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Mycorrhiza

Initial and residual toxicity of soil-applied thiram on the vesicular-arbuscular mycorrhizal symbiosis*

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Abstract. The effect of the non-systemic fungicide thiram on the vesicular-arbuscular mycorrhizal (VAM) symbiosis and on *Leucaena leucocephala* was evaluated in a greenhouse experiment. In the uninoculated soil treated with P at a level optimal for mycorrhizal activity, mycorrhizal colonization of roots was low, and did not change as the concentration of thiram in the soil increased from 0 to 1000 mg/kg. When this soil was inoculated with the VAM fungus *Glomus aggregatum*, VAM colonization was enhanced significantly, but decreased with increase in thiram concentration until it coincided with the level observed in the uninoculated soil. Similarly, symbiotic effectiveness was reduced, its expression delayed or completely eliminated with increase in the concentration of thiram. Amending soil to a P level sufficient for non-mycorrhizal host growth fully compensated for thiram-induced loss of VAM activity if the thiram levels did not exceed 125 mg/kg. In soil treated with 50 mg thiram/kg, the toxicity of the fungicide dissipated within 66 days of application. At higher concentrations, the toxicity of the chemical on the mycorrhizal symbiosis appeared to be enhanced.

Key words: *Glomus aggregatum* – Phytotoxicity – Pesticide – Residual toxicity – Vesicular-arbuscular mycorrhiza

Introduction

Pesticides applied to agricultural crops as well as those introduced into the soil environment as waste products of industrial manufacturing could have severe impacts on non-pathogenic soil populations, and hence on biochemical processes involved in nutrient acquisition and cycling (Pimentel and Levitan 1986; Hicks et al. 1990). The concern that pesticides could interfere with the formation and function of vesicular-arbuscular mycorrhizal (VAM) symbiosis dates back over 20 years (Nesheim and Linn 1969); numerous papers have been published since that time (Smith 1978; Menge 1982; Trappe et al. 1984). From the synthesis prepared by Trappe and colleagues (1984), it is apparent that the literature on the subject is inconsistent and confusing. They believed that the problems were caused, among other things, by the lack of a common denominator in the studies reported, the diversity in experimental variables and the lack of focus on physiological mechanisms. These problems could in part be alleviated if pesticide impacts on mycorrhizal formation and function were evaluated under conditions optimal for the mycorrhizal symbiosis, and if experiments were conducted under conditions allowing a distinction between pesticide effects on endophyte activity and host growth.

The objective of the current investigation was to determine the initial and residual impacts of the non-systemic fungicide thiram [bis(dimethylthiocarbamoyl) disulfide] on VAM symbiosis in *Leucaena leucocephala* in an oxisol with a soil-solution P level optimal for VAM activity or sufficient for non-mycorrhizal host growth. Thiram is used as seed and soil dressing for the control of fungal pathogens and is also industrially employed in the vulcanization of rubber.

Materials and methods

The soil used in this study was a subsurface sample (15–30 cm) of the Wahiawa silty clay loam (clayey, kaolinitic, isohyperthermic, Tropeptic Eutrustox). It was crushed to pass through a 4-mm-aperture sieve. The pH of the soil was 5.8. A phosphorus sorption isotherm (Fox and Kamprath 1970) was used to establish target soil solution P levels of 0.02 and 0.6 mg/l. The former P level is considered optimal for VAM activity while the latter level is sufficient for 95% maximum yield of non-mycorrhizal *L. leucocephala* (Habte and Manjunath 1987). Other nutrients were added as previously specified (Habte and Manjunath 1987). Portions of the soil (2.8 kg dry wt.) were transferred into 15-cm plastic pots. Thiram was supplied as a red dust (50% active ingredient; E. I. du-

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Pont de Nemours and Co., Midland, Mich.). Different quantities of the dust were thoroughly mixed with air-dried soil to obtain concentrations of the active ingredient ranging from 0 to 1000 mg/kg soil.

The mycorrhizal inoculum used was *Glomus aggregatum*. The inoculum was produced and incorporated into soil as described previously by Habte and Manjunath (1987).

Seeds of *L. leucocephala* (Lam.) de Wit var. K8 were scarified with concentrated H_2SO_4 for 30 min and washed six times in sterile water. The scarified seeds were germinated in water agar (0.9% agar), and two germinated seeds were planted per pot.

Treatment groups were arranged on greenhouse benches in a randomized complete block design with three replicates per treatment. Plants were grown under natural light (21° 19′ N, 157° 57′ W) from 27 June to 28 August 1989 (initial effects of thiram) and from 13 September to 28 November 1989 (residual effect of thiram). Pots were watered as needed to maintain the soil at approximately 60% water holding capacity.

Plant height was measured from the soil line to the tip of a plant. The development of VAM effectiveness was monitored as described by Habte and colleagues (1987).

At harvest, shoots and roots were removed from the soils, and soils were placed back into their respective pots to be used for the determination of residual toxicity of thiram on VAM activity and on growth of *L. leucocephala*. After 3 weeks of standing, the soils received blanket nutrients as above, and were planted with pregerminated *L. leucocephala* seeds. Arrangement of treatment groups and growth conditions were maintained as described above.

The extent of VAM colonization of roots was determined by the grid-line intersect method (Giovannetti and Mosse 1980) after clearing and staining roots by employing a modification of the procedure described by Kormanik et al. (1980) (0.15% instead of 0.01% acid fuchsin). We also followed a no-heat procedure in which roots were kept in a clearing, staining or destaining solution at room temperature for 48 h at each step of the staining process.

Dry matter yield was determined after plant samples were dried to constant weight at 70° C. P contents of pinnules, shoots and roots were estimated by the molybdenum-blue method (Murphy and Riley 1962) after samples were dry-ashed at 500° C and the ash dissolved in water.

Results

Initial toxicity of thiram

At both levels of soil solution P tested, a low but constant level of VAM colonization was maintained in the uninoculated soil irrespective of thiram treatment (Fig. 1). At the P level optimal for VAM activity, the infection level observed in the inoculated soil decreased as the concentration of thiram increased, but the level of infection never declined below that observed in the uninoculated soil. The trend observed at high P level was similar to that observed above, except that the values were lower and declined at a faster rate in response to thiram.

The influence of thiram on VAM symbiotic effectiveness indicated by pinnule P content of *L. leucocephala* leaves is summarized in Figs. 2 and 3. At low P level, the time required for the initiation of VAM effectiveness was prolonged as the concentration of thiram increased from 0 to 125 mg/kg (Fig. 2). At 250 mg thiram/kg, the fungicide not only delayed the expression of VAM effectiveness but also reduced the maximum level of effectiveness observed. Phytotoxicity was also evident at this concentration of thiram, since pinnule P content observed in the uninoculated soil was lower than that observed in the untreated soil. At concentrations of thiram higher than 250 mg/kg, differences in pinnule P content of *L. leucocephala* grown in inoculated and uninoculated soils were eliminated and the adverse effect of the fungicide on P uptake by the unaided root became more pronounced.

At a soil solution P level sufficient for nonmycorrhizal host growth, the pinnule P status of plants grown in the inoculated and uninoculated soils were essentially indistinguishable (Fig. 3).

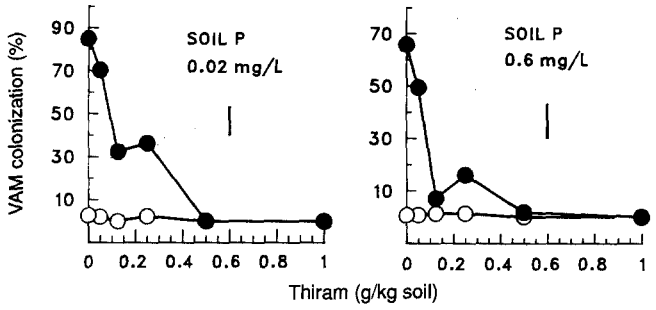

Fig. 1. Influence of thiram on mycorrhizal colonization of roots at a soil P level optimal for mycorrhizal activity or sufficient for non-mycorrhizal host growth. ○, Uninoculated; ●, inoculated. *Bar* = LSD at the 5% level

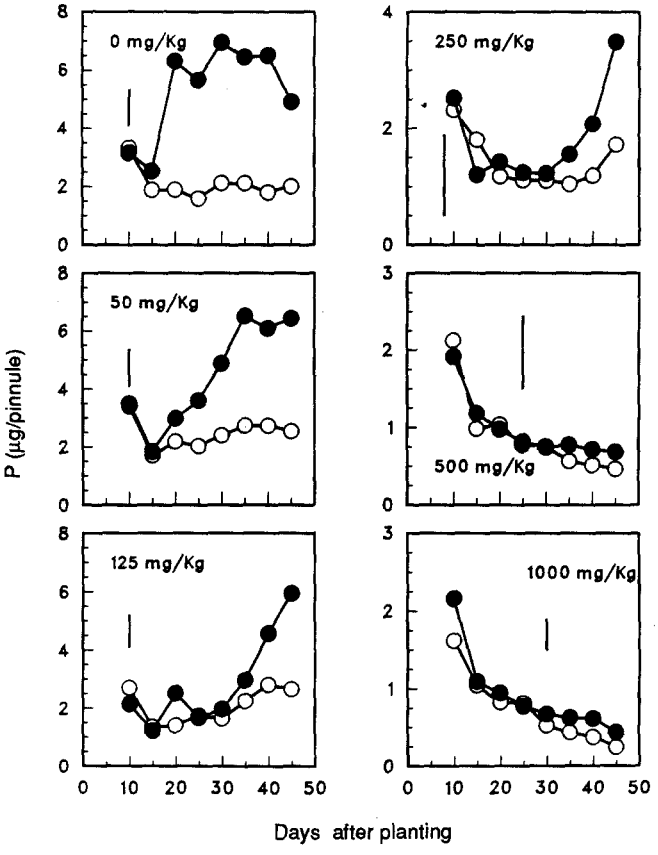

Fig. 2. Mycorrhizal effectiveness measured as pinnule P content of *Leucaena leucocephala* in the presence or absence of thiram at a soil P level optimal for mycorrhizal activity or sufficient for non-mycorrhizal host growth. Symbols as in Fig. 1

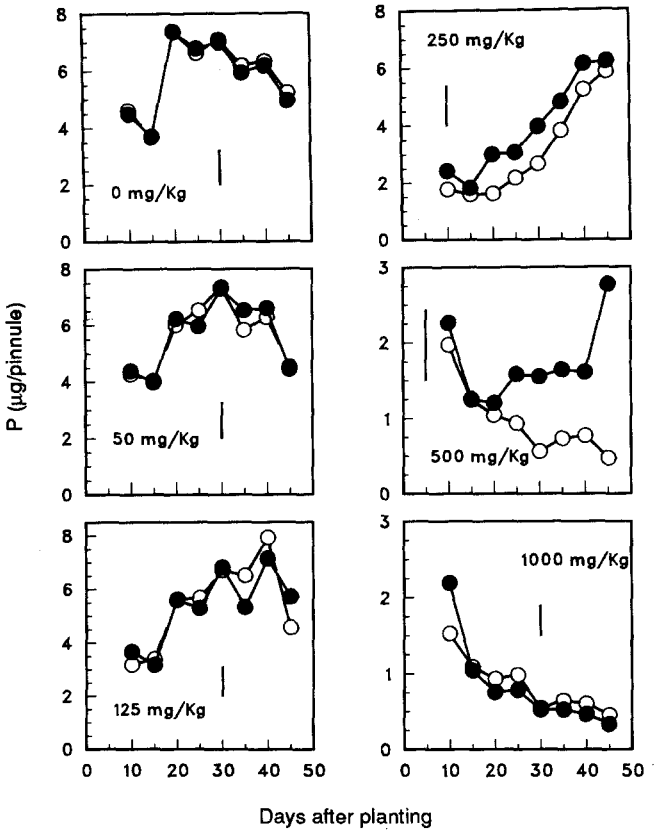

Fig. 3. Pinnule P content of *L. leucocephala* in the presence or absence of thiram and mycorrhizal inoculum in soil with a P level sufficient for non-mycorrhizal host growth. Symbols as in Fig. 1

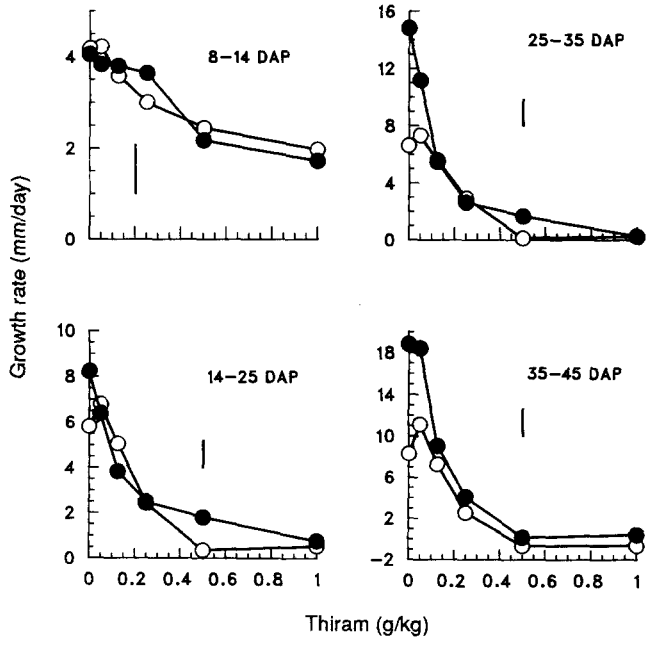

Fig. 4. Influence of thiram and mycorrhizal inoculation on the growth of *L. leucocephala* at a soil P level optimal for mycorrhizal activity. *DAP*, Days after planting; other symbols as in Fig. 1

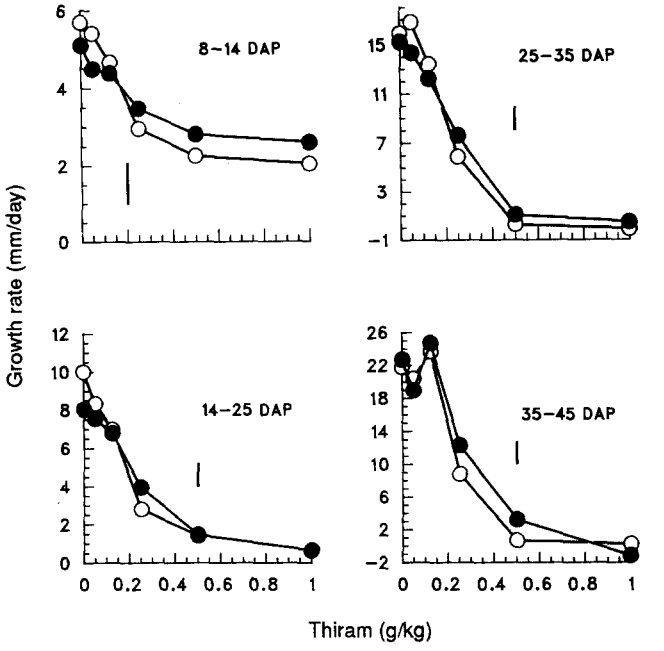

Fig. 5. Influence of thiram and mycorrhizal inoculation on the growth of *L. leucocephala* at a soil P level sufficient for non-mycorrhizal host growth. *DAP*, Days after planting; other symbols as in Fig. 1

The growth rate of plants in soil with a P level optimal for VAM activity declined with increase in thiram concentration (Fig. 4). The effect of VAM inoculation was not evident from day 8 to day 14 irrespective of thiram treatment. During the 14–25 day period, *L. leucocephala* responded significantly to inoculation if the soil was not treated with thiram. Subsequently, plants grown in the soil treated with 50 mg thiram/kg also responded to VAM inoculation. At higher thiram levels, plants grown in inoculated and uninoculated soils exhibited similar growth rates. Growth rate patterns observed at a P level sufficient for non-mycorrhizal host growth were similar to those described above (Fig. 5), except that plants grown at the higher P level had generally higher growth rates and did not respond to VAM inoculation.

At thiram concentrations below 250 mg/kg, plants grown in the soil with a P level optimal for VAM activity had higher root and shoot P levels if the soils were inoculated with VAM fungus than if they were not (Fig. 6). Above this level of thiram, shoot P concentration tended to increase with increase in thiram concentration, but VAM inoculation had no influence on this variable. At a P level sufficient for non-mycorrhizal host growth, tissue P levels did not respond to mycorrhizal inoculation and the values observed were comparable to the ones observed in the inoculated soil with a P level optimal for VAM activity.

Shoot and root dry matter yields of plants declined as the concentration of thiram increased (Fig. 7). Response to mycorrhizal inoculation was evident only in plants grown in the soil not treated with thiram or treated with 50 mg thiram/kg. Similarly, dry matter yield declined as a function of thiram concentration at the high P level,

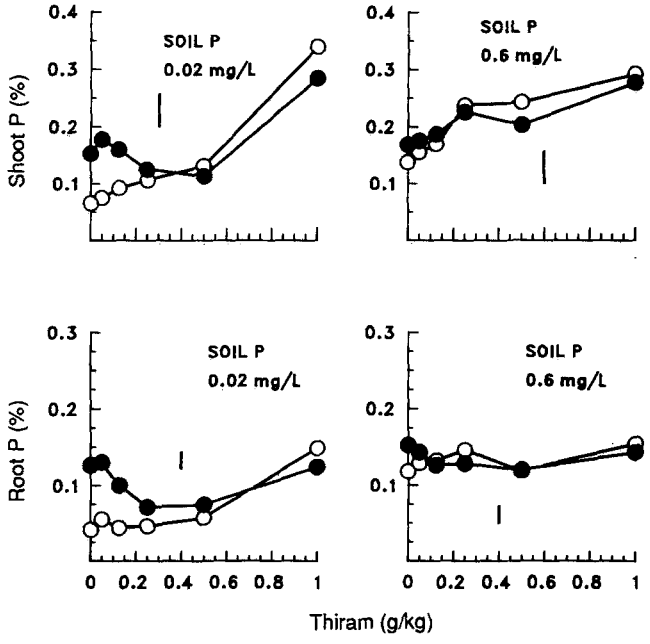

Fig. 6. Influence of thiram and mycorrhizal inoculation on shoot and root P content at a soil P level optimal for mycorrhizal activity or sufficient for non-mycorrhizal host growth. Symbols as in Fig. 1

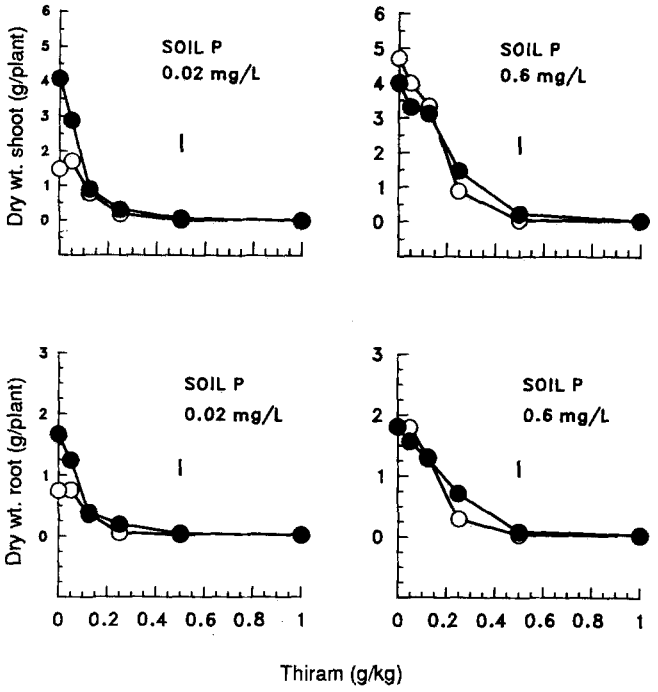

Fig. 7. Influence of thiram and mycorrhizal inoculation on shoot and root dry weight at a soil P level optimal for mycorrhizal activity or sufficient for non-mycorrhizal host growth. Symbols as in Fig. 1

but VAM inoculation had no significant influence on the variable.

Residual toxicity

The infection level in uninoculated soil remained roughly constant at about 5% irrespective of initial thiram dose (Fig. 8). It was significantly increased by VAM inoculation in the soil with a P level optimal for VAM activity either untreated or treated with up to 125 mg thiram/kg. No residual toxicity was evident in the soil exposed to 50 mg thiram/kg. However, VAM infection was completely inhibited in the soil previously treated with 250 mg thiram/kg. At a soil P level sufficient for non-mycorrhizal host growth, VAM infection was completely suppressed in soil previously treated with 125 or 250 mg thiram/kg. The inhibition of VAM colonization by residual thiram was more pronounced in this soil compared to that observed in the soil with a P level optimal for VAM activity.

VAM effectiveness measured in terms of pinnule P content of *L. leucocephala* leaves was not sensitive to residual thiram in the uninoculated soil with a P level optimal for VAM activity (Fig. 9). In the inoculated soil, residual thiram delayed the expression of VAM effectiveness and progressively lowered the level of effectiveness after fungicide treatments of 0 to 125 mg/kg. VAM effectiveness was completely suppressed in the soil treated with 250 mg thiram/kg. At a soil solution P level sufficient for non-mycorrhizal host growth, the pinnule

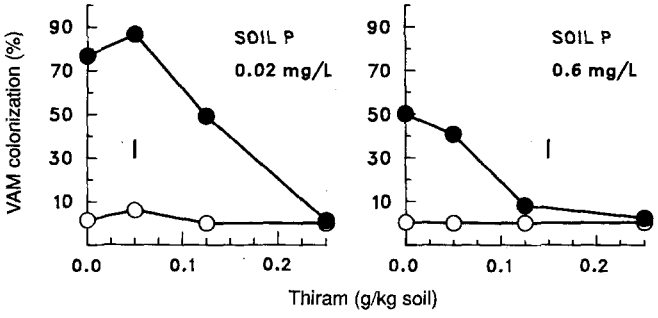

Fig. 8. Residual effect of thiram on mycorrhizal colonization of roots at two soil P levels. Symbols as in Fig. 1

P contents of plants grown in inoculated and uninoculated soils were indistinguishable (Fig. 10).

In the soils previously exposed to thiram concentrations up to 250 mg/kg, VAM inoculation led to significant stimulation of P accumulation in shoots and roots if the soil was amended with P at a level optimal for mycorrhizal activity (Fig. 11). At a P level sufficient for non-mycorrhizal host growth, root and shoot P content did not respond to VAM inoculation if the soil had previously been treated with thiram at concentrations exceeding 50 mg/kg.

VAM inoculation did not stimulate dry matter yield in soils treated with thiram in excess of 50 mg/kg and supplied with P at a level optimal for VAM activity (Fig. 12). In the soil with a P level sufficient for non-mycorrhizal host growth, the dry matter yields of roots and

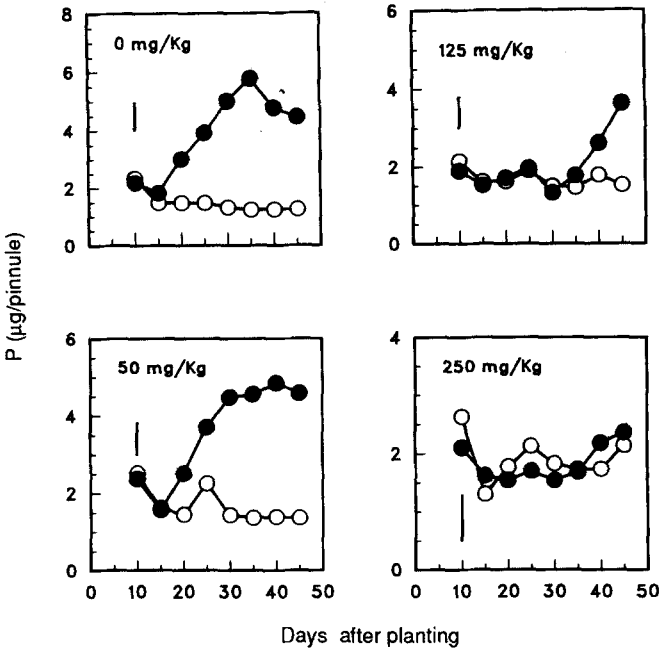

Fig. 9. Residual effect of thiram on mycorrhizal effectiveness at a soil P level optimal for mycorrhizal activity. Symbols as in Fig. 1

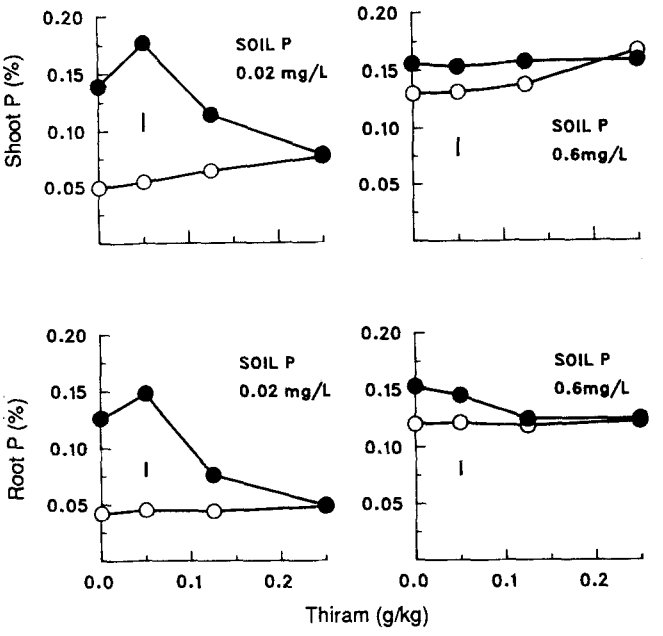

Fig. 11. Residual effect of thiram on shoot and root P content at a soil P level optimal for mycorrhizal activity or sufficient for non-mycorrhizal host growth. Symbols as in Fig. 1

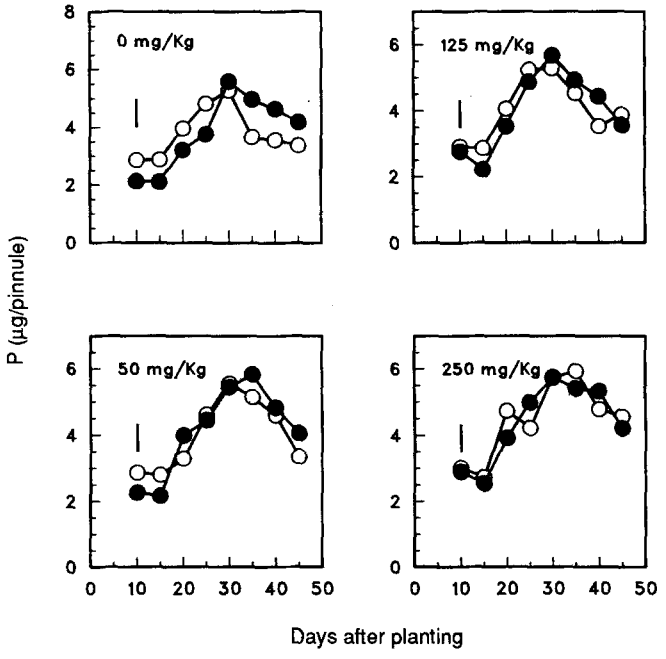

Fig. 10. Residual effect of thiram on pinnule P content of *L. leucocephala* at soil P levels sufficient for non-mycorrhizal host growth. Symbols as in Fig. 1

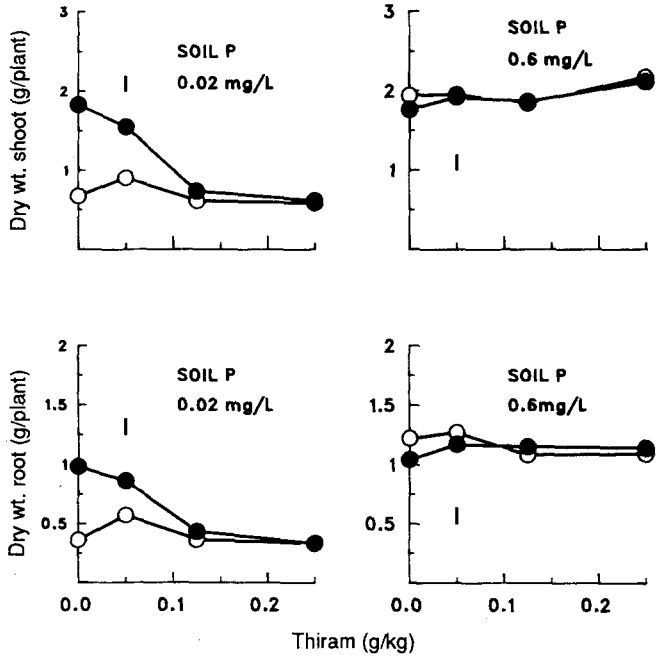

Fig. 12. Residual effect of thiram on shoot and root dry weight at a soil P level optimal for mycorrhizal activity or sufficient for non-mycorrhizal host growth. Symbols as in Fig. 1

shoots were not influenced by inoculation or previous thiram treatment of soil.

Discussion

It is clear from our results that addition of thiram to soil at concentrations of 50 mg/kg or higher resulted in decreased VAM infectivity and VAM symbiotic effectiveness (Figs. 1, 2). The failure of P amendment to fully compensate for lost VAM activity in the soil treated with 250 mg thiram/kg or higher suggests that the chemical was phytotoxic at these concentrations. The positive response to inoculation observed in the soil treated with a high P level and 500 mg thiram/kg is perplexing, since

VAM infection in this soil was reduced to the level observed in the uninoculated soil (Fig. 1).

Although an infection level of 10% or less has no serious consequences for host growth (Habte and Aziz 1985), the failure of thiram to completely suppress VAM colonization is intriguing. This low level of infection may reflect the resistance to thiram of a fraction of the indigenous VAM population. Recently, Lu and Habte (unpublished work) exposed a surface Wahiawa soil to up to 200 mg thiram/kg and observed a drop in infection level from 47% to 29% in uninoculated soil. The decline in infection level in inoculated soil was twice as high, but the final infection levels observed in the two soils were similar. These observations support the hypothesis of Trappe et al. (1984) that there is genetic variability among VAM fungi with respect to pesticide tolerance.

While data on the influence of thiram on VAM symbiotic effectiveness are generally sparse, our findings are in good agreement with published data regarding the adverse influence of thiram on the formation of VAM symbiosis (Nesheim and Linn 1969; Sutton and Sheppard 1976; Jalali 1979). The tendency of thiram to hamper mycorrhizal formation to a greater extent in soil with a P level sufficient for non-mycorrhizal host growth than in soil with a P level optimal for VAM activity is due to the combined inhibition of VAM colonization by thiram and high P. High soil P levels generally lead to high tissue P levels and the latter are known to be detrimental to the infection process (Ratnayake et al. 1978; Habte and Manjunath 1987).

By evaluating the toxicity of thiram at a soil solution P concentration optimal for VAM activity or sufficient for non-mycorrhizal host growth, we have succeeded in separating the effect of thiram on VAM activity from its effect on the host. Our results clearly show that at thiram concentrations of up to 125 mg/kg soil, the adverse effect of the chemical was restricted to VAM activity. This follows from the fact that at these concentrations of thiram the adverse effect of the fungicide on plant P status and host growth was eliminated if plants were supplied with P levels sufficient for non-mycorrhizal host growth (Figs. 2, 3). By the same token, the failure of high P to compensate for the adverse effect of 250 mg thiram/kg or higher suggests the onset of phytotoxicity. In the few studies reported on the interaction of thiram with VAM symbiosis, it is difficult to separate the effect of thiram on VAM activity from its effect on host plants. However, some inferences could be made from two of the published reports. Nesheim and Linn (1969) noted a reduction in the number of root hairs, root-hair deformation and root-hair stunting in sterilized sand-soil medium inoculated with VAM-infected tulip roots and treated with 50 or 100 mg thiram/kg. On the other hand, Hong (1976) found no evidence of phytotoxicity in *Pinus caribea* grown in soil saturated with a 0.2% thiram suspension twice a week for 6 months. Neither of these results is supported by our findings. These discrepancies perhaps reflect differences in the tolerance to thiram of the host species involved.

Evaluation of residual toxicity of thiram resulting from initial soil applications of 50–250 mg of the active ingredient/kg yielded some interesting results (Figs. 8–12). While the inhibitory effect of the fungicide had dissipated by 66 days in soil treated with 50 mg thiram/kg, its toxicity to VAM symbiosis in the soil treated with 125 or 250 mg/kg was enhanced. On the other hand, the initial phytotoxicity in the soil treated with 250 mg thiram/kg had dissipated. Thiram is reported to be degraded quite rapidly in aerobic soils (Sinah et al. 1988). The lack of toxicity to VAM activity in the soil treated with 50 mg thiram/kg and the loss of phytotoxicity in the soil treated with 250 mg thiram/kg suggests that the fungicide was being degraded in soil. The enhanced toxicity to VAM activity of the fungicide in the soil treated with 125 or 250 mg thiram/kg suggests the release of thiram degradation products which were detrimental to VAM activity.

Our results demonstrate that in soils recently treated with thiram VAM activity could be seriously hampered. However, the detrimental effect of thiram concentrations up to 50 mg/kg soil appear to be transitory. Where higher levels of the chemical are introduced to soil, thiram and its degradation products are likely to suppress the formation and function of the VAM symbiosis for an extended period.

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