Acta Scientiarum 22(2):305-310, 2000.
ISSN 1415-6814.

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

Rosilaine Carrenho[1]*, Vera Lúcia Ramos Bononin[2] and Luiz Antônio Graciolli[3]

*[1]Departamento de Biologia, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900, Maringá-Paraná, Brazil. [2]Instituto de Botânica, Seção de Micologia, C.P. 4005, 01061-970, São Paulo-São Paulo, Brazil. [3]Departamento de Biologia, Faculdade de Engenharia de Ilha Solteira, Universidade Estadual Paulista, C.P. 31, 15378-000, Ilha Solteira-São Paulo, Brazil. *Author for correspondence. e-mail: rcarrenho@uem.br*

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.

The soil, a red-yellow latosoil, dystrophic, sandy textured, presented the following chemical characteristics: pH ($CaCl_2$) = 5.5; M.O = 15 g/dm^3; P (resin) = 6 mg/dm^3, and in mmol/dm^3: K^+ = 12, Ca^{2+} = 20, Mg^{2+} = 6, Al^{3+} + H^+ = 28, S = 27.

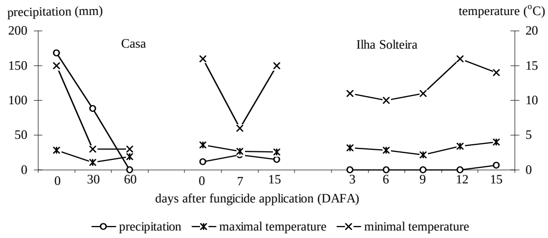

Figure 1. Air temperature and precipitation in Casa Branca and Ilha Solteira (São Paulo state, Brazil) during the sampling periods. Maximal and minimal temperatures expressed in average values and precipitation are values accumulated

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./m^2; T4 = Metalaxyl 2 g a.i./m^2; T5 = Fosetyl-Al 2 g/L + Metalaxyl 1 g a.i./m^2.

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 (20º25'S, 31º21'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 31.1ºC and 12.5ºC (Figure 1).

The experiment was carried out in a distrophic, sandy textured, yellow latosoil, with the following chemical and chemical-physical characters: pH ($CaCl_2$) = 4; O.M. = 10 g.dm^{-3}; P (resin) = 44 g.dm^{-3}, and in mmol.dm^{-3}: K^+ = 1.3, Ca^{2+} = 8, Mg^{2+} = 2, Al^{3+}+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 adition, 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 (30 cm), inside a nursery, where they remained for 40 days. In March/1992, they were transferred to the field, where they were installed 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 5ºC) 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 percentage[1] 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).

Treatments	Root Colonization (%)[2] days after fungicide application				
Casa Branca 1	0		30		60
Control	67.0 a		61.1 a		48.2 a
Fosetyl-Al	56.7 a		52.3 a		37.4 ab
Metalaxyl 1 g	66.5 a		50.1 a		36.7 ab
Metalaxyl 2 g	69.7 a		48.3 a		28.3 b
Fosetyl-Al + Metalaxyl 1 g	54.5 a		51.9 a		25.8 b
	LSD[3] = 21.1		LSD = 14.8		LSD = 13.5
Casa Branca 2	0		7		15
Control	59.5 a		45.6 b		54.8 a
Fosetyl-Al	54.1 a		47.0 ab		52.2 a
Metalaxyl 1 g	59.7 a		50.8 ab		42.6 a
Metalaxyl 2 g	67.8 a		54.2 ab		43.2 a
Fosetyl-Al + Metalaxyl 1 g	64.8 a		61.3 a		31.4 a
	LSD = 23.6		LSD = 15.1		LSD = 23.4
Ilha Solteira	3	6	9	12	15
Control	44.6 a	42.6 a	52.0 a	43.6 a	45.7 a
Fosetyl-Al	39.7 a	44.6 a	46.6 ab	42.4 a	39.1 ab
Metalaxyl 1 g	46.7 a	36.1 a	41.7 bc	38.4 ab	33.2 b
Metalaxyl 2 g	44.6 a	37.3 a	35.1 c	32.9 b	36.9 ab
Fosetyl-Al + Metalaxyl 1 g	39.9 a	37.0 a	33.8 c	32.3 b	33.7 b
	LSD = 0.88	LSD = 0.67	LSD = 0.79	LSD = 0.97	LSD = 0.67

[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 $P \leq 0.05$; [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 favourable 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 and r = -0.91, 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 + Metalaxyl 1g and Control, respectively (Table 1). Nevertheless, the values were generally lower than those obtained in the field experiments at Casa Branca.

Such lower values may be due to different conditions affecting the experiments at Casa Branca and Ilha Solteira. In the last experiment several fertilizations based on nitrogen, potassium and micronutrients were applied in addition and the humidity of the soil was controlled. Applications of fertilizers increase leaf area and the photosynthetic rates of the host plants, and they can decrease or increase the mycorrhizal colonization in many tropical arable plants (Sieverding, 1991; Toro, 1984). The behavior of the root colonization by AMF during the experimental periods is shown in Figure 2c.

The control plants (treatment 1) showed high percentages of root colonization in almost all the sampled periods, without significant variations between averages. These values were significantly higher than those of treatments 3 (Metalaxyl 1 g), 4 (Metalaxyl 2 g) and 5 (Fosetyl + Metalaxyl 1 g), starting from 9th DAFA (Table 1). In treatments 1 and 2 (Control and Fosetyl-Al, respectively), the variations in the percentages of root colonization during the five sampling periods were not statistically significant, r = 0.26 and r = -0.14, respectively (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 (22ºC), as shown in Figure 1. Sieverding (1983) observed that the most favorable temperatures for the development and effectiveness of AMF varied between 25ºC to 30ºC and suggested this was probably related to the physiological behavior of the host plant and soil microbiota. With a decrease of temperature, the metabolical 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.

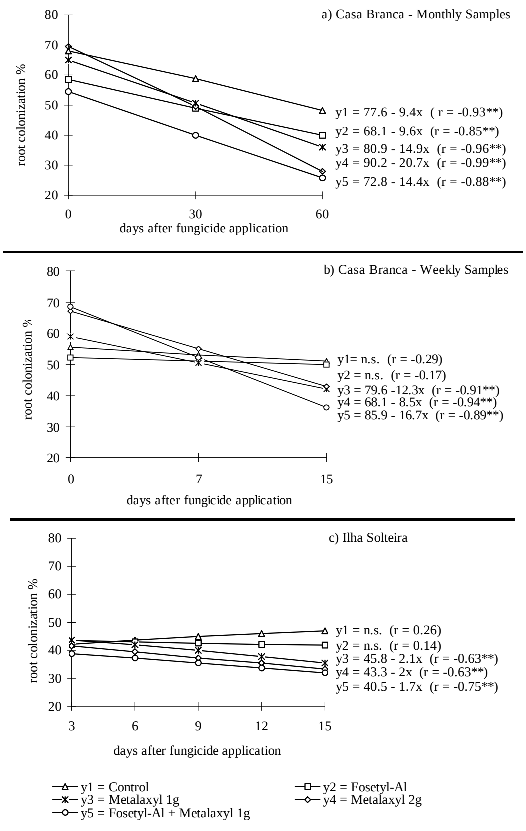

Figure 2. Effect of sampling periods on root colonization by AMF in citric plants cultivated under different fungicidal

treatments, in Casa Branca and Ilha Solteira (São Paulo state, Brazil)
** significant at $P \leq 0.05$

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.

Acknowledgements

We thank Dr. Eduardo Feichtenberger for helping with the experiment of Casa Branca (SP), and to Ciba-Geigy for supplying the fungicide Ridomil 50G.

References

Afek, V.; Menge; J.A.; Johnson, E.L.V. The effect of *Pythium ultimatum* and treatments with metalaxyl on root length and mycorrhizal colonization of cotton, onion, and pepper. *Plant Dis., 4*:117-120, 1990.

Baylis, G.T.S. Root hairs and phycomycetous mycorrhizas in phosphorus deficient soil. *Plant Soil, 33*:713-716, 1970.

Cardoso, E.J.B.N.; Lambais, M.R. Efeito de aldicarb e fosetil-Al no desenvolvimento e na colonização micorrízica de tangerina cleópatra. *Rev. Bras. Cienc. Solo, 17*:179-184, 1993.

Feichtenberger, E. Gomose de *Phytophthora* dos citros. *Laranja, 11*(1):97-122, 1990.

Giovanneti, M.; Mosse, B. An evaluation of techniques for measuring vesicular-arbuscular mycorrhizal infection in roots. *New Phytol., 84*:489-500, 1980.

Graham, J.H. Effect of citrus root exsudates on germination of chlamidospores of the vesicular-arbuscular mycorrhizal fungus. *Glomus epigaeum. Mycologia, 74*(5):831-835, 1982.

Guillemin, J.P.; Gianinazzi, S. Fungicide interaction with VA fungi in *Ananas comosus* grown in a tropical environment. In: Read, D.J.; Lewis, D.H.; Fitter, A.H.; Alexander, I. (eds.). *Mycorrhizas in ecosystems*. Wallingford: CAB International, 1992. p.381-382.

Hetrick, B.A.D.; Wilson, G.W.T. Effect of mycorrhizal fungus species and metalaxyl application on microbial suppression of mycorrhizal symbiosis. *Mycologia, 83*(1):97-102, 1991.

Jabaji-Hare, S.; Kendrick, B. Effects of fosetyl-Al on root exudation and on composition of extracts of mycorrhizal leek roots. *Can. J. Plant Pathol.,* 7:118-126, 1985.

Kough, J.L.; Gianinazzi-Pearson, V.; Gianinazzi, S. Depressed metabolic activity of vesicular-arbuscular mycorrhizal fungi after fungicide applications. *New Phytol., 106*:707-715, 1987.

Krikun, J.; Levy, Y. Effect of vesicular-arbuscular mycorrhiza on citrus growth and mineral composition. *Phytoparasitica, 8*:195-200, 1980.

Lopes, A.S. O meio ambiente dos cerrados. In: Lopes, A.S. (ed.). *Solos sob cerrado:* características, propriedades e manejo. Piracicaba: Associação Brasileira para pesquisa da Potassa e Fosfato, 1984. p.3-9.

Mc Gonigle, T.P.; Miller, M.H.; Evans, D.G.; Fairchild, G.L.; Swan, J.A. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytol., 115*:495-501, 1990.

Musumeci, M.R.; Feichtenberger, E.; Ruegg, E.F.; Campacci, C.A. Absorção e translocação sistêmica de metalaxil ^{14}C por plântulas de *Citrus sinensis* Osbeck após aplicações em diferentes solos. *Fitopatol. Bras.* 7:393-400, 1982.

Ouimette, D.G.; Coffey, M.D. Phosphonate levels in avocado (*Persea americana*) seedlings and soil following treatment with fosetyl-Al or potassium phosphonate. *Plant Dis., 73*:212-215, 1989.

Phillips, J.M.; Hayman, D.S. Improved procedures for clearing roots for rapid assessment of infection. *Trans. Brit. Mycol. Soc. 55:*158-161, 1970.

Pimentel, M.F.; Christofidis, D.; Pereira, F.J. Recursos hídricos no cerrado. In: Ferri, M.G. (coord*.).* SIMPÓSIO SOBRE CERRADO: BASES PARA UTILIZAÇÃO AGROPECUÁRIA, 4, 1997, Belo Horizonte. *Anais...* São Paulo: Ed. Universidade de São Paulo, 1977. p.121-154.

Sieverding, E. *Manual de m*étodos para la *investigación* de *la micorriza vesiculo-arbuscular en el laborat*ório. Cali: CIAT, 1983.

Sieverding, E. Plant protection practices with pesticides. In: Sieverding, E. (ed.). *Vesicular-arbuscular mycorrhiza management in tropical agrosystems.* Eschborn: Technical Cooperation Federal Republic of Germany, 1991. p.165-182.

Toro, T. *Estudio* sobre *la* presencia de *los hongos formadores* de *micorriza vesiculo arbuscular* en*n la caña* de *azucar (*Saccharum *spp.) en el Valle del Cauca*. Manizales, 1984. (Doctoral Thesis) - Universidad of Caldas.

Trappe, J.M.; Molina, R.; Castellano, M. Reactions of mycorrhizal fungi and mycorrhizal formation to pesticides. *Ann. Rev. Phytopathol*., *22*:331-359, 1984.

Trouvelot, A; Abdel-Fattah, G.M.; Gianinazzi, S.; Gianinazzi-Pearson, V. Differential effects of fungicides on VA fungal viability and efficiency. In: Read, D.J.; Lewis, D.H.; Fitter, AH.; Alexander, I.I. (eds.). *Mycorrhizas in ecosystems.* Wallingford: CAB International, 1992. p.404-405.

Wellings, N.P.; Thompson, M.F.; Fiske, M.L. Phytotoxicity of phosphonic (phosphorus) acid and fosetyl-alumminum to the host of mycorrhizal cultures on maize. *Aust. Plant Pathol., 19*(4):141-142, 1990.

Received on December 07, 1999.

Accepted on May 18, 2000.