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Effect of Benomyl on the Formation of Mycorrhiza in Roots of Tomato (*Lycopersicon esculentum* Mill.) Seedlings

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Systemic fungicides are effective for the treatment of leguminous, cereals and vegetables seeds. They are absorbed during germination and control pathogens located deep inside the seeds. These fungicides act by killing or preventing the development of pathogens originating from infected seeds, thus preventing systemic seedling infection and also protecting the plants for a long time from the attack of soil pathogens (Dhingra et al. 1980).

Benomyl is particularly outstanding as one of the systemic fungicides most frequently utilized for the treatment of different crop seeds. By penetrating the fungus in minimal quantities, benomyl moves and inhibits the formation of microtubules in the cell division spindle (Davidse and Waard 1984). Due to this mechanism of action specifically directed at a vital cellular process, benomyl also affects other species of fungi, including beneficial ones such as those which form vesicular-arbuscular mycorrhiza (VAM).

VAM represents a mutualistic association formed by fungi of the family Endogonaceae with plant roots. The ecological importance of these associations is shown by their occurrence in the most diverse ecosystems and in almost all types of vascular plants (Lopes et al.1983). Externally formed mycelium functions as an extension of the root system of the host plant, significantly increasing the root-soil interface. Thus, the mycelium acts by providing a larger area for water and nutrient absorption from soil to plant (Mosse 1981).

As is also the case for other agricultural practices, few studies have been performed on the effects of

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pesticides on mycorrhiza infection in controlled field experiments (Abbot and Robson 1991). In greenhouse experiments, insecticides, fungicides and herbicides applied at the doses recommended often have shown minor effects (Trappe et al. 1984). Spore formation in the soil and formation of vegetative VAM structures has been found to be inversely proportional to the benomyl dose applied. The effects observed when using higher or lower doses varied from reductions to increases in these parameters in the roots of various plant species (Trappe et al. 1984). Rouchaud et al. (1974) reported that 70 to 90% of the benomyl absorbed was rapidly translocated and concentrated in the leaves of seedlings. Benomyl was linearly reduced in the root system, reaching, by 20 days, 11.7% of the concentration observed 5 days after treatment. Unestam et al. (1989) reported that benomyl did not affect the growth rate of ectomycorrhyza-forming fungi or the rate of infection of roots of **Pinus** sp seedlings 55 days after being applied in a nutrient solution.

The objective of the present study was to assess the effect of increasing benomyl doses used to treat tomato seeds on the initial infection of roots by VAM forming fungi.

MATERIAL AND METHODS

The experiment was conducted on "Latossolo Vermelho Escuro" (Dark Red Latossol) soil of the following physical and chemical composition: 32% sand, 22% silt, 46% clay, 2.1% organic matter, pH 5.1, 9 µg/mL P_2O_5 (resin), and with the following values of meq/100 mL soil: 0.3 K, 2.5 Ca, 1.0 Mg, 2.8 H+Al, 3.8 base sum, and 6.6 cation exchange capacity (CEC).

Seeds of Santa Cruz type tomato, "Rio Grande" cultivar, were treated by mixing with doses of Benlate 500 fungicide in the soluble powder formulation corresponding to 0, 25, 50 and 100 g benomyl/100 kg. Immediately after treatment, the seeds were sown in soil previously prepared for cultivation. In September 1988, approximately 150 seeds/m were sown in rows spaced 0.5 m apart without fertilizer and covered with a 2.0 cm thick layer of soil. A fully randomized experimental design with four treatments and six replications was used. The experimental plots consisted of 3.0 m long rows. The source of inoculum consisted only of the natural fungal propagula of the soil, since this soil has been cultivated for several years. After sowing, the soil was irrigated daily by sprinkling. Weeds were removed manually once a week and disposed of outside the trial area.

Infection of tomato seedling roots by VAM-forming fungi was assessed 10, 15, 20 and 30 days after sowing. On each occasion, five plants per plot were carefully removed with their root systems. At the laboratory, they were washed in tap water, separated and stained by the method of Phillips and Hayman (1970). Sixty 0.5 cm long root fragments were collected at random from the entire root material, fixed on three glass slides and observed under the light microscope according to the technique of Giovannette and Mosse (1980), to calculate the percentage of those infected with VAM. The percentages were analyzed statistically by a 4 x 4 factorial analysis, where factor A = benomyl doses (0, 25, 50, 100 g/kg), and factor B = days after sowing (10, 15, 20, 30), using Tukey test for comparison of the means.

RESULTS AND DISCUSSION

The mean percentages of infection of root fragments from tomato seedlings are presented in Table 1. It can be seen that, on the occasion of the first assessment 10 days after sowing, benomyl significantly reduced mycorrhiza formation in the seedlings in a dose-related manner.

Table 1. Mean percentages of tomato seedling root fragments with vesicular-arbuscular mycorrhiza obtained from benomyl-treated seedlings.

Treatment	Days after sowing			
	10	15	20	30
1. Check	^{b}36.0 a	^{b}34.0 a	^{ab}42.0 a	^{a}60.0 a
2. 25 g/100 kg	^{b}14.0 b	^{b}28.0 ab	^{b}32.0 ab	^{a}62.0 a
3. 50 g/100 kg	^{b}14.0 b	^{b}18.0 ab	^{b}26.0 ab	^{a}76.0 a
4. 100 g/100 kg	^{ab}16.0 ab	^{b}2.8 b	^{ab}18.0 b	^{a}34.0 b
F Factor A				16.26**
Factor B				46.76**
A x B interaction				3.27**
LSD (0.05) Factors A and B				9.73%
A x B interaction				19.47%

Means preceded by the same letter, compared horizontally, and followed by the same letter, compared vertically, did not differ from one another by the Tukey test at the 5% probability level.

In the treatment with 100 g/100 kg seeds, corresponding to twice the dose recommended by the manufactures, the percentage of root fragments with mycorrhiza was significantly lower throughout the period of evaluation. However, mycorrhiza formation after the use of 25 and 50 g/100 kg, respectively corresponding to half the dose recommended and the dose recommended, was significantly lower than observed in the check only on the occasion of the first assessment. On the occasion of the remaining assessments, the three treatments not only did not differ amongst themselves, but the percentage of root fragments with mycorrhiza formation returned to the infection level observed in the check.

However, may be seedlings tomato have yours nutrient uptake decreased without mycorrhizae throughout this initial period. This could cause reduced plant growth due to fungicide application, after which plants rapidly try to reestablish the symbiosis. From total nutrient uptaken, the uptake of tomato plants, cvs Vemone and Marglobe, throughout the first for weeks period, respectively, was: 1.9 and 3.8% at N; 3.4 and 1.9% at P; 1.3 and 2.1% at K; 0.8 and 1.0% at Ca; 3.0 and 3.4% at Mg; 5.3 and 3.8% at Fe; 4.4 and 2.5% at Mn; 4.6 and 11.2% at Zn; and 3.1 and 2.7% at B (Carpena et al. 1988).

Thus, at the doses used benomyl did not impair mycorrhiza formation in tomato seedlings one month after sowing, in agreement with results obtained in studies on other plant species (Trappe et al. 1984 and Unestam et al. 1989).

The effect of benomyl dose on the rate of mycorrhiza formation in tomato seedling roots between 10 and 30 days after sowing can be observed in Fig. 1. It can be seen that mycorrhiza formation in the treatments with the two lower doses was more rapid than in the check starting from the 15-day assessment.

This effect was confirmed by the higher angular coefficient of the line estimated with the data for the dose of 25 g/100 kg (2.33) and by the significant quadratic effect estimated with the data for the dose of 50 g/100 kg. The recovery in mycorrhiza formation with these two lower benomyl doses starting from the 20th day after sowing and the lack of effect on the 30th day agree with the results reported by Unestam et al (1989), in the greenhouse or growth chamber conditions.

The recovery of mycorrhiza formation in tomato seedlings seems to be proportionally related to the dissipation and degradation of benomyl and of its

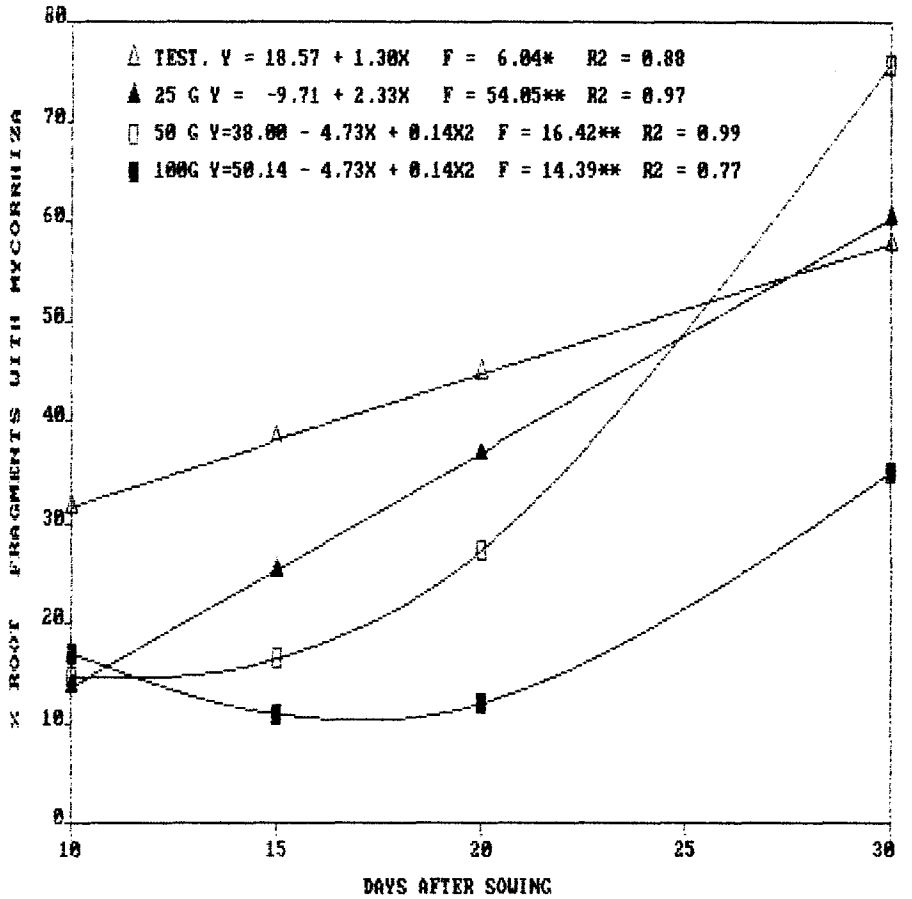

Figure 1. Mean percentages of the number of tomato seedling root fragments infected with mycorrhiza after treatment of seeds with benomyl.

transformation products with fungicidal activity, as observed by Rouchaud et al. (1974) in melon seedlings. These investigators noted that, after root absorption, 70 to 90% of the benomyl administered was rapidly transferred to the leaves and the amount remaining in the root system dissipated within 20 days.

The increase in rate of mycorrhiza formation of tomato seedlings may be explained also by the selective control of organisms which antagonize VAM-forming organisms (Ross and Ruttencotter 1977; Daniels and Menge 1980; Baath and Hayman 1983 and Siqueira et al. 1984). Thus, after a certain time of dissipation, the

decreasing fungicide concentrations may not be sufficient to inhibit mycorrhiza-forming fungi. Thus, benomyl may act by reducing or eliminating the competitive effects of these antagonists, since benomyl is a fungicide with a broad spectrum of activity (Davidse and Waard 1984).

The treatment of tomato seeds with benomyl at the recommended dose of 50 g/100 kg had no long term significantly harmful effect on the infection of the root system of seedlings by VAM fungi under field conditions. Infection levels were initially reduced by half, compared to the check treatment, but infection recovered to the level of the check after 30 days. As VAM increases the nutrient uptake and water, and since the initial two week establishment period is important in crop establishment against weeds, or in low P soils, mycorrhizae could be very useful during this period. However, when a two-fold dose was used, 100 g/100 kg, a significant reduction in the rate of root mycorrhiza formation was observed.

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