Effect of the fungicides Fosetyl-Al and Metalaxyl on arbuscular mycorrhizal colonization of seedlings of *Citrus sinensis* (L.) Osbeck grafted onto *C. limon* (L.) Burmf

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ABSTRACT. We investigated the effect of the fungicides Fosetyl-Al and Metalaxyl, recommended for prevention and control of gummosis, on the development of arbuscular mycorrhizal fungi (AMF) in *Citrus sinensis* Osbeck grafted onto *C. limon* (L.) Burmf. Three experiments were performed in 1990 and 1992, in Casa Branca and Ilha Solteira, State of São Paulo, Brazil, respectively. Root samples were collected after fungicide application at long (monthly) and short (weekly) periods at Casa Branca and every three days at Ilha Solteira, in order to evaluate the percentage of root colonization by AMF. Fosetyl-Al generally did not show a deleterious effect on the development of mycobionts. In contrast, Metalaxyl reduced root colonization by AMF, and when both fungicides were applied together, mycorrhizal colonization was decreased, particularly in the shorter time experiments.

Key words: arbuscular mycorrhizal fungi, citrus, fungicides, gummosis.

RESUMO. Efeito dos fungicidas Fosetil-Al e Metalaxil na colonização de mudas de *Citrus sinensis* (L.) Osbeck enxertadas em *C. limon* (L.) Burmf. O efeito dos fungicidas Fosetil-Al e Metalaxil, recomendados para prevenção e controle da gomose, sobre o desenvolvimento de fungos micorrízicos arbusculares (FMA) em *Citrus sinensis* Osbeck enxertado em *C. limon* Burmf foi investigado. Três experimentos foram realizados em 1990 e 1992, em Casa Branca e Ilha Solteira, estado de São Paulo, Brasil, respectivamente. Coletas de raízes foram feitas após a aplicação dos fungicidas, em intervalos mensais e semanais em Casa Branca, e a cada três dias em Ilha Solteira, para avaliar-se a percentagem de colonização radical por FMA. De modo geral, Fosetil-Al não apresentou efeito deletério no desenvolvimento dos micobiontes. Ao contrário, Metalaxil reduziu a colonização radical; e quando ambos os fungicidas foram aplicados juntos, a colonização radical foi diminuída, particularmente nos experimentos mais curtos.

Palavras-chave: fungos micorrízicos arbusculares, citrus, fungicidas, gomose.

Arbuscular mycorrhizal fungi (AMF) are frequently found associated with roots of citric plants, increasing the content of nutrients in leaves and roots, and improving the growth and development of plants (Krikun and Levy, 1980).

It is known that the roots of *Citrus* spp. have low capacity for absorption of nutrients, which has been attributed to the small number of absorbent hairs. Thus, according to Baylis’s theory *Citrus* spp. would have a greater dependence on mycorrhiza (Baylis, 1970). Citrus roots exude sugars, amino acids and volatile compounds, which stimulate the germination of the spores of AMF, as well as stimulate the growth of the fungal hyphae, during the period of root penetration (Graham, 1982). Such root exudates can also attract and provide nutrients for other rhizospheric microorganisms, such as bacteria, actinomycetes and fungi. These microorganisms, if established in the rhizosphere, may cause modifications that may be harmful to the host plant.

Most agricultural and particularly the perennial cultures are treated with large amounts of different biocidal agents. Besides affecting pathogenic
microorganisms, these agents may also affect detrimentally the microorganisms that are beneficial to the plants.

Gummosis, caused by Phytophthora citrophthora (SM, & SM.) Leonian and P. nicotianae Haan var. parasitica (Dastur) Waterh, is a major problem affecting Citrus spp. in many regions. Once gummosis has become established in one orchard it is very difficult to control. Several fungicides with different modes of action are approved and registered for use by the Agriculture Ministry in Brazil. Among them Fosetyl-Al is one of the most effective (Feichtenberger, 1990; Ouimette and Coffey, 1989). Fungicides based on Metalaxyl (an acilalanin group) act on several oomycetes, but tend to be very specific, which can lead to the selection of resistant races among the target microorganisms.

Several studies on the effects of different fungicides on the formation of arbuscular mycorrhiza and sporulation of AMF have been published (Hetrick and Wilson, 1991; Kough et al., 1987; Trappe et al., 1984). Sieverding (1991) suggested that fungicides generally affect the process of infection by AMF; however, different effects may be obtained depending upon different microbial populations, soil types, host plants, mycobionts, as well as fungicide concentration, chemical nature, persistence, mode of action and translocation.

The objective of this study was to investigate the effects of the fungicides Metalaxyl (Ridomil 50G) and Fosetyl-Al (Alliette) used for controlling gummosis on root colonization by AMF in Citrus limon under C. sinensis.

Materials and methods

Field experiment

Site description and preparation of research area. The experiment was carried out in Casa Branca, in the northeast of the state of São Paulo (21°46'S, 47°05'W), in an area with a typical warm climate, with defined dry and humid stations, being classified as of the type CWa in the classification of Köppen (Lopes, 1984). The annual averages of maximal and minimal temperatures are 26°C and 13°C, respectively, with annual medium precipitation varying from 1,250 to 1,500 mm (Lopes, 1984; Pimentel et al., 1977). The climatological data for the experimental period are shown in Figure 1.

After the results of the chemical analysis (December/89), the plot was fertilized with 150 g of NPK per linear meter, applied as urea, simple superphosphate and potassium chloride, respectively.

Installation and maintenance of the experiment. The experiment was installed in an area surrounded by citric plants, in December of 1989. Two hundred two-year-old seedlings of C. sinensis grafted on C. limon were transplanted to the field. These seedlings were bought in commercial pots, each one holding approximately 5 kg of nonsterile soil from Limeira (SP), which constituted the primary source of inoculum of AMF. In April and September of 1990, 30 plants received applications of the fungicides. Other ten plants were maintained as controls and did not receive any fungicidal treatment.

Experimental design and treatments. The experimental design was of completely randomized blocks with 5 treatments and 10 replicates. The treatments were: T1 = Control plants; T2 = Fosetyl-Al 2 g/L (e.g. 0.16 mg.dm-3/ plant); T3 = Metalaxyl 1 g a.i./m2; T4 = Metalaxyl 2 g a.i./m2; T5 = Fosetyl-Al 2 g/L + Metalaxyl 1 g a.i./m2.

Sample collection. Samples of approximately 200 g of soil with roots were collected from the plants at monthly intervals (April, May and June/1990 at 0, 30, and 60 days after the fungicide application - DAFA - respectively) and weekly (19/September, 26/September, and 04/October/1990 at 0, 7 and, 15 DAFA, respectively). The samples were packed in plastic bags and taken to the laboratory for processing.

Root colonization. Fresh root samples of 1 g were separated from the soil by sieving, were cleared with KOH 10%, and stained with Trypan blue and lactophenol (Phillips and Hayman, 1970). The
quantification of root colonization by AMF followed the approaches established by McGonigle et al. (1990). Roots from each sample were mounted on microscope slides with coverslips. The field of view was moved across the slide, and a hairline graticule inserted into the eyepiece acted as the line of intersection with each root. The percentage of root colonization (RC%) was obtained by examining 100 intersections for each root sample.

**Experiment in the nursery**

**Site description.** A further experiment was established at the Estação Experimental de Agronomia, UNESP, district of Ilha Solteira, located at the extreme west of the State of São Paulo, Brazil (20°25'S, 31°21'W).

The climate is characterized, according to Köppen, as being of the type CWa (mesothermic) with little hydric deficiency. The medium temperatures (maximal, minimal) obtained during the period studied were respectively 31.1°C and 12.5°C (Figure 1).

The experiment was carried out in a dystrophic, sandy textured, yellow latosol, with the following chemical and chemical-physical characters: pH (CaCl2) = 4; O.M. = 10 g.dm-3; P (resin) = 44 g.dm-3; and in mmol.dm-3: K+ = 1.3, Ca2+ = 8, Mg2+ = 2, Al3++H+ = 31, S = 11.

During the experimental period the plants received basic fertilization (NPK) and micronutrients (Hoagland solution). The macronutrients (NPK) were dissolved in distilled water, in the form of ammonium sulphate (100 mg.dm-3/plant), simple superphosphate (128 mg.dm-3/P/plant) and potassium chloride (130 mg.dm-3 K/plant), respectively, and applied to the surface of the soil, in February/1992. In addition, complementary applications of ammonium sulphate were made in March, May, and June with 150, 100, 100 mg.dm-3/plant, respectively. The micronutrient solution (B= 0.020; Mn = 0.020; Cu = 0.001; Zn = 0.002; Mg = 0.002 mg.dm-3/plant) was applied in a similar way, in March/1992.

**Installation and maintenance of the experiment.** The experiment was carried out between January and July 1992. Two hundred seedlings of C. sinensis grafted onto rootstock of C. limon were transplanted in perforated bags with approximately 4 kg of natural soil from Limeira, State of São Paulo, Brazil. The seedlings were placed on wooden supports. Fertilization occurred during the whole experimental period. The fungicidal treatments were made in the middle of July. The soil and root sampling started three days after the fungicidal applications.

**Experimental design and treatments.** The experimental design was completely randomized, with eight replicates per treatment; the treatments were described previously for the field experiment.

**Sample collection.** In July/1992, simple samples of roots from 200 seedlings were collected during a period up to 15 DAFA with intervals of 3 days between the samplings. The samples were packed in transparent plastic bags and taken to the laboratory, where they were kept under refrigeration (approximately 5°C) until processing.

**Root colonization.** Root colonization (arbuscule, intraradical hyphae, coils, vesicles, and intraradical spores) was assessed under a stereomicroscope after staining using the gridline intersect method (Giovannetti and Mosse, 1980) with 1.5g samples of roots (fresh weight). The percent values were transformed into arcsin prior to statistical analysis.

**Data analysis.** The transformed data obtained for root colonization were submitted to analysis of variance (ANOVA) taking the least significant difference (LSD) determined at P = 0.05. Regression analysis was carried out, where each experimental unit was evaluated at 0, 30 and 60 and 0, 7 and 15 days after fungicide applications at Casa Branca, and 3, 6, 9, 12, and 15 days after fungicide applications, at Ilha Solteira. For evaluating the fungicide treatments, the values were compared using the Tukey test (P = 0.05) for each sampled period.

**Results and discussion**

**Field experiment**

In the monthly samplings from Casa Branca, a generally high percentage of root colonization by AMF was observed, varying from 19.9 to 82.3% between treatments 5 (Fosetyl-Al+ Metalaxyl) and 3 (Metalaxyl 1 g), respectively. The average values are given in Table 1.

In all fungicidal treatments there was a continuous decline in the colonization percentage. However, the variations were generally small between the first two sampling periods, 0 and 30 DAFA, respectively (Table 1). At 60 DAFA, significant decreases were observed mainly in
treatments 4 (Metalaxyl 2 g) and 5 (Fosetyl-Al + Metalaxyl 1 g) when compared to the percentages of root colonization from the control plants. In that period, variations of the climatic conditions were observed (Figure 1), including reduction in temperature, precipitation and photoperiod which are characteristic of Autumn-Winter. Possibly, these variations influenced, directly or indirectly, the physiological activities of the plants, imposing modifications on their growth and development.

Table 1. Average percentage1 of root colonization by AMF in seedlings of Citrus sinensis grafted on C. limon, in three experiments (Casa Branca 1 - monthly sampling; Casa Branca 2 - weekly sampling; Ilha Solteira - samples collected each three days).

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Root Colonization (%)2</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Casa Branca 1</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Fosetyl-Al</td>
</tr>
<tr>
<td></td>
<td>Metalaxyl 1 g</td>
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<td></td>
<td>Metalaxyl 2 g</td>
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<tr>
<td></td>
<td>Fosetyl-Al + Metalaxyl 1 g</td>
</tr>
<tr>
<td></td>
<td>LSD3 = 23.6</td>
</tr>
<tr>
<td>Casa Branca 2</td>
<td>Control</td>
</tr>
<tr>
<td></td>
<td>Fosetyl-Al</td>
</tr>
<tr>
<td></td>
<td>Metalaxyl 1 g</td>
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<td></td>
<td>Metalaxyl 2 g</td>
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<tr>
<td></td>
<td>Fosetyl-Al + Metalaxyl 1 g</td>
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<tr>
<td></td>
<td>LSD = 23.6</td>
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<tr>
<td>Ilha Solteira</td>
<td>Control</td>
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<td></td>
<td>39.7 a</td>
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<td></td>
<td>Metalaxyl 1 g</td>
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<td>Metalaxyl 2 g</td>
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<td></td>
<td>Fosetyl-Al + Metalaxyl 1 g</td>
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<tr>
<td></td>
<td>LSD = 0.67</td>
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</tbody>
</table>

1 Replicates: 10 in Casa Branca experiments; 8 in Ilha Solteira experiment; 2 Numbers followed by the same letter in each column are not significantly different by the Tukey’s test at P ≤ 0.05; 3 LSD = least significant difference.

Fosetyl-Al did not show a deleterious effect on AMF. Such observation agrees with previous data (Cardoso and Lambais, 1993; Sieverding, 1991). The use of Fosetyl-Al has been related to the increase in root exudates (Jabaji-Hare and Kendrick, 1985), which seems to favor formation and penetration of the spore germinative tube into the root, facilitating its establishment inside the root cortex (Sieverding, 1991). However, the percentages of colonization observed in the Fosetyl-Al treatment were not higher or significantly different from those observed in the control plants (P = 0.05).

Application of 1 or 2 g of Metalaxyl (treatments 3 and 4) depressed the percentage of root colonization at 30 and 60 DAFA (Table 1). However, the decrease was only significant (r = -0.96 and r = -0.99, respectively) in the last sampling period (60 DAFA), as shown in Figure 2a. The association of Fosetyl-Al and Metalaxyl (treatment 5) did not seem to influence root colonization in the first 30 days. During this period, climatic conditions were not favorable to nutrient and Metalaxyl absorption. With less absorption, the effects of Metalaxyl on RC were reduced. This, associated with the positive influence of Fosetyl-Al on RC, meant that AMF development was not restricted, and thus similar percentages of RC between treatment 5 and control plants were observed. With the increased temperature in the last period (60 DAFA), it is possible that more Metalaxyl was absorbed, and this was high enough to reduce the intraradical development of the mycobionts (r = -0.88). Data obtained by Musumeci et al. (1982) showed that the absorption and translocation of Metalaxyl occurred over approximately 60 days, and that after this period, it decreased due to its degradation in the soil and in the tissues of the plant. Previous studies have reported that Metalaxyl and its metabolites did not have negative effects on the development of AMF, and generally it increased colonization (Afek et al., 1990; Hetrick and Wilson, 1991). In contrast, in treatments 3, 4, and 5 (Metalaxyl 1g, Metalaxyl 2g and Fosetyl-Al + Metalaxyl 1g, respectively) there was a considerable reduction in percentage of colonization (Table 1).

In the second experiment, the percentage of root colonization varied from 29.2 to 80.6 (untransformed data). The percentages of colonization in control plants and in those that received Fosetyl-Al were similar during the whole experimental period (Table 1). Up to 7 DAFA there was a significant decrease, with subsequent increase up to 15th DAFA (r = -0.29 and r = -0.17, respectively). A decrease was also observed in the samples from the other treatments suggesting it may be related to the low temperatures experienced during this period (Figure 2b). The colder temperature probably decreased the photosynthetic rate of the plant and reduced the production of sugars and organic acids, thus limiting the development of AMF. It was also apparent for treatments 2 and 5, both with Fosetyl-Al, that the decreases were less accentuated (Table 1) and these may be related to the action of Fosetyl-Al on root exudation (Jabaji-Hare and Kendrick, 1985; Sieverding, 1991). In the following period (15 DAFA), increases in the percentages of mycorrhizal colonization were observed in the plants treated with Fosetyl-Al and in the roots of the control plants. There was apparently no significant action of Fosetyl-Al on the arbuscular mycorrhiza, since the percentages of colonization were similar in both
treatments (Table 1). Similar data were obtained by Guillemim and Gianinazzi (1992), Trouvelot et al. (1992) and Cardoso and Lambais (1993). In contrast, Wellings et al. (1990) observed the opposite effect in maize, and they considered the decrease in root colonization as resulting from a phytotoxic action from a too high dosage (0.148 g/plant) of Fosetyl-Al, rather than from a fungitoxic effect.

Constant decreases were observed in the percentages of root colonization in the plants treated with 1 or 2 g of Metalaxyl \( (r = -0.94 \text{ and } r = -0.91, \text{ respectively}) \) during the whole experimental period (Figure 2b). In treatment 5 (Fosetyl-Al + Metalaxyl 1g) there was accentuated reduction of root colonization in the last sampling period (15 DAFA; \( r = -0.89 \)). It is possible that up to 7th DAFA, the stimulating action of the Fosetyl-Al counteracted the deleterious action of Metalaxyl on AMF, minimizing the decrease in root colonization. Up to 15th DAFA, with a probable increased absorption of Metalaxyl, stimulated by higher temperature and larger exposure, the depressive effect became more evident.

**Experiment in nursery**

In nursery, the variation in the percentage of root colonization was high, from 26.9 to 62.1% (untransformed data) for the treatments Fosetyl-Al colonization was high, from 26.9 to 62.1% (untransformed data) for the treatments (Figure 2c). Up to 9th DAFA there was a small increase in the colonization percentages in both treatments, and this coincided with the coldest period of the experiment (22°C), as shown in Figure 1. Sieverding (1983) observed that the most favorable temperatures for the development and effectiveness of AMF varied between 25°C to 30°C and suggested this was probably related to the physiological behavior of the host plant and soil microbiota. With a decrease of temperature, the metabolic activity of the host plant diminished, resulting in reduced production of photoassimilates. Depending on the AMF species present in the mycorrhizal association and its stage of development, its nutritional requirements can vary. If the AMF species established in the root cortex are poor competitors for organic compounds, then they can only capture a small amount of free photoassimilates for their development in conditions of high temperatures. These same species may be favored by the decreased metabolic rhythm of the host plant in conditions of lower temperature, allowing the AMF to capture more organic compounds, due to the smaller requirement of the host plant.

![Figure 2. Effect of sampling periods on root colonization by AMF in citric plants cultivated under different fungicidal](image-url)
treatments, in Casa Branca and Ilha Solteira (São Paulo state, Brazil) 
** significant at P ≤ 0.05

The application of 1 or 2 g of Metalaxyl (treatments 3 and 4) to the rhizospheres of the host plants exercised continuous negative effects on the percentage of mycorrhizal colonization (r = -0.63; r = -0.66, respectively), starting from 9th DAFA (Figure 2c). Similar effects were observed in treatment 5 (r = -0.75). In these three treatments, there was a more or less regular decrease during the whole experimental period.

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References


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