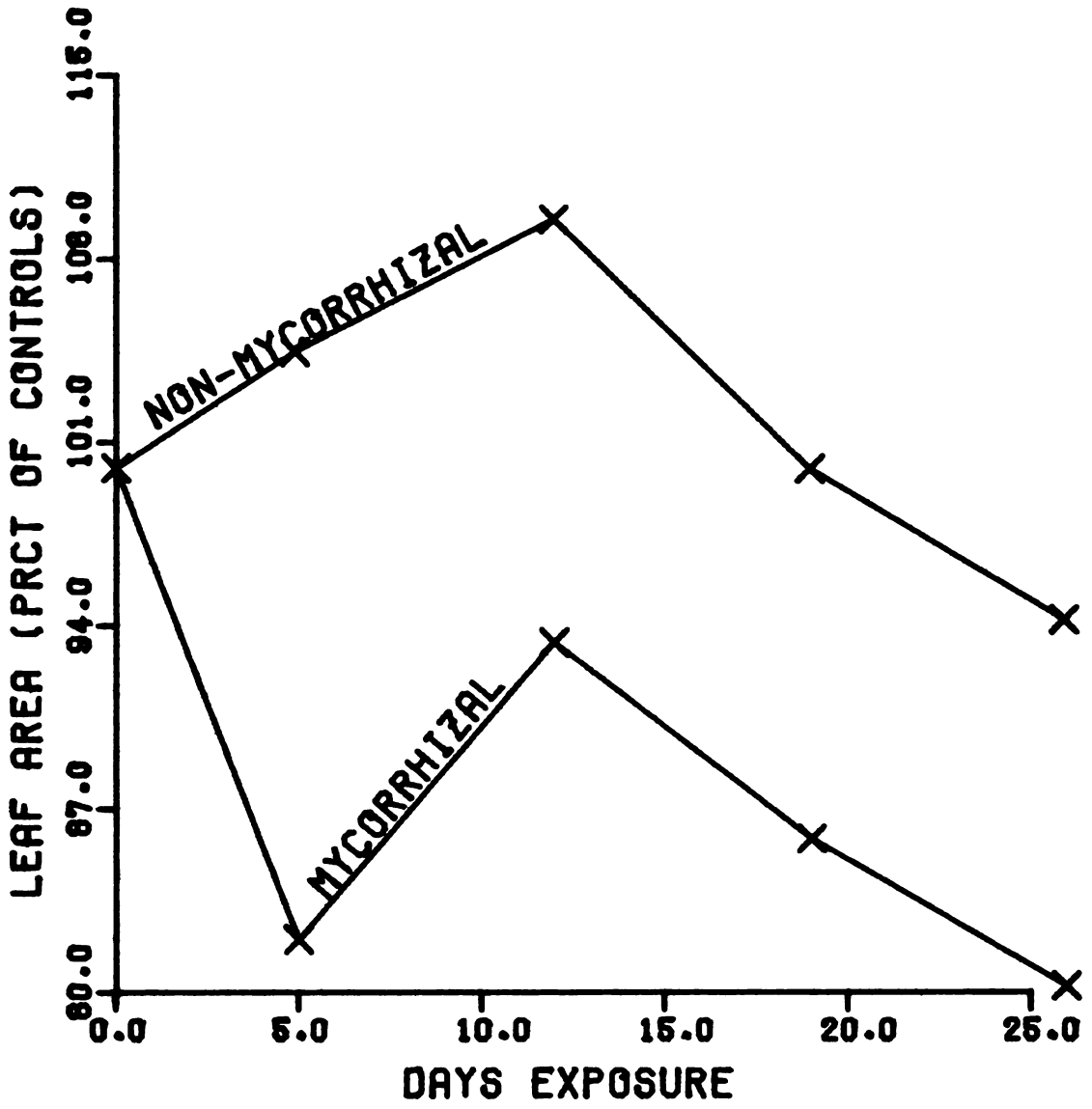


Figure 7. Leaf area as a percentage of non-treated controls vs. exposure time to 25.0 µg/g benomyl. Each point represents the average of five plants.

DISCUSSION

This paper showed that mycorrhizal spores germinated and infected soybean plants when up to 250 µg/g soil benomyl was applied. The infection process was hindered however, as evidenced by the decrease in mycorrhizal infection after benomyl application. The reduction in infection could be due to an overall reduced germination of the chlamydospores and/or hyphal growth in the presence of benomyl or to an effect on infection per se. Even if 250.0 µg/g of benomyl was initially too high for infection to occur dilution via watering, binding to organic matter, degradation, etc., could have reduced the effective dose to a level allowing germination and infection. No significant change in growth occurred when benomyl was applied to NM plants, thus, phytotoxicity or the reduction of an unnoticed disease cannot account for these differences. Therefore, the increases in growth were attributable to the mycorrhizal associations and the reductions in growth were attributed to the ill effects of benomyl on the host fungus relationship.

The fact that 50% infection did not increase plant growth may preclude "percentage-type" measures of infection as valid indices of symbiotic efficiency. Future studies on soil-plant ecology must

include both infection rating and growth response measurements.

Total transpiration usually increases with increasing leaf area, which was expected to result in more benomyl uptake. However, the larger leaf area also provides additional tissue to be supplied from a finite pool of benomyl. Under the conditions used in this work there was no discernible relationship between leaf area or root wieght with concentration of the compound in the leaves. Therefore, any true differences in sizes of zones of inhibition in the experiments would probably have been a result of increased efficiency of the root systems to take up benomyl. Since there were no consistent differences in uptake of benomyl by M and NM plants in any of the experiments, mycorrhizal infection apparently can increase growth without increasing the uptake of benomyl.

An interesting trend was seen in growth of M plants when exposed to benomyl for various lengths of time. With increasing exposure, the M plants showed decreasing rates of leaf area formation. Here, the mycorrhizal system may no longer function normally resulting in slower growth rates.

In Sutton's and Sheppard's work (79) sand was used as the planting medium, which would not have retained benomyl to any great extent. Unless the chlamydospores took up the fungitoxicant there should have been an extremely low concentration of benomyl 73 days after a 15 ug/g soil drench. Nevertheless, after this period of time, there was no infection of the plants in their experiments. Several genera of fungi which are known to be sensitive to benomyl (17,10)

were found abundantly in their treated soil. These were species of Cladosporium, Fusarium, Penicillium, Colletotrichum, and Humicola. Evidently, the benomyl had fungistatic effects on these organisms in that they were able to grow once removed from the soil and placed on artificial media. It would have been interesting had they removed the Glomus chlamydospores and applied them to untreated plants to test for viability. Sutton's and Sheppard's results, along with those of this paper, indicate that there may be a wide difference in susceptibility of Glomus species to benomyl. Possibly a less sensitive fungus was used in this work than in theirs. Comparisons of these two works are complicated by differences in soil type, method of benomyl application and the mycorrhiza-forming fungus used.

The results of this work indicate that mycorrhizal plants do not have an increased ability to take up benomyl, at least under the conditions tested. However, extensive colonization of the root cortex by VA fungal hyphae did not hinder benomyl uptake. In addition it was shown that G. fasciculatus can withstand a 250 µg/g soil drench of benomyl, a concentration much higher than normally used, and still infect soybean roots. A most important finding is that a seemingly healthy mycorrhiza loses its ability to increase plant growth when benomyl is added to the soil.

LITERATURE CITED

LITERATURE CITED

1. Alexopoulous, C. J. 1962. Introductory mycology. John Wiley & Sons, Inc., New York. 613 pp.

2. Bartels-Schooley, J. and B. H. MacNeil. 1971. A comparison of the modes of action of three benzimidazoles. Phytopathology 61: 816-819.

3. Bartha, R., R. P. Lanzilotta, and D. Pramer. 1967. Stability and effects of some pesticides in soil. Appl. Microbiol. 15: 67-75.

4. Baude, F. J., J. A. Gardiner, and J. C. Y. Han. 1973. Characterization of residues on plants following foliar spray applications of benomyl. J. Agr. Food Chem. 21: 1084-1090.

5. Baude, F. J., H. L. Pease, and R. F. Holt. 1974. Fate of benomyl on field and soil turf. J. Agr. Food Chem. 22: 413-417.

6. Baylis, G. T. S. 1959. Effect on vesicular-arbuscular mycorrhizae on growth of Grisclinia littoralis (Cornaceae). New Phytol. 58: 274-280.

7. Baylis, G. T. S. 1967. Experiments on the ecological significance of phycomycetous mycorrhizas. New Phytol. 66: 231-243.

8. Berg, G. A. Van Den, and G. J. Bollen. 1971. Effect of benomyl on incidence of wilting of Callistephus chinensis, caused by Phytophthora cryptogea. Acta. Bot. Neerl. 20: 256-257. (Abstract).

9. Biehn, W. L. and A. E. Dimond. 1970. Reduction of tomato Fusarium wilt symptoms by benomyl and correlation with a bioassay of fungitoxicant in benomyl-treated plants. Plant Dis. Rep. 54: 12-14.

10. 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.

11. Clark, F. B. 1969. Endotrophic mycorrhizal infection of tree seedlings with Endogone spores. Forest Sci. 15: 134-137.

12. Clemons, G. P. and H. D. Sisler. 1969. Formation of a fungitoxic derivative from benlate. Phytopathology 59: 705-706.

13. Corbett, J. F. 1974. Pesticides thought to inhibit biosynthetic reactions. pp. 221-249. In: The biochemical mode of action action of pesticides. Academic Press. London, New York.

14. Daft, M. J. and T. H. Nicolson. 1966. Effect of Endogone mycorrhiza on plant growth. New Phytol. 65: 343-350.

15. Daft, M. J. and T. H. Nicolson. 1969. Effect of Endogone mycorrhiza on plant growth. II. Influence of soluble phosphate on endophyte and host in maize. New Phytol. 68: 945-952.

16. Delp, C. J. and H. L. Klopping. 1968. Performance attributes of a new fungicide and mite ovicide candidate. Plant Dis. Rep. 52: 95-99.

17. Edgington, L. V., K. L. Khew, and G. L. Barron. 1971. Fungitoxic spectrum of benzimidazole compounds. Phytopathology 61: 42-44.

18. Ellis, M. A. and J. B. Sinclair. 1976. Effect of benomyl field sprays on internally borne fungi, germination, and emergence, of late-harvest soybean seeds. Phytopathology 66: 680-682.

19. Enyinnia, T. and J. K. Springer. 1974. Effect of fungicidal seed treatment on emergence of Diaporthe-infected soybeans. Phytopathology 64: 580.

20. Erwin, D. C. 1968. The systemic effect of 1-(butylcarbamoyl)-2-Benzimidazole carbamic acid, methyl ester, on Verticillium wilt of cotton. Phytopathology 58: 528-529.

21. Erwin, D. C. 1969. Methods of determination of the systemic and fungitoxic properties of chemicals applied to plants with emphasis on control of Verticillium wilt with Thiabendazole and benlate. World Rev. Res. Control 8: 6-22.

22. Erwin, D. C. 1973. Systemic fungicides: disease control, translocation, and mode of action. Annu. Rev. Phytopathol. 11: 389-422.

23. Filer, T. H. and E. R. Toole. 1968. Effect of methyl bromide on mycorrhizae and growth of sweet gum seedlings. Plant Dis. Rep. 52: 483-485.

24. Fuchs, A., D. L. Fernandes, and F. W. DeVries. 1974. The function of and MBC-releasing deposits of benomyl and thiophanates in plant roots and soil. Neth. J. Plant Path. 80: 7-18.

25. Fuchs, A., G. A. Van Den Berg, and L. C. Davidse. 1972. A comparison of benomyl and thiophanates with respect to some chemical and systemic fungitoxic characteristics. Pestic. Biochem. and Physiol. 2: 191-205.

26. Gerdemann, J. W. 1964. The effect of mycorrhiza on the growth of maize. Mycologia 56: 344-349.

27. Gerdemann, J. W. 1965. Vesicular-arbuscular mycorrhizae formed on maize and tulip-tree by Endogone fasciculata. Mycologia 57: 562-575.

28. Gerdemann, J. W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. Annu. Rev. Phytopathol. 6: 397-418.

29. Gerdemann, J. W. 1974. The Endogonaceae in the pacific northwest. Mycologia Memoir No. 5. 76 pp.

30. Gerdemann, J. W. 1975. Vesicular-arbuscular mycorrhizae. pp. 575-591. In: The Development and Function of Roots.

31. Gray, L. E. and J. W. Gerdemann. 1969. Uptake of phosphorus-32 by vesicular-arbuscular mycorrhizae. Plant and Soil 30: 415-421.

32. Gray, L. E. and J. W. Gerdemann. 1972. Uptake of sulphur-35 by vesicular-arbuscular mycorrhizae. Plant and Soil 39: 687-689.

33. Gray, L. E. and J. B. Sinclair. 1970. Uptake and translocation of systemic fungicides by soybean seedlings. Phytopathology 60: 1486-1488.

34. Greenway, W. 1973. Growth of helminthosporia on media containing the fungicide benomyl and the assay of benomyl using Botrytis cinerea. Ann. Appl. Biol. 73: 319-324.

35. Hammerschlag, R. S. and H. D. Sisler. 1972. Benomyl and methyl-2-benzimidazolecarbamate (MBC): biochemical, cytological and chemical aspects of toxicity of Ustilago maydis and Saccharomyces cerevesiae. Pesticide Biochem. Physiol. 3: 42-54.

36. Hammerschlag, R. S. and H. D. Sisler. 1972. Differential action of benomyl and methyl-2-benzimidazolecarbamate in Saccharomyces pastorianus. Potomac Abstracts. p. 669 (Abstract)

37. Hatting, M. J., L. E. Gray, and J. W. Gerdemann. 1973. Uptake and translocation of ^{32}P-labeled phosphate to onion roots by endomycorrhizal fungi. Soil Sci. 116: 383-387.

38. Hayman, D. S. 1970. Endogone spore numbers in soil and vesicular-arbuscular mycorrhiza in wheat as influenced by season and soil treatment. Trans. Brit. Mycol. Soc. 54: 53-63.

39. Hayman, D. S. 1975. The occurrence of mycorrhiza in crops as affected by soil fertitily. pp. 495-509. In: Endomycorrhizas. (F. E. Sanders, Barbara Mosse, and P. B. Tinker, eds.) Academic Press, New York.

40. Hayman, D. S. and B. Mosse. 1970. Plant growth responses to vesicular-arbuscular mycorrhiza. New Phytol. 70: 19-27.

41. Hayman, D. S. and B. Mosse. 1971. Plant growth responses to vesicular-arbuscular mycorrhizae. III. Increased uptake of labile P from soil. New Phytol. 71: 41-47.

42. Helling, C. S., D. G. Dennison, and D. D. Kaufman. 1974. Fungicide movement in soils. Phytopathology 64: 1091-1100.

43. Hook, W. K., L. R. Schreiber, and B. R. Roberts. 1970. Factors influencing uptake, concentration, and persistence of benomyl in American elm seedlings. Phytopathology 60: 1619-1622.

44. Jackson, N. 1970. Evaluation of some chemicals for control of stripe smut in Kentucky bluegrass turf. Plant Dis. Rep. 54: 168-170.

45. Jackson, N. E., R. A. Miller, and R. E. Franklin. 1973. The influence of vesicular-arbuscular mycorrhiza on uptake of ^{90}Sr from soil by soybeans. Soil Biol. Biochem. 5: 205-212.

46. Jalali, B. L. and K. H. Domsch. 1975. Effect of systemic fungitoxicants on the development of endotrophic mycorrhiza. pp. 619-626. In: Endomycorrhizas. (F. E. Sanders, Barbara Mosse, and P. B. Tinker, eds.) Academic Press, New York.

47. Kanouse, B. B. 1936. Studies of two species of Endogone in culture. Mycologia 28: 47-62.

48. Kaastra-Howeler, L. H. and W. Gams. 1973. Preliminary study on the effect of benomyl on the fungal flora in greenhouse soil. Neth. J. Plant Pathol. 79: 156-158.

49. Khan, A. G. 1972. The effect of vesicular-arbuscular mycorrhizal associations on growth of cereals. I. Effects on maize growth. New Phytol. 71: 613-619.

50. Khan, A. G. 1973. The effect of vesicular-arbuscular mycorrhizal association on growth of cereals. II. Effects on wheat growth. Ann. Appl. Biol. 80: 27-36.

51. Kirkland, Joseph J., Richard E. Holt, and Harlan L. Pease. 1973. Determination of benomyl residues in soils and plant tissues by high-speed cation exchange liquid chromatography. J. Agr. Food Chem. 21: 368-371.

52. 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.

53. Leach, L. D., E. M. Huffman, and W. C. Reische. 1969. Some factors influencing protection of cotton from Verticillium wilt in benomyl-treated soil based on bioassay and performance. Proc. Nat. Cotton Council, 29th Cotton Disease Council. pp. 36-40.

54. Meyer, W. A., J. F. Nicholson, and J. B. Sinclair. 1971. Translocation of benomyl in creeping bentgrass. Phytopathology 61: 1198-1200.

55. Mosse, B. 1973. Plant growth responses to vesicular-arbuscular mycorrhizae. IV. In soil given additional phosphate. New Phytol. 72: 127-136.

56. Mosse, B. 1974. Advances in the study of vesicular-arbuscular mycorrhizae. Annu. Rev. Phytopathol. 12: 171-197.

57. Mosse, B. and J. M. Phillips. 1971. The influence of phosphate and other nutrients on the development of vesicular-arbuscular mycorrhiza in culture. J. Gen. Microbiol. 69: 157-166.

58. Murdoch, C. L., J. A. Jackobs, and J. W. Gerdemann. 1967. Utilization of phosphorous sources of different availability by mycorrhizal and non-mycorrhizal maize. Plant Soil 27: 329-334.

59. Nesheim, Norman O. and M. B. Linn. 1969. Deletarious effects of certain fungitoxicants on the formation of mycorrhizae on corn by Endogone fasciculatus and corn root development. Phytopathology 59: 297-300.

60. Nicholson, J. F., J. B. Dinclair, J. C. White, and B. L. Kirkpatr i ck. 1972. Upward and lateral translocation of benomyl in strawberry. Phytopathology 62: 1183-1185.

61. Patblado, R. E. and L. V. Edgington. 1972. Movement of benomyl in field soils as influenced by acid surfacants. Phytopathology 62: 513-516.

62. Peeples, J. L. 1974. Microbial activity in benomyl-treated soils. Phytopathology 64: 857-860.

63. Peterson, Carol A. and L. V. Edington. 1970. Transport of the systemic fungicide benomyl, in bean plants. Phytopathology 60: 475-478.

65. Peterson, Carol A. and L. V. Edington. 1971. Transport of benomyl into various plant organs. Phytopathology 61: 91-92.

66. Ponchet, J. and R. Tramier. 1971. Effets du benomyl sur la croissance de l'ceillet et al microflore des sols traites. Ann. Phytopathol. 3: 401-406.

67. Rhodes, L. H. and J. W. Gerdemann. 1975. Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol. 75: 555-561.

68. Rhodes, L. H. and J. W. Gerdemann. 1976. Effect of vesicular-arbuscular mycorrhizae on sulfate uptake by onion plants. Proc. Am. Phyto. Soc. 3: 274-275.

70. Ross, J. P. 1971. Effect of phosphate fertilization on yield of mycorrhizal and non-mycorrhizal soybeans. Phytopathology 61: 1400-1403.

71. Ross, J. P. and J. A. Harper. 1970. Effect of Endogone mycorrhizae on soybean yields. Phytopathology 60: 1552-1556.

72. Safir, G. R., J. S. Boyer and J. W. Gerdemann. 1971. Mycorrhizal enhancement of water transport in soybean. Science 172: 581-583.

73. Safir, G. R., J. S. Boyer and J. W. Gerdemann. 1972. Nutrient status and mycorrhizal enhancement of water transport in soybean. Plant Physiol. 49: 700-703.

74. Schreiber, L. R., W. K. Hack and B. R. Roberts. 1971. Influence of planting media and soil sterilization on the uptake of benomyl by American elm seedlings. Phytopathology 61: 1512-1515.

75. Siegel, M. R. 1975. Benomyl-soil microbial interactions. Phytopathology 65: 219-220.

76. Sims, J. J., Henry Mee and D. C. Erwin. 1969. Methyl-2-benzimidazole-carbamate, a fungitoxic-compound isolated from cotton plants treated with methyl-1-(butylcarbamoyl)-2-benzimidazole-carbamate (benomyl). Phytopathology 59: 1775-1776.

77. Smith, A. M., B. A. Stynes and K. J. Moore. 1970. Benomyl stimulates growth of a basidiomycete on turf. Plant Dis. Rep. 54: 774-775.

78. Sutton, J. C. 1973. Development of vesicular-arbuscular mycorrhizae in crop plants. Can. J. Bot. 51: 2487-2493.

79. Sutton, J. C. and B. R. Sheppard. 1976. Aggregation of sand-dune soil by endomycorrhizal fungi. Can. J. Bot. 54: 326-333.

80. Thapliyal, P.B. and J. B. Sinclair. 1970. Uptake of three systemic fungicides by germinating soybean seed. Phytopathology 60: 1373-1375.

81. Thornberry, H. A. 1950. A paper-disk plate method for the quantitative evaluation of fungicides and bacteriocides. Phytopathology 40: 419-429.

82. Tinker, P. B. H. 1975. Effects of vesicular-arbuscular mycorrhizas on higher plants. pp. 325-349. In: Symposia of the Society for Experimental Biology.

83. Walker, L. B. 1923. Some observations on the development of Endogone malleola Hark. Mycologia 15: 245-257.

84. Weeks, R. E. and H. G. Hedrick. 1975. Influence of a systemic fungicide on oxygen uptake by soil microorganisms. Soil Sci. 119: 280-284.

APPENDICES

APPENDIX A

Mycorrhizal Infection Rating Systems

Growth Observation Method

The entire ball of dirt containing the roots was carefully removed from the pots and the free soil was removed by submerging in water and gently massaging. The M roots remained black with organic matter in areas where M hyphae were dense. A rating system of 0 to 3 was used to designate the amount of organic matter clumping. A 1 represented the amount of organic matter normally adhering to NM plants with up to 25% of the root system being covered, 2 was from 25 to 75% and a 3 from 75 to 100% of the root system covered by dark clumps.

Clump Method

A clump was defined as an area of root which had organic matter attached, yet was able to have limited movement in running water. These areas were found through microscopic observation to be approximately 98% due to mycorrhizal hyphae.

Such areas were not always distinct. When a continuous length of root had clumping for more than 0.5 cm, each 0.5 cm of length was

counted as one clump.

Roots were washed as above and three samples of the root system were removed. The samples were taken from the top left, lower left, and right center of the root system. The samples were then placed in a large petri dish (150 mm diameter) which was divided into 2 cm square sections. A small amount of water was placed in the dish and the roots were distributed as evenly as possible. Once distributed, a piece of paper with ten dots was slid under the dish. Every square which corresponded with a dot was rated for clumps on a lighted slide viewing tray at 10 X magnification. Ten squares were counted and the total clumps for the whole sample were calculated. The sample was then patted dry, weighed, and the infection was reported as clumps per gram of root.

APPENDIX B

Bioassay Procedures

Leaf Plug Method

To sample plant tissue a paper punch (6.0 mm diameter) was used to remove a circle of tissue from the second trifoliate leaf of each plant. The plugs were removed from the center leaflet with the right margin forming a tangent to the punched circle. The plugs of tissue were frozen on dry ice for 60 seconds, and placed in petri plates containing approximately 8 ml of (1.5%) PDA. The plates were sprayed with equal amounts of a spore suspension as used in the acetone extract method (see methods). Zones of inhibition were measured after three days.

The effect of varying the location of the sample plug and the effect of freezing leaf plugs were tested. To accomplish this, benomyl was added as a soil drench to four soybean plants thirty days after planting. One plant was left untreated to serve as a control. Two days later, four plugs were taken from the middle leaflet of the second trifoliate leaf of each plant as illustrated in Figure 8.

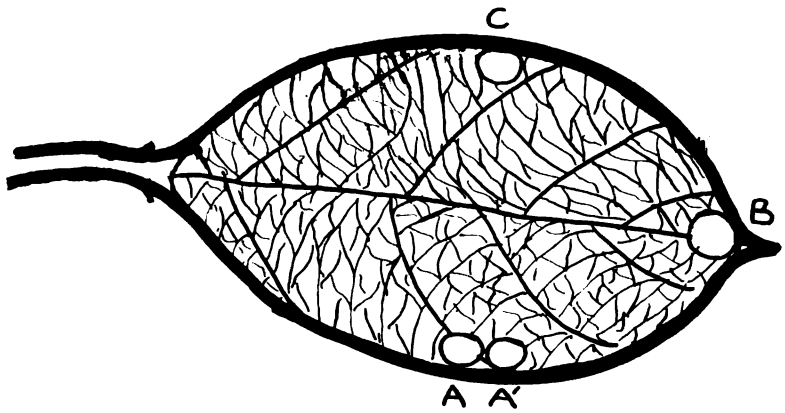


Figure 8. Illustration of middle leaflet of the second trifoliate leaf. A, A', B, C mark the locations for the plugs taken two days after drenching with benomyl.

One of the "A" plugs was frozen by holding it to a block of dry ice for 60 seconds. The rest of the plugs were left unfrozen. All plugs were bioassayed as above. This experiment was done twice (Table 1).

No statistical differences in zone of inhibition were found (t test $P < .05$) between the frozen and non-frozen leaf plugs taken from various locations (Table 1). Leaf tissue alone had no fungitoxic effect on the P. digitatum spores.

Comparison of the Leaf Plug and Acetone Disk Methods

To determine if the uptake of one leaflet was proportional to total plant uptake, the leaf plug and acetone extraction methods were compared for different benomyl concentrations and times of application. Benomyl at 0.2, 2.0, 5.0, 10.0, and 25.0 µg/g soil was applied 24 hours before and two to four weeks after seeding. This gave exposure times of 35, 21, and 7 days respectively, before the assay.

Table 1. Effect of leaf plug location and freezing on area of zone of inhibition.

Experiment		Zone of Inhibition[a]				
		Frozen[b]		Non-Frozen		Average
Test	Treatment	A[c]	A	B	C	ABC
1 & 2	Benomyl	11.0[d]	9.6	9.1	10.1	9.6
	No Benomyl	0.0[e]	0.0	0.0	0.0	0.0

[a]Zones measured in square centimeters.

[b]Frozen by holding to dry ice for 60 seconds.

[c]Plug location on leaf (Figure 8).

[d]Average of eight leaf plugs from the middle leaflet of the second trifoliate leaf (one leaf plug/plant).

[e]Average of two leaf plugs from the middle leaflet of the second trifoliate leaf (one leaf plug/plant).

Both methods showed maximum recoverable benomyl at seven days after treatment. When exposure periods were longer than seven days, recovery was greatly reduced with no recoverable benomyl at 35 days (Figure 9 and 10). The leaf plug method was able to detect benomyl applied at 2 µg/g soil whereas the acetone method could detect benomyl at 5 µg/g soil. The acetone disk method better differentiated higher concentrations of benomyl as reflected by the disproportionally larger increases in zone areas over the leaf plug method. The variance remained small enough with the acetone disk method to show statistical differences between the zones at 14 days exposure whereas this does not hold true for the leaf plug method at the same exposure time.

When qualitative indications of fungitoxicity in the leaves was sought, the leaf plug biomass method was utilized due to its ease and quick results. However, when quantitative measurements were needed, the acetone extract method was used. As was seen in the experiment comparing the two methods (Figures 9 and 10), the leaf plugs gave zones of inhibition at low concentrations of benomyl. As soil drench concentrations increased, the leaf plug method rapidly lost resolution. The acetone method represented a larger amount of tissue and consequently produced larger zones at the same tissue/fungitoxicant ratios. This resulted in a more definitive system for measuring total protection of the leaves.

Other sorts of inaccuracy may be encountered using the leaf plug method. It has been shown (65) that the movement of benomyl within a leaf follows the transpiration stream. This results in a clearing of the

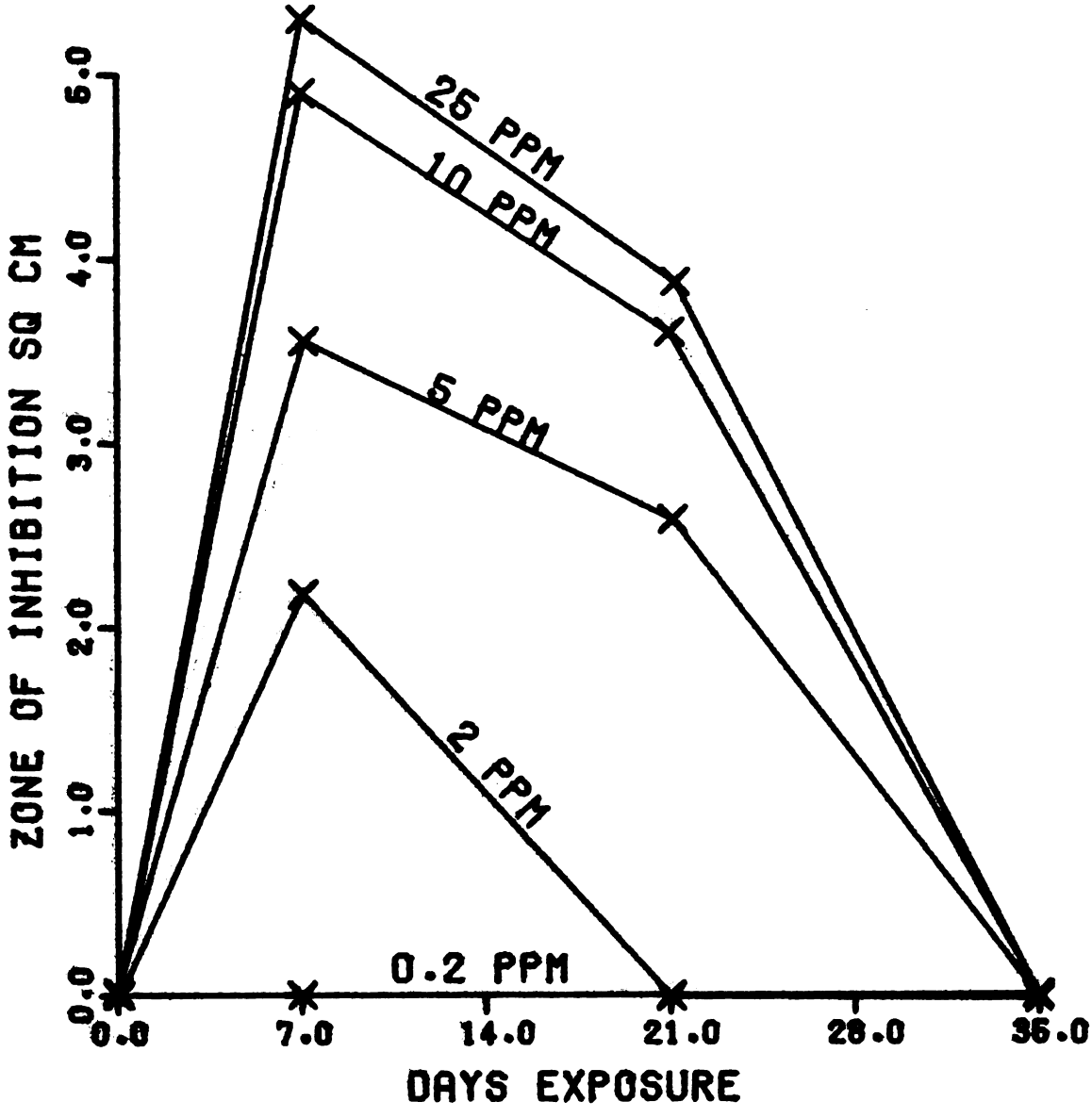
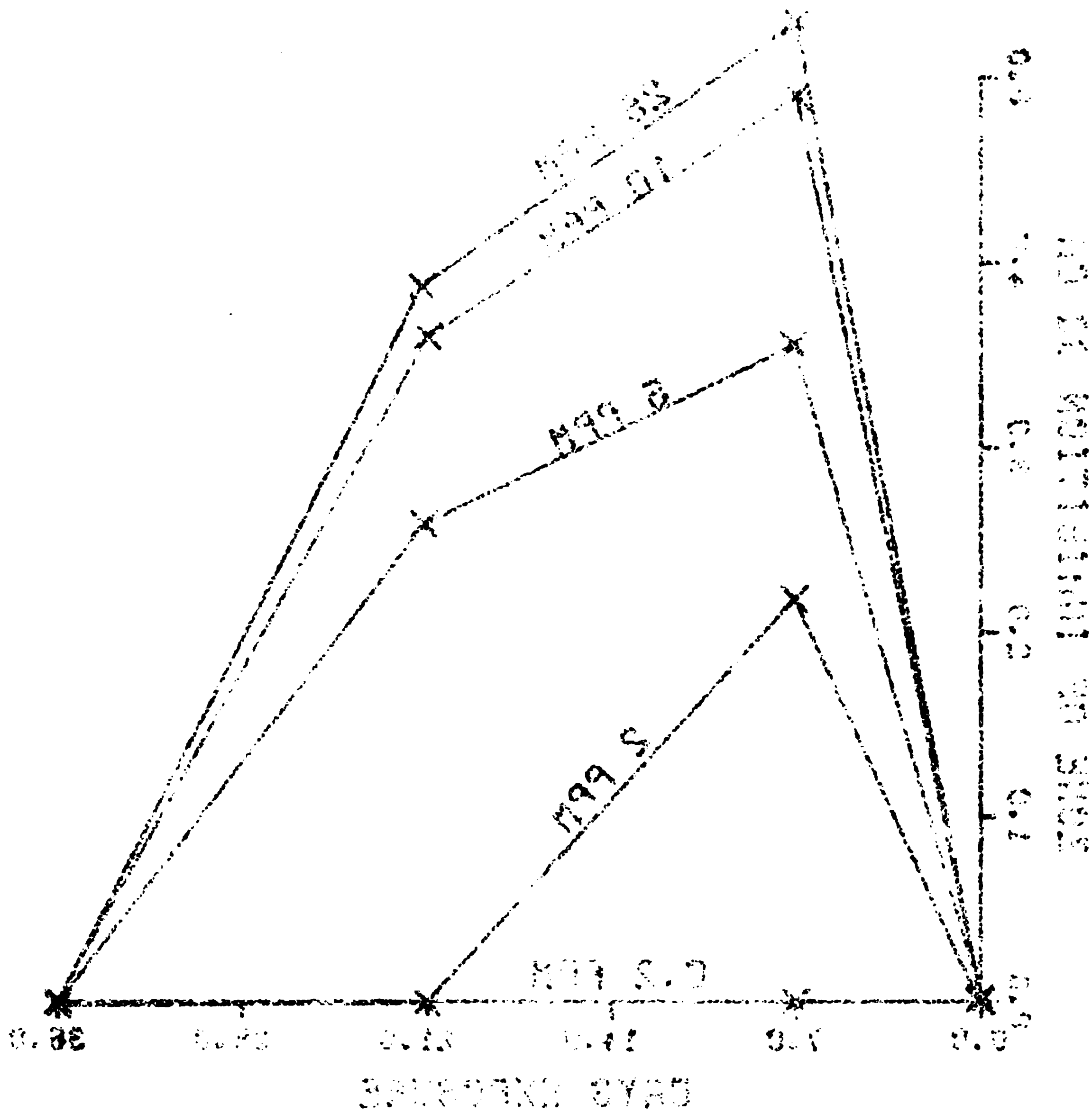


Figure 9. Zone of inhibition (cm^2) about leaf plugs with relation to exposure time and benomyl concentration. Each point represents the average of five plants.



10 PPM
5 PPM
2 PPM
0.2 PPM

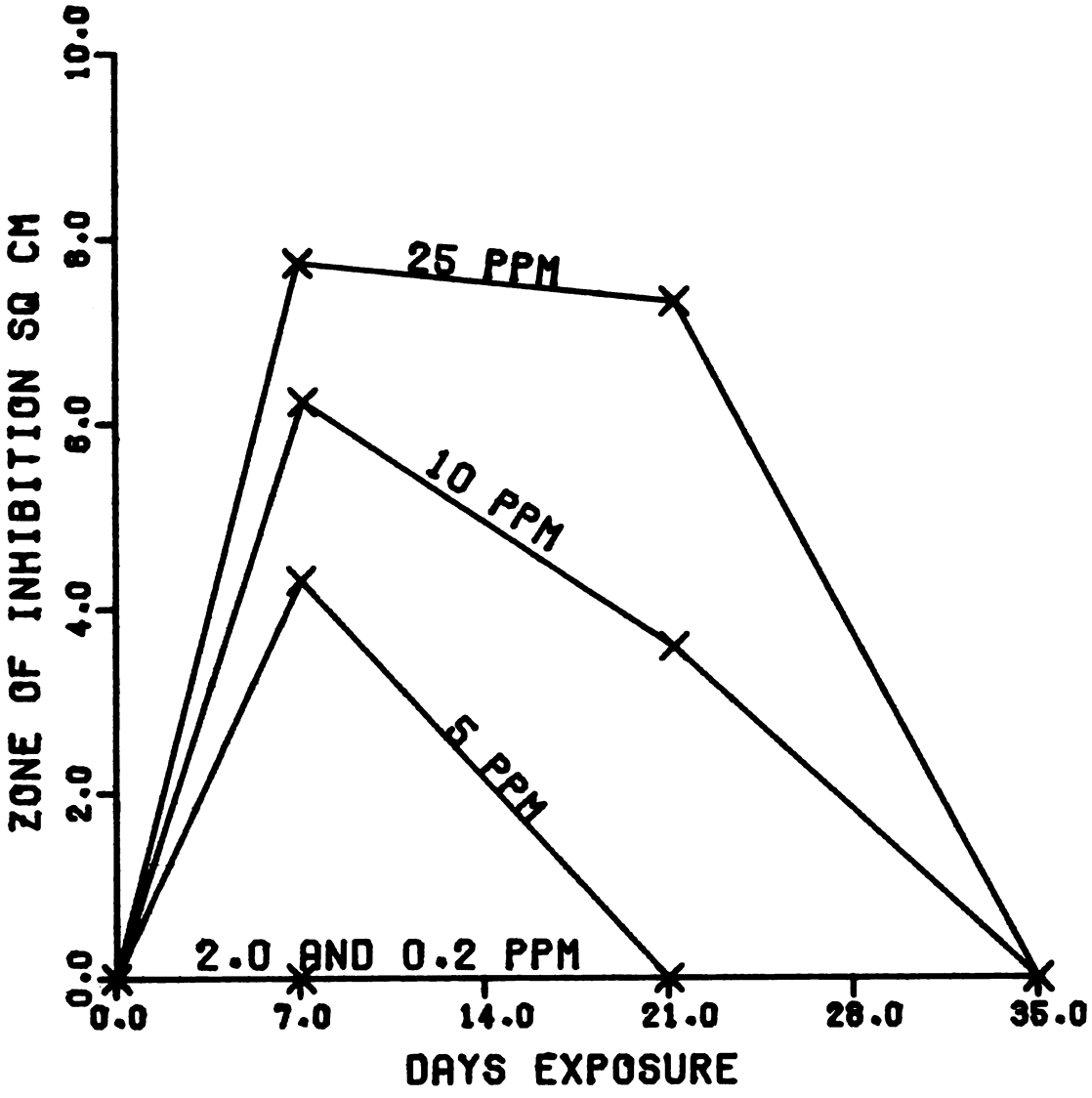


Figure 10. Zone of inhibition (cm^2) about acetone disks with relation to exposure times and benomyl concentrations. Each point represents the average of three plants.

base and mid-vein and concentrating along the margins of the leaf. There are differences in the amount of transpiration with different ages of leaves, thus different rates of benomyl accumulation would be expected according to the age of the leaf. Finally, the form of the fungitoxicant (i.e., benomyl or MBC) alters uptake characteristics (51, 25, 63, 24). Benomyl is taken up into the roots and slowly released as MBC which travels to the leaf tissues, which are transpiring (24, 63). This process is gradual and results in distribution to new leaves which were not present at the time of application as well as to leaves already present. MBC, however, is rapidly translocated to existing leaves, depleting the source thusly rendering leaves formed later unprotected. The ability of the acetone method to reflect the fungitoxic status of the entire leaf area rather than just a minute portion of one leaf (i.e., leaf plug) is desirable in any study equating inhibition with theoretical disease resistance.

The acetone method has a major advantage for analytical work, its ease of comparison to a standard curve. During zone of inhibition formation, benomyl or MBC diffuses from a leaf plug through a maze of cell wall material. To compare the zones of inhibition of this system to those of a compound diffusing from filter paper disks is at best an extremely crude comparison. It is known that not all of the fungitoxicant in plant tissue is removed by acetone. However, the ratios of extractable to non-extractable compound can be determined (65), and a correction factor can be applied. An additional advantage of

acetone extraction is its versatility in that any part of a plant can be assayed. This eliminates variation due to differences in thickness of the parts to be assayed. Moreover, small concentrations may be analyzed with simple concentration procedures.

APPENDIX C

Preliminary Testing of Benomyl Uptake

Variable Time

Twenty five µg/g soil benomyl was added to each pot at various times to give 21, 14, 7, and 3 days exposure. Two days later, plants were analyzed using the acetone disk method and infection was measured by the visual estimation method (Table 2).

No consistent growth stimulation occurred in the M plants. There did, however, appear to be a difference in uptake patterns (Figures 11 and 12). M plants tended to accumulate more benomyl in leaves per unit time than did NM plants. The uptake continued to increase until 14 through 21 days after treatment. At 21 days, the M plant leaves had accumulated 2.5 times more of the fungitoxic compound than the NM plants. The root bioassay results were quite different (Figure 12), in that NM roots appeared to retain the compound at a higher level for a longer period of time than did the M plants. The concentrations found in the roots were quite low when compared to the leaves.

Table 2. Benomyl distribution within plants vs. time after soil treatment.

Days Exposure[a] to Benomyl	Type of Plant	Leaf Area[b] (cm^2)	Root Weight (g)
21	M	184.7	3.6
	NM	248.0	4.8
14	M	267.0	6.2
	NM	189.3	5.0
7	M	171.3	4.5
	NM	206.0	5.2
3	M	206.5	4.4
	NM	244.3	5.6
0	M	244.8	4.6
	NM	287.5	7.0
Average	M	219.1	4.7
	NM	238.3	5.6

[a]Time after a 25.0 µg/g soil drench.

[b]Average of three M and NM plants.

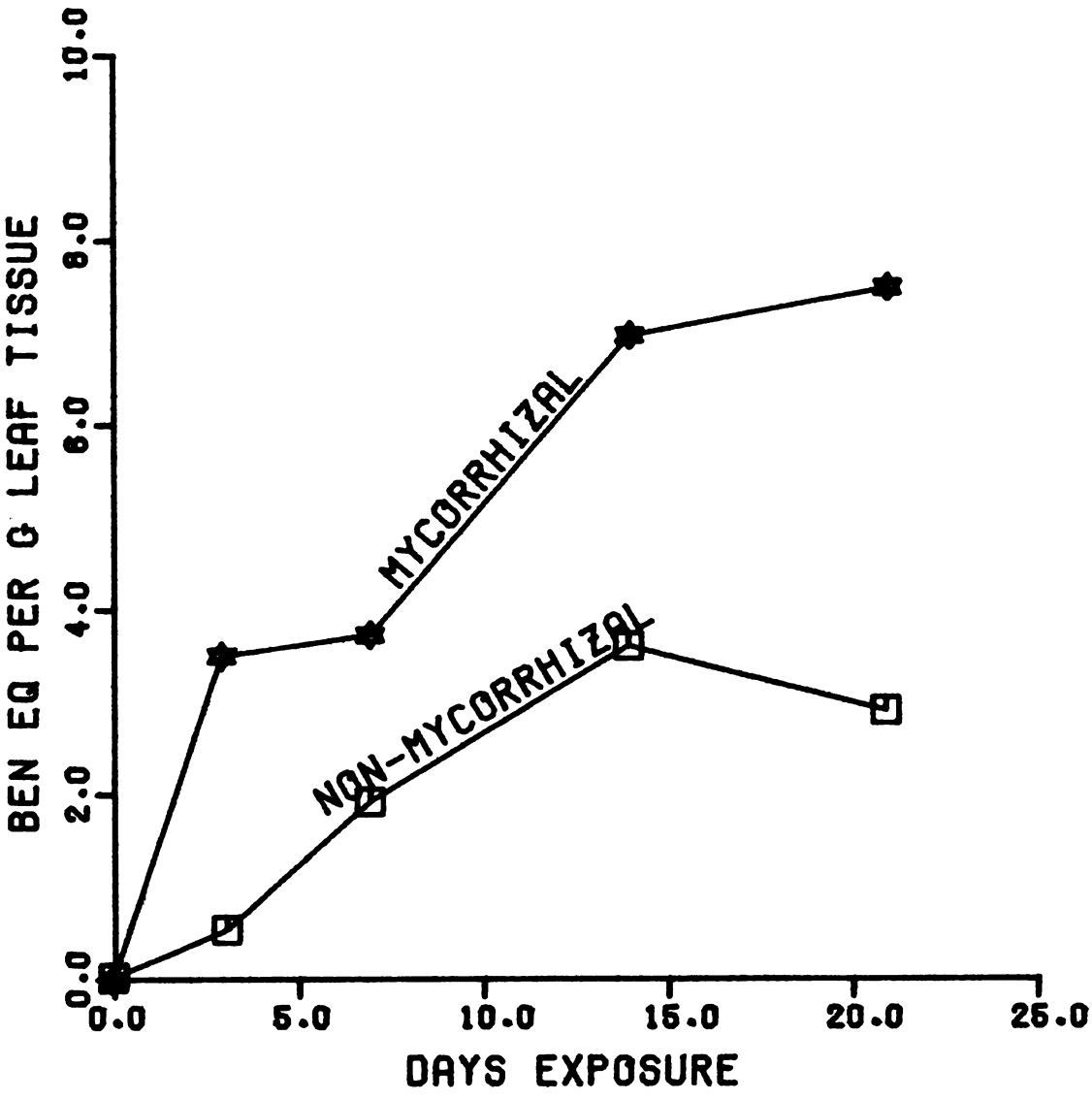


Figure 11. Benomyl concentration in leaves vs. days exposure to a 25.0 µg/g soil drench. Each point represents the average of five soybean plants either with (mycorrhizal) or without (non-mycorrhizal) a G. fasciculatus infection.

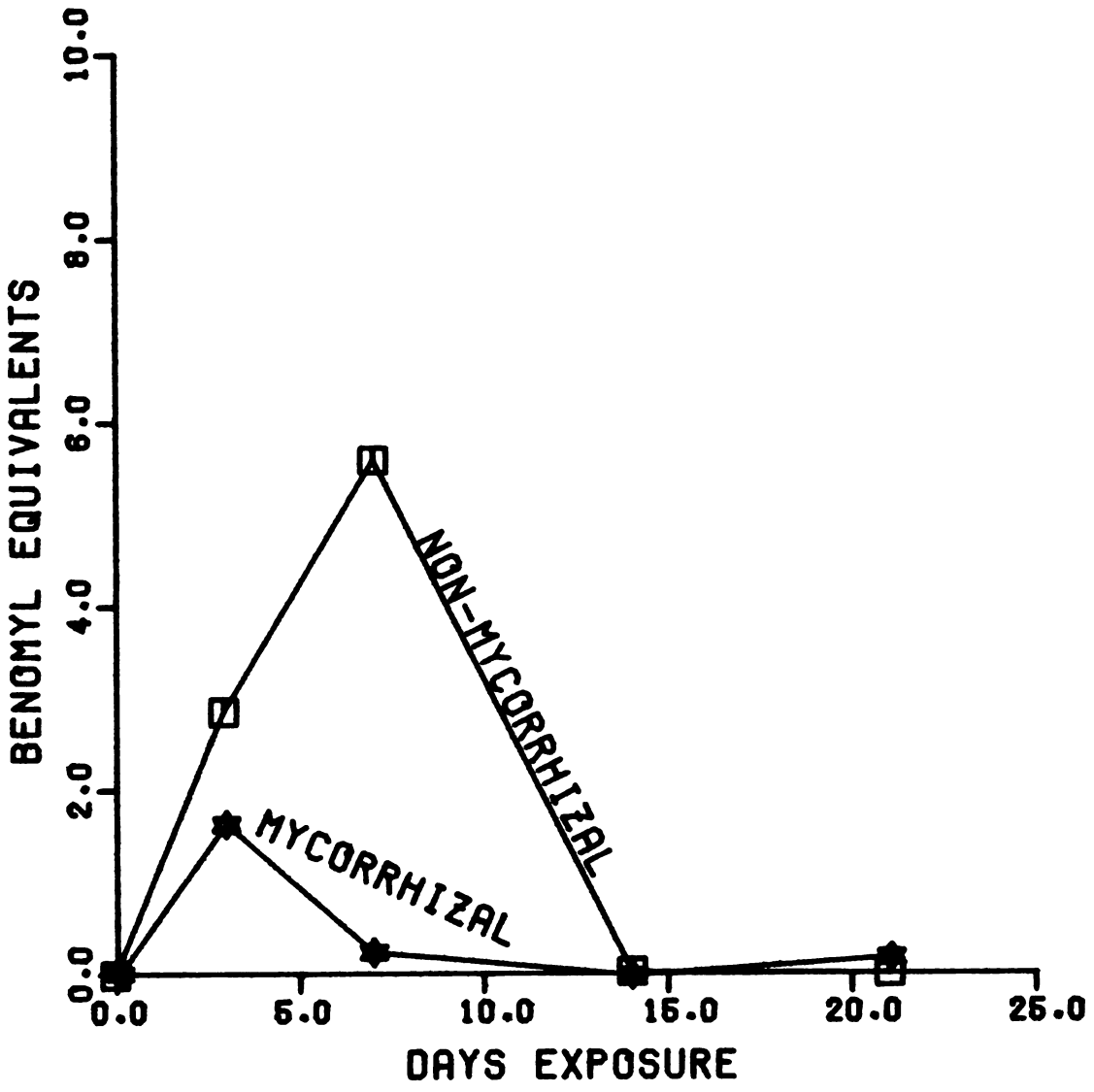


Figure 12. Benomyl concentration in roots vs. days exposure to a 25.0 µg/g soil drench. Each point represents the average of five soybean plants either with (mycorrhizal) or without (non-mycorrhizal) a G. fasciculatus infection.

Multiple Concentration

To compare the ability of mycorrhizal and non-mycorrhizal plants to take up benomyl over a wide range of concentrations, six M and NM plants were grown for 44 days before benomyl was added. Benomyl was applied to individual plants at 8.5, 17.0, 34.3, 68.5, and 137.0 µg/g soil. One concentration was used per plant and the experiment was conducted twice. One M and NM plant served as controls and were drenched with an equal amount of distilled water. Plants were assayed two days later using the leaf plug method.

The M shoots were found to be significantly larger (P .10) than NM shoots in both tests. Root growth of M and NM plants were not significantly different. In the bioassays of the first experiment the M plants showed a greater amount of uptake at all concentrations. In the second experiment, the NM plants showed either the same or a greater amount of uptake than did the M plants (Figure 13).

Single Concentration

To test the abilities of M and NM plants to take up and translocate benomyl from soil five to ten M and NM plants were grown for 30 to 40 days. Soil was treated with 45 µg/g soil benomyl leaving one to two plants of each type untreated to serve as controls. Two to three days after benomyl treatment the plants were bioassayed using the leaf plug method. This experiment was done twice.

In these preliminary experiments no growth differences occurred in any of the treatments (Table 3). M plants took up more benomyl than NM plants in every test, however, the differences were not significant.

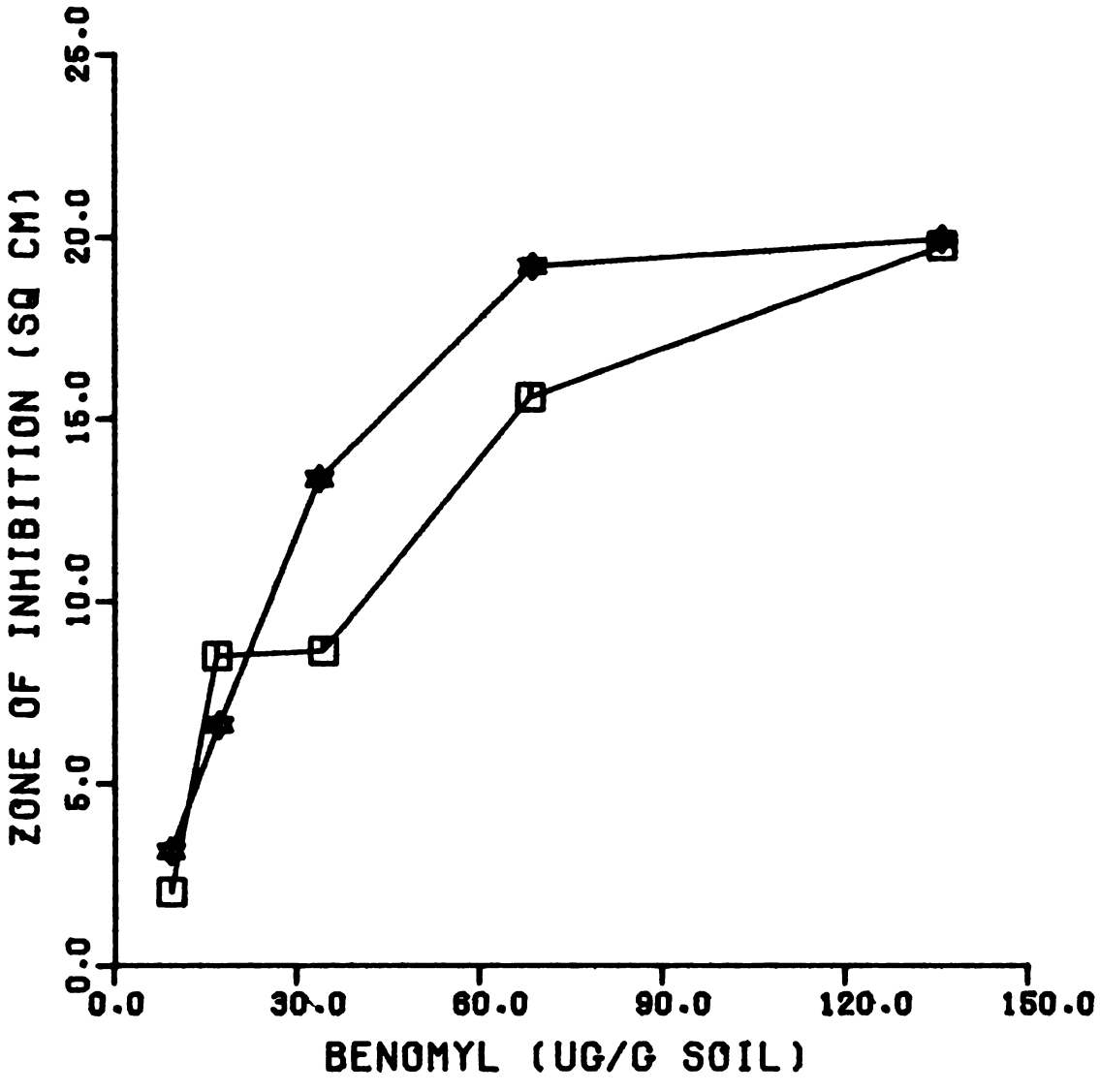


Figure 13. Distribution of benomyl in leaves two days after application. Each point represents the average of two experiments at one plant/experiment/concentration.

Table 3. Growth parameters and uptake of benomyl by M and NM plants after a soil drench of benomyl. This is the average of three experiments in which four to eight plants were bioassayed (leaf plug method) two to three days after benomyl treatment. One to two M and NM plants served as non-treated controls.

Treatment[a]	Height (cm)	Weight (g)			Area of Inhibition (cm^2)
		Root	Shoot	Total	
M + B	44.4	5.3	4.7	10.0	8.1
M	34.3	2.3	2.9	5.2	0.0
NM + B	39.6	6.7	4.7	11.4	7.5
NM	21.0	3.2	2.8	6.0	0.0

[a] M + B = mycorrhizal with benomyl (45 µg/g soil; M = mycorrhizal without benomyl; NM + B = non-mycorrhizal with benomyl, NM = non-mycorrhizal without benomyl.

APPENDIX D

Supplemental Data From Results

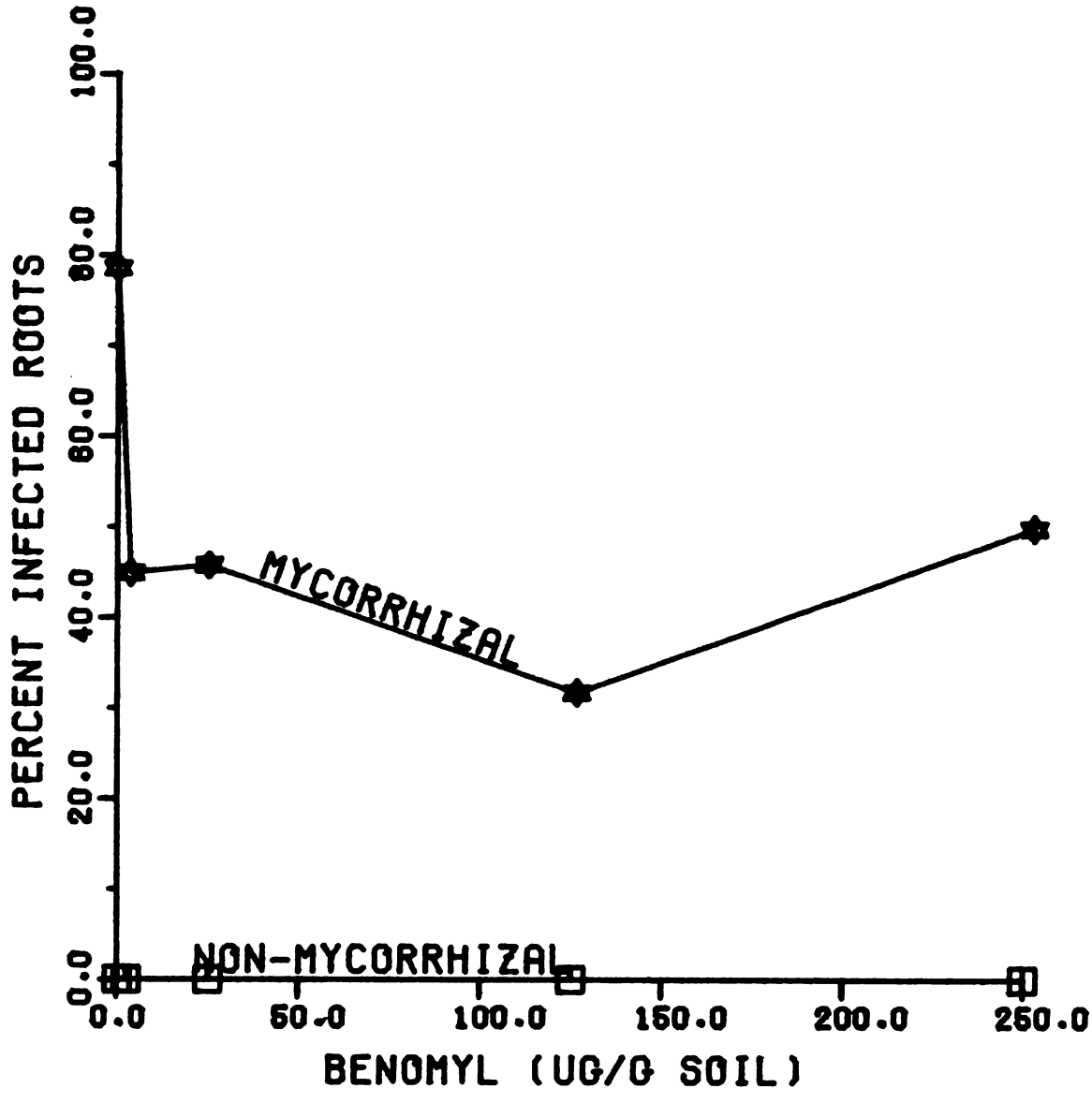


Figure 14. Effect of various concentrations of benomyl applied to soil on G. fasciculatus infection of soybean. Each point represents the average infection of four to six plants.

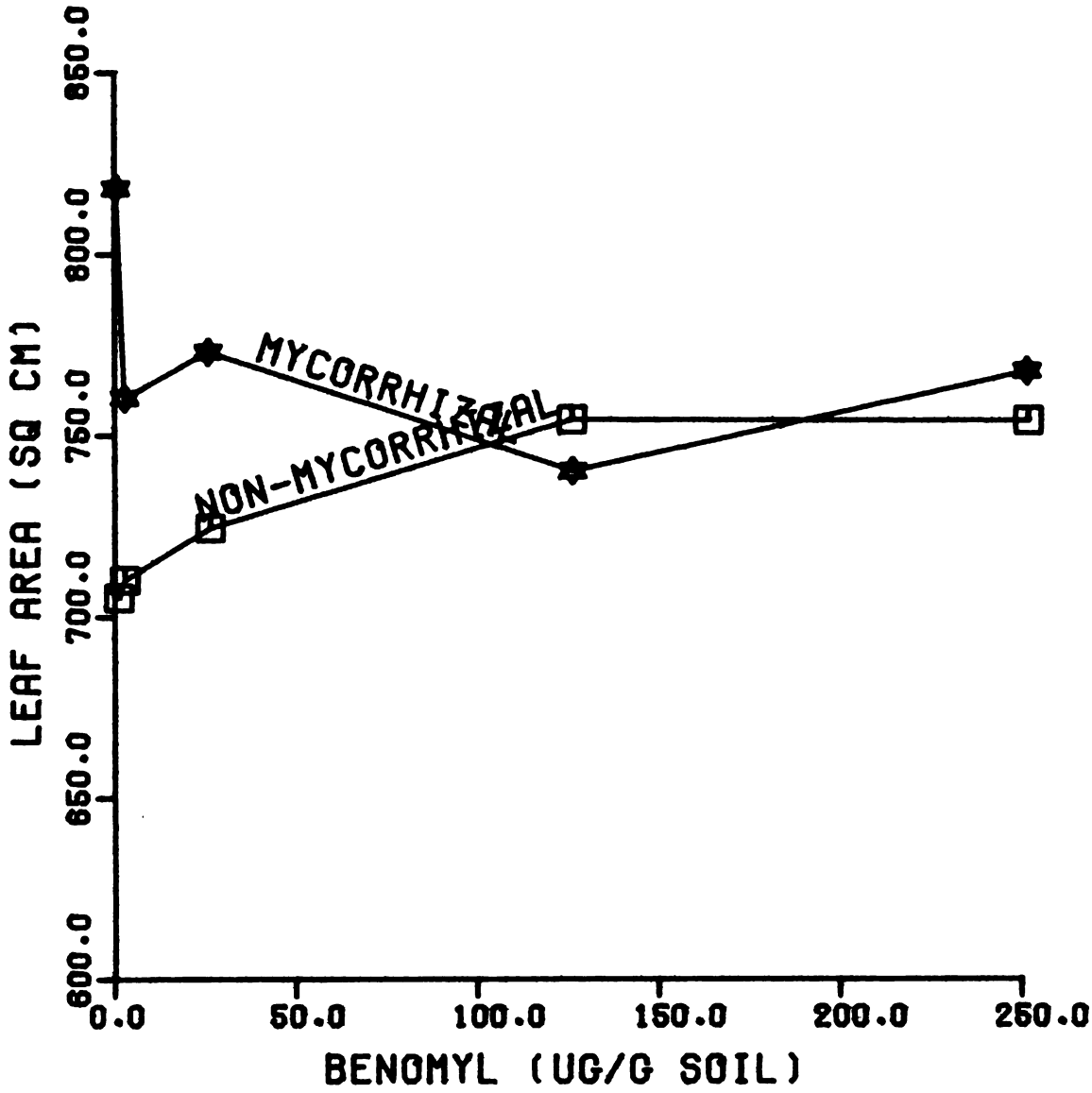


Figure 15. Effect of various concentrations of benomyl applied to soil on the leaf area of soybean plants with (mycorrhizal) or without (non-mycorrhizal) a G. fasciculatus infection. Each point represents the average leaf area (cm^2) of four to six plants.

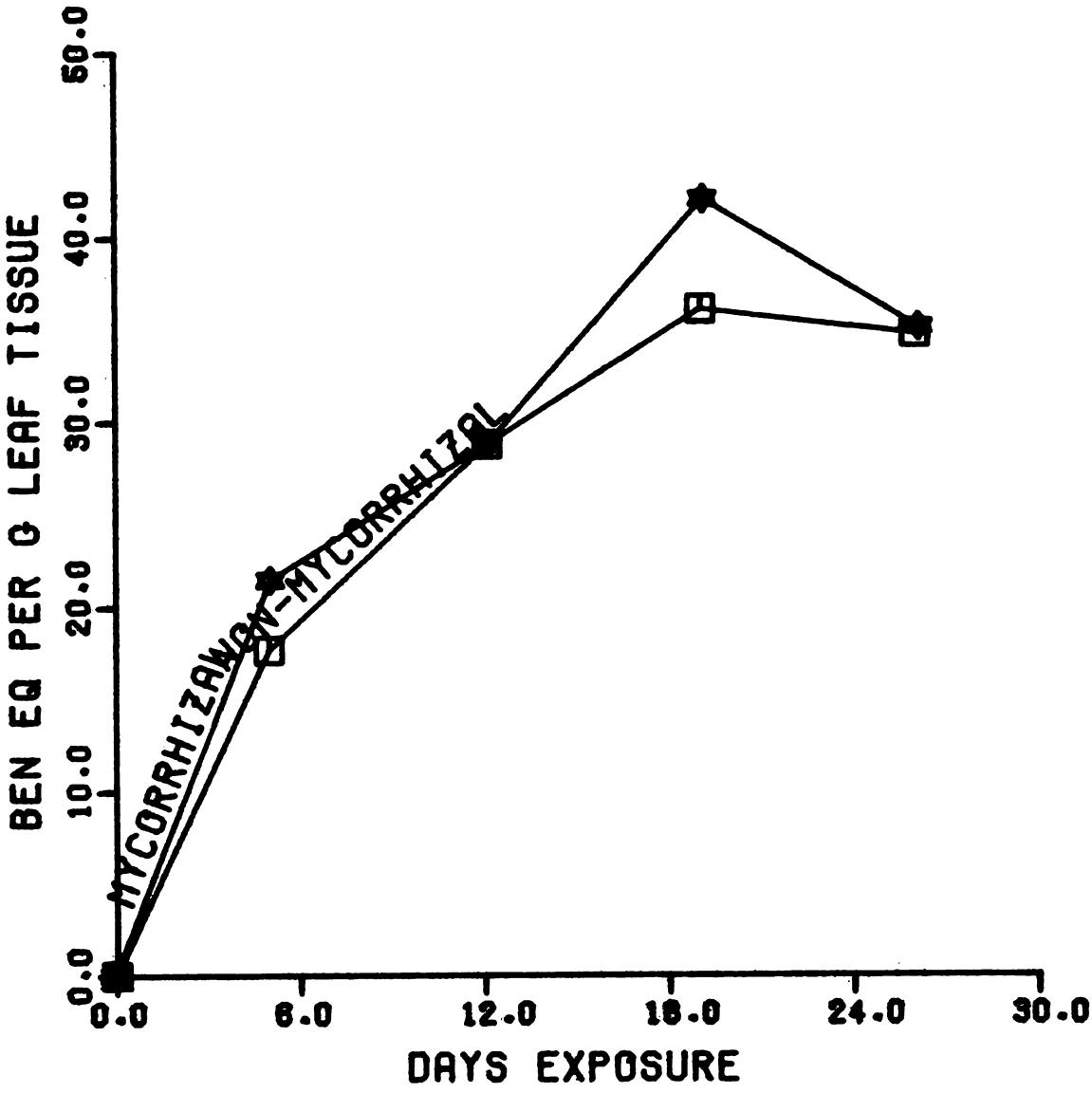


Figure 16. Benomyl concentration in leaves vs. days exposure to a 25.0 µg/g soil drench. Each point represents the average of five soybean plants either with (mycorrhizal) or without (non-mycorrhizal) a G. fasciculatus infection.

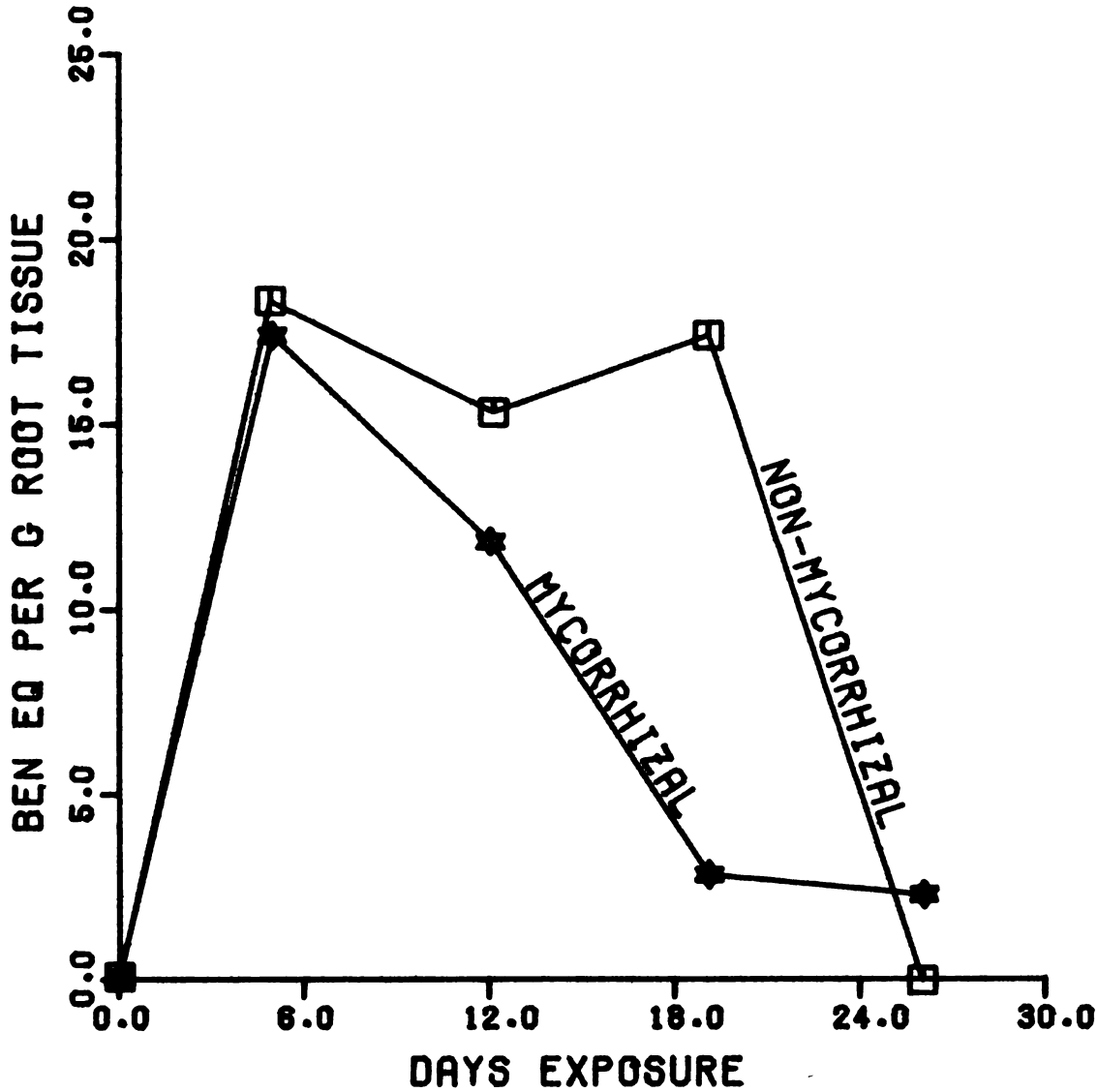
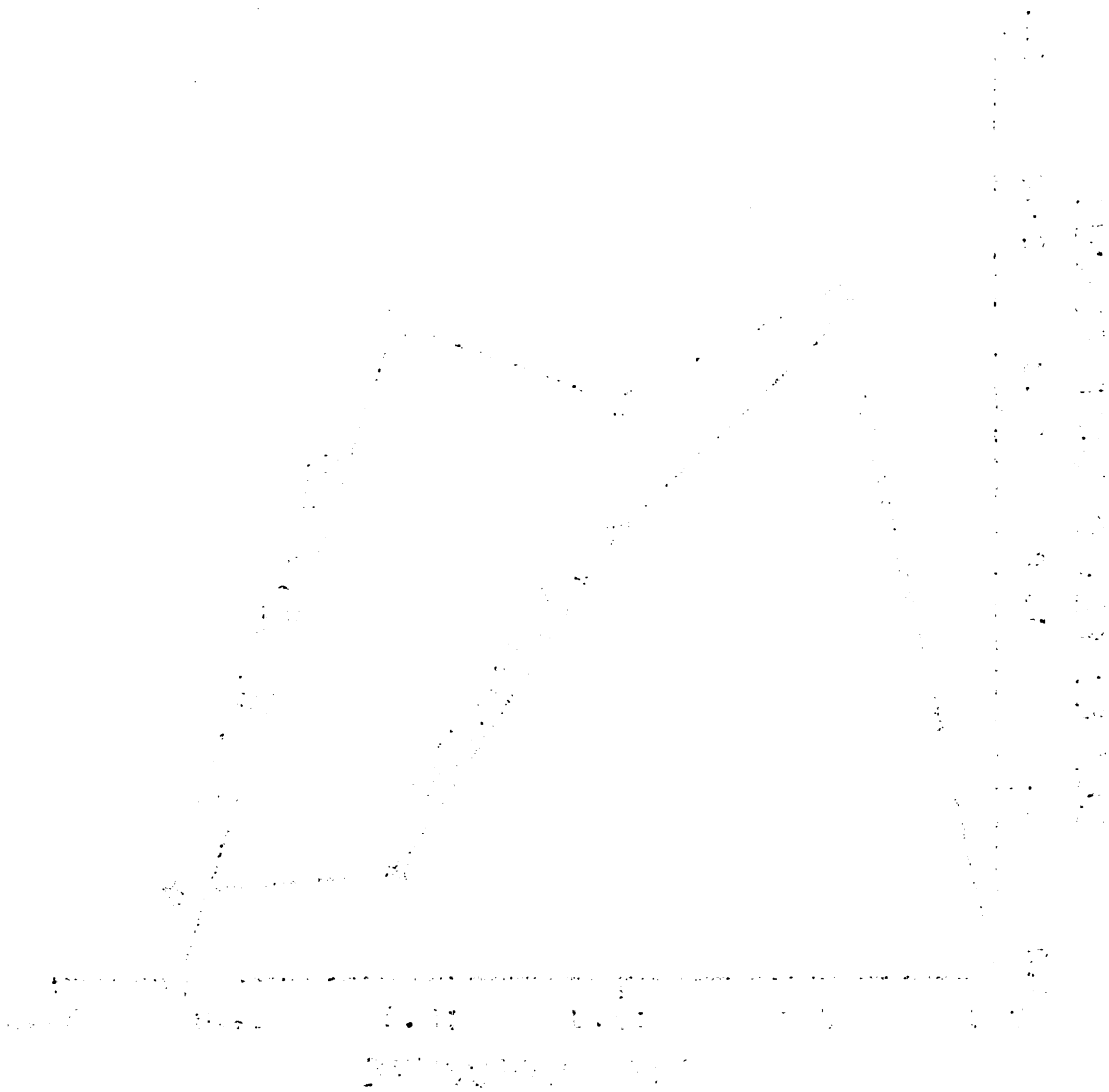


Figure 17. Benomyl concentration in roots vs. days exposure to a 25.0 µg/g soil drench. Each point represents the average of five soybean plants either with (mycorrhizal) or without (non-mycorrhizal) a G. fasciculatus infection.



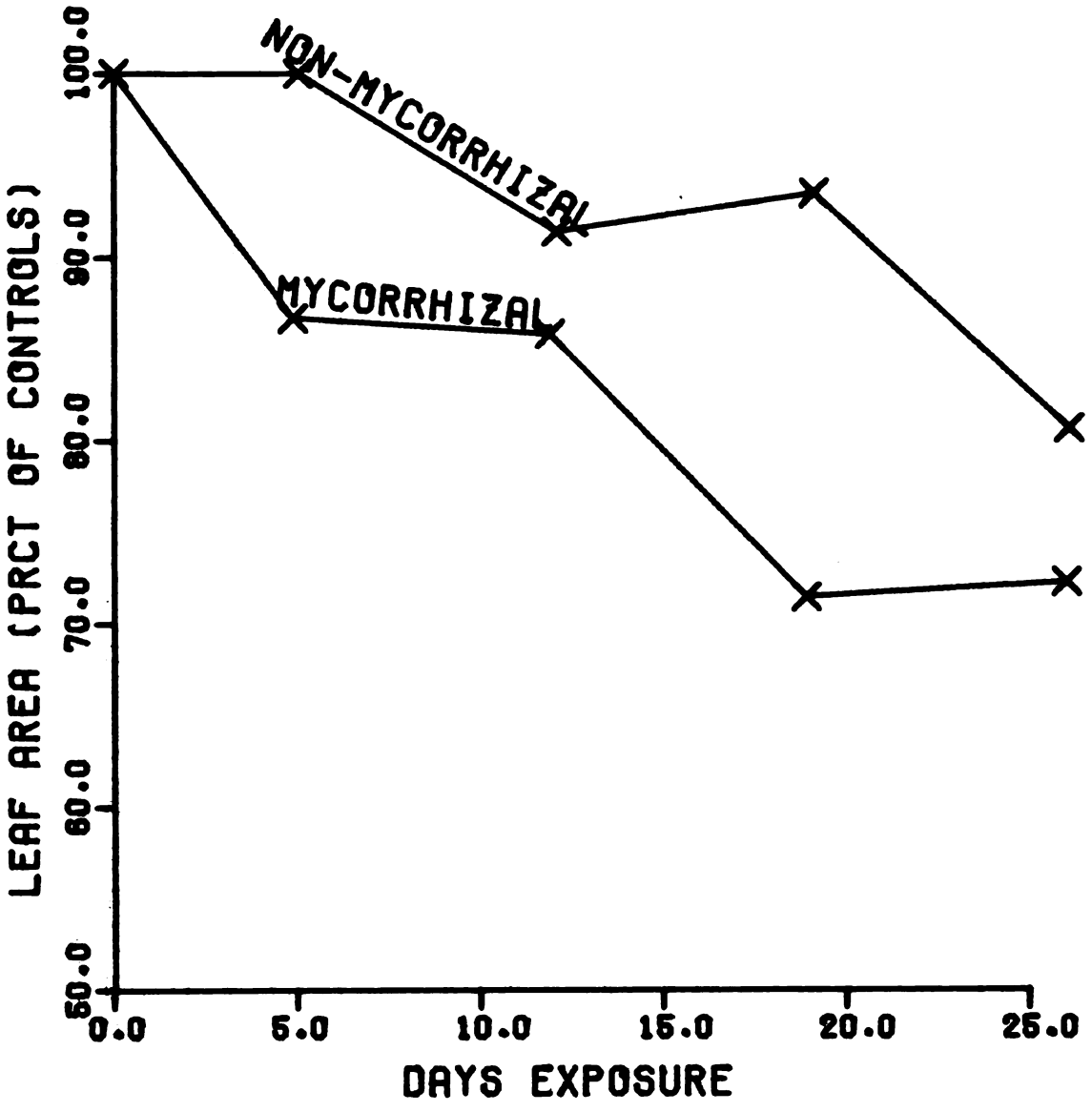


Figure 18. Leaf area as a percentage of non-treated controls vs. exposure time to 25.0 µg/g benomyl. Each point represents the average of five plants.

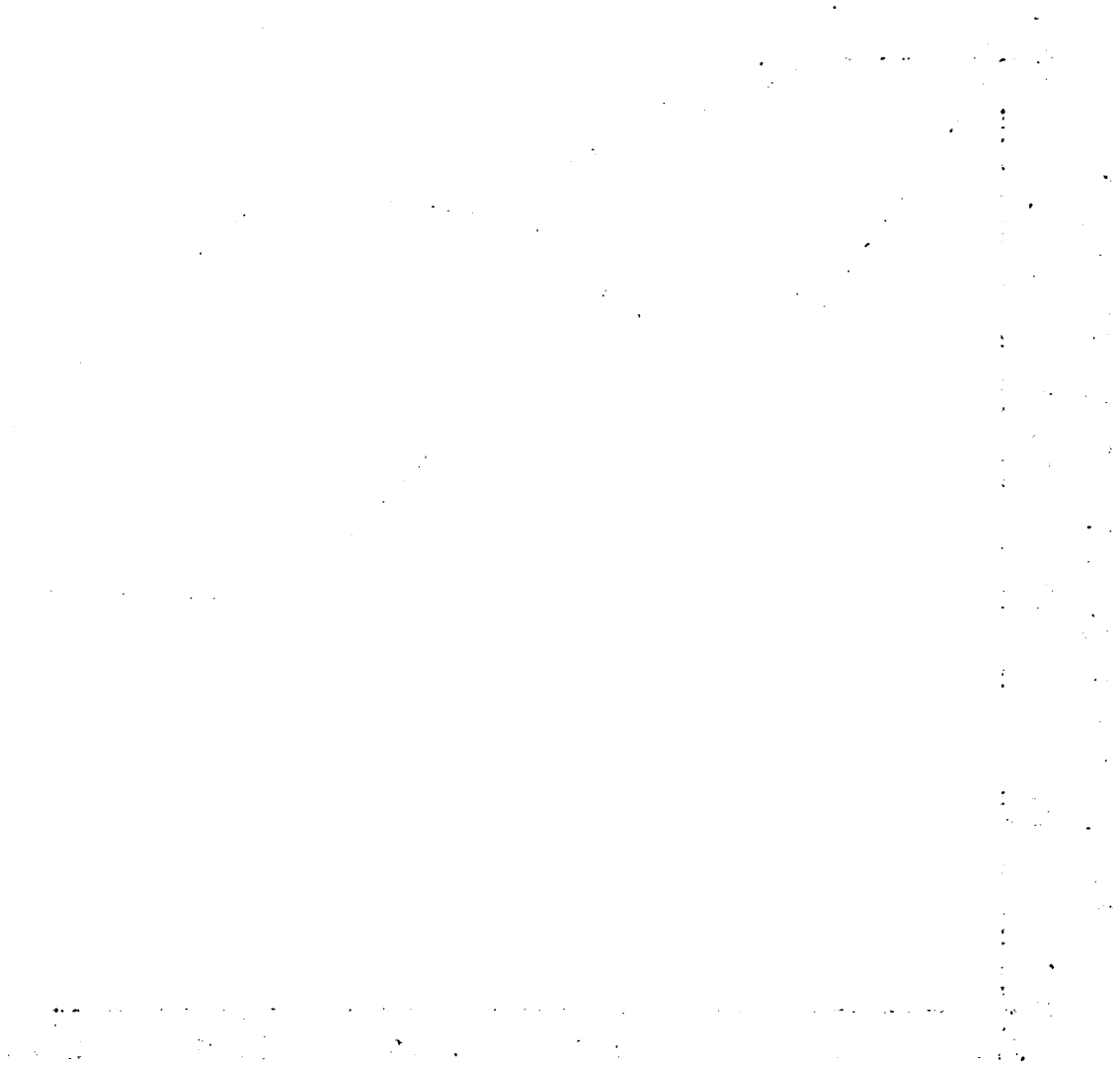


Table 4. Relationship of plant growth parameters, benomyl uptake, and time. This is the average of two experiments in which five M and NM plants from each exposure time were tested except that there were only four M and NM controls (no benomyl).

Type of Plant	Days Exposure	Leaf Area (cm^2)	Root Weight g	Percent Infection	Benomyl Equivalents	
					Roots	Leaves
M	0	239.7	4.7	22.5	0.0	0.0
NM	0	177.0	4.3	0.0	0.0	0.0
M	5	202.7	4.4	20.3	8.9	11.6
NM	5	181.1	4.8	0.0	9.5	10.4
M	12	214.0	4.7	29.5	6.3	16.3
NM	12	176.1	4.7	0.0	8.1	17.1
M	19	187.1	4.2	14.0	1.7	24.5
NM	19	170.8	4.3	0.0	9.0	21.8
M	26	181.8	4.4	13.7	1.4	21.6
NM	26	153.4	4.6	0.0	0.1	21.5

Table 5. Relationship of plant growth parameters to benomyl uptake. This is the average of three experiments in which four M and NM plants received 25.0 µg/g soil benomyl and one M and NM plant was left untreated.

Treatment[a]	Leaf Area (cm^2)	Height (cm)	Weight (g)				Infection[b]	Benomyl Equivalents[c]	
			Root	Leaves	Stem	Total		Root	Leaf
25.0 µg/g M	458.1	57.5	14.6	6.5	6.5	27.5	+	3.2	14.3
25.0 µg/g NM	438.1	54.2	9.6	6.3	5.9	21.8	-	3.5	16.9
0.0 µg/g M	545.9	59.3	16.8	7.6	6.9	31.2	+	0.0	0.0
0.0 µg/g NM	402.0	66.3	8.9	5.9	6.0	15.4	-	0.0	0.0

[a]Soil drench of benomyl or a distilled water control.

[b]+ = infected, i.e. presence of hyphae and/or vesicles; - = no fungus present in roots.

[c]Result of comparing the zones of inhibition to standard benomyl dilutions.

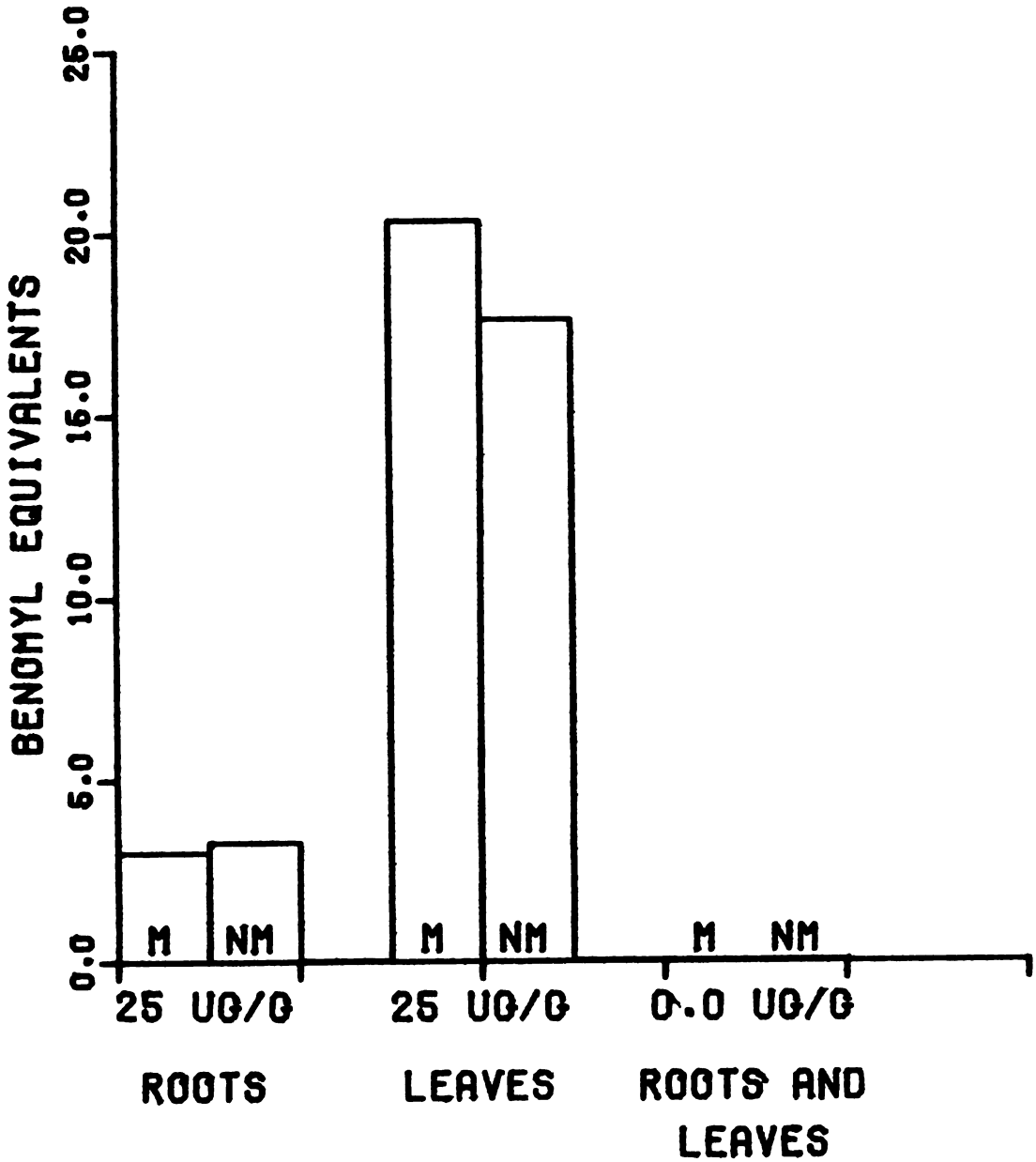
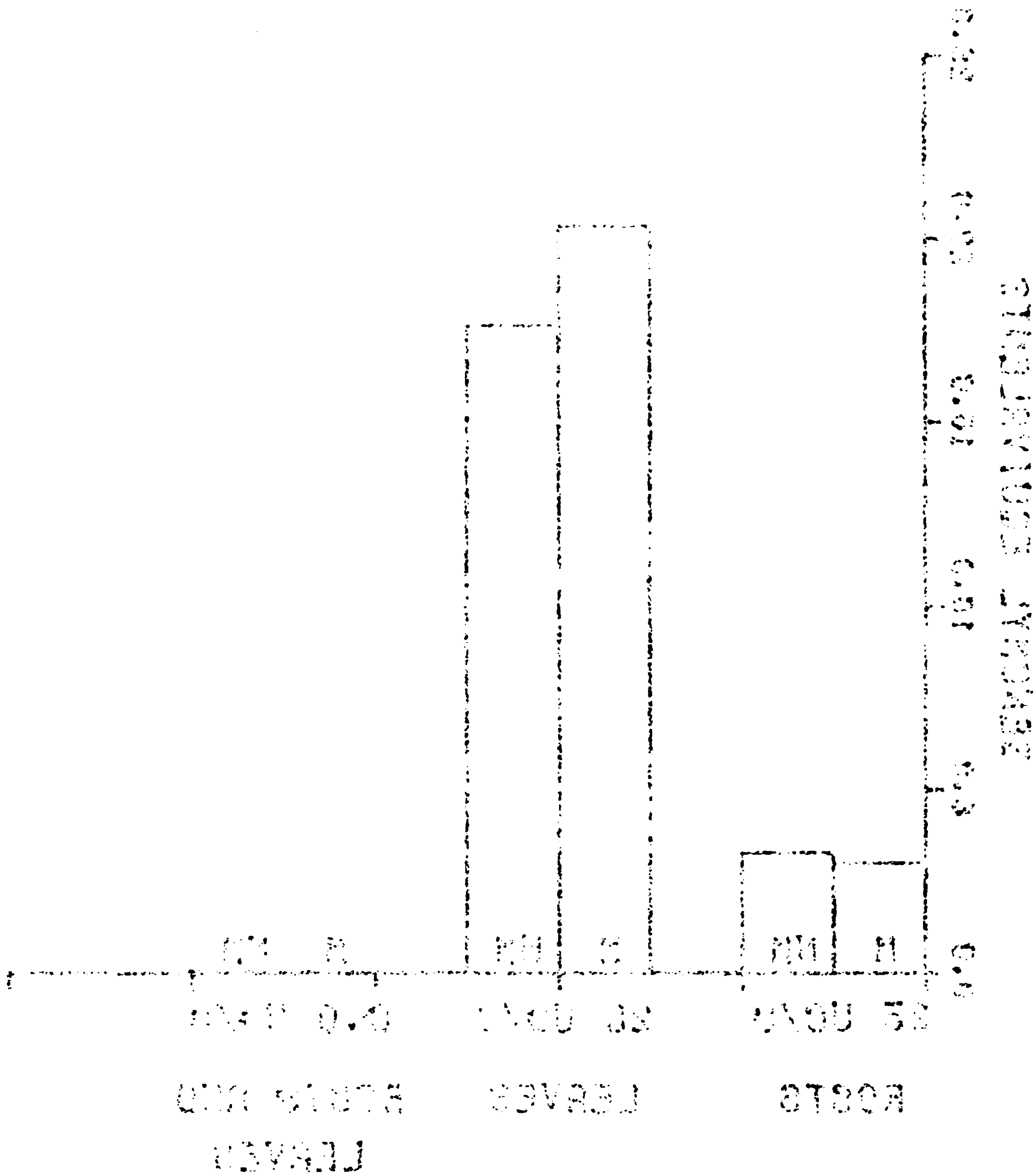


Figure 19. Benomyl distribution in roots vs. leaves of soybeans with (M) or without (NM) a G. fasciculatus infection. Each bar represents the mean of five plants except the 0.0 µg/g bar which represents one plant.



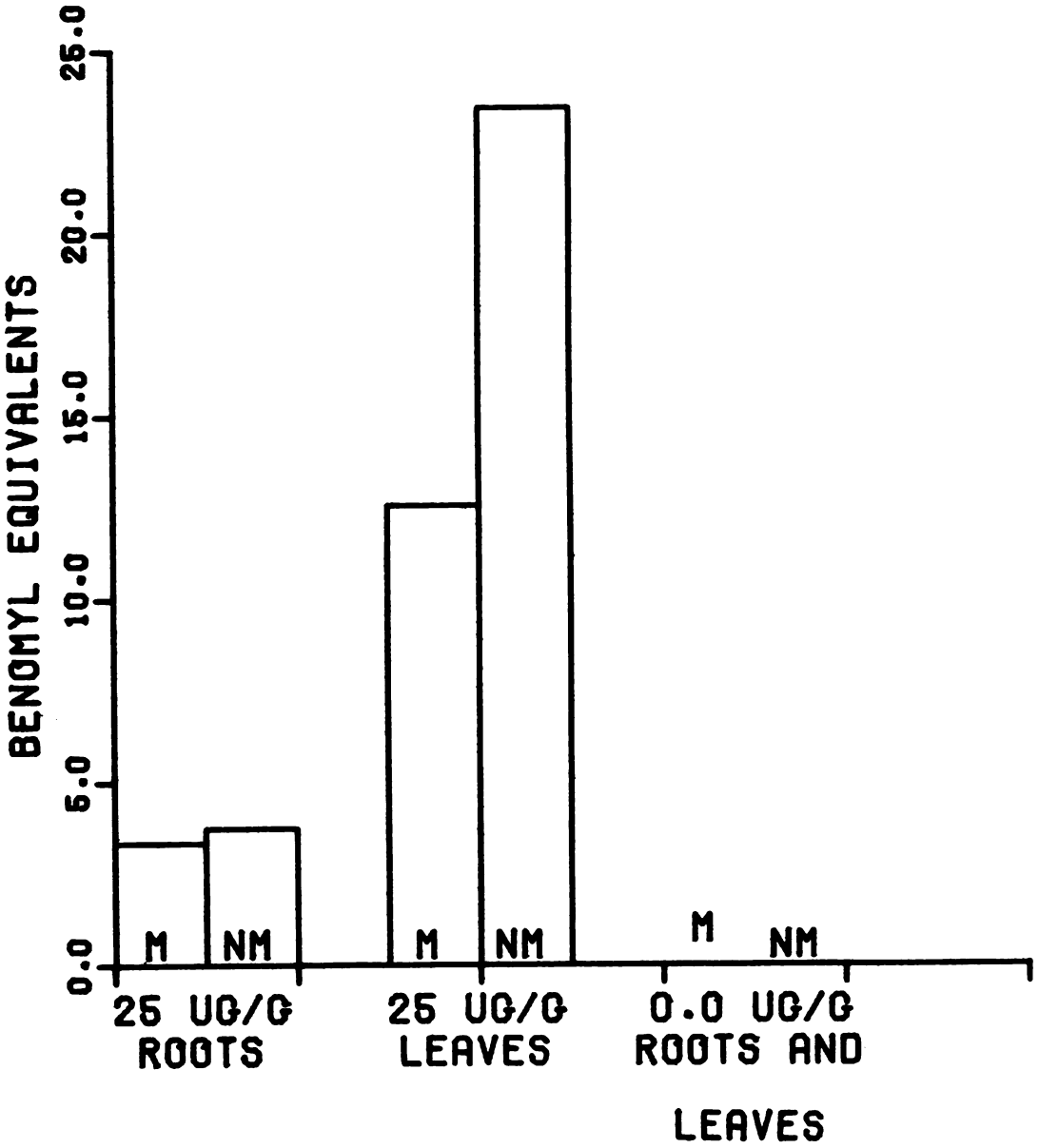


Figure 20. Benomyl distribution in roots vs. leaves of soybeans with (M) or without (NM) a G. fasciculatus infection. Each bar represents the mean of five plants except the 0.0 µg/g bar which represents one plant.