

SEÇÃO III - BIOLOGIA DO SOLO

GROWTH AND METABOLIC ACTIVITY OF THE EXTRAMATRICAL MYCELIUM OF ENDOMYCORRHIZAL MAIZE PLANTS⁽¹⁾

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SUMMARY

The objective of this experiment was to quantify the extramatrical mycelium of the arbuscular mycorrhizal (AM) fungus *Glomus etunicatum* (Becker & Gerdemann) grown on maize (*Zea mays* L. var. Piranão) provided with various levels of phosphate fertilizer and harvested at 30, 60 and 90 days after planting (DAP). Total extramatrical mycelium (TEM) was extracted from soil using a modified membrane filtration method, followed by quantification using a grid intersection technique. Active extramatrical mycelium (AEM) proportion was determined using an enzymatic method which measured dehydrogenase activity by following idonitrotetrazolium reduction. At low levels of added P, there was relatively less TEM than at high levels of added P, but the AEM proportion at low soil P availability was significantly greater than at high soil P.

Index terms: arbuscular mycorrhizae, *Glomus etunicatum*, extramatrical hyphae, metabolically active mycelium.

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RESUMO: *DESENVOLVIMENTO E ATIVIDADE DO MICÉLIO EXTRAMATRICIAL EM PLANTAS DE MILHO COLONIZADAS COM FUNGO MICORRÍZICO*

*O objetivo do trabalho foi quantificar o micélio extramatricial do fungo micorrízico arbuscular (AM) *Glomus etunicatum* (Becker & Gerdemann) e medir sua atividade 30, 60 e 90 dias após o plantio (DAP), utilizando-se a cultura do milho (*Zea mays* L. var. Piranão) com quatro doses de adubação fosfática. O micélio extramatricial total (TEM) foi extraído do solo pelo método da membrana de filtração modificado e foi quantificado pela técnica da grade de interseção. A proporção de micélio extramatricial ativo (AEM) foi determinada pelo método que mede a atividade das enzimas desidrogenases com coloração de iodotetrazolol. Em baixas doses de P, o TEM foi menor do que a quantidade em altas doses de P, mas a proporção de AEM foi maior nas baixas doses de P.*

Termos de indexação: *Glomus entunicatum, hifas externas, micélio ativo, micorriza arbuscular.*

INTRODUCTION

Root colonization by arbuscular mycorrhizal (AM) fungi occurs in two phases, one internal (intramatricial) and one external (extramatricial). There is a very good relationship between internal root colonization and active AM biomass within a colonized plant root (Bethlenfalvay et al., 1982), but there are marked differences between AM species in terms of extramatricial hyphal length and of the correlation with internal infection or effectiveness (Abbott et al., 1984). The growth and distribution of the extramatricial phase have received less attention than the growth of the fungus within the host root (Pacovsky & Bethlenfalvay, 1982; Miranda & Harris, 1994), as well as the internal structures that the fungus produces intramatricially (Smith & Walker, 1981; Sanders & Sheik, 1983). However, there is evidence that the stimulation of host growth by mycorrhizal fungal colonization cannot be explained solely by the quantification of internal colonization (Graham et al., 1982; Silveira & Cardoso, 1987).

Phosphorus amendment is capable of inhibiting spore germination, hyphal branching and growth in laboratory medium (Nagahashi et al., 1996), but the P effect on soil is more complex (Lambais & Cardoso, 1988; Miranda et al., 1989). Abbott et al. (1984) demonstrated that small amounts of P were stimulatory and large amounts of P inhibitory to extramatricial growth of mycorrhizal hyphae, while Miranda & Harris (1994) proposed that high concentrations of soil P would inhibit spore germination and germ tube growth at the lag phase of mycorrhiza formation.

The extramatricial hyphae of the AM fungus is the primary structure responsible for nutrient and water uptake, stimulation or inhibition of the soil

microbiota in the mycorrhizosphere, and may play a role in altering soil structure (Bethlenfalvay & Ames, 1987). An evaluation of the growth and development of these extramatricial structures is therefore essential in physiological studies involving this host-endophyte association. The enhanced absorption of phosphorus in soils of low available P content by mycorrhizal plants has been well documented (Lambert et al., 1979; Harley & Smith, 1983). It is the extramatricial hyphae of the fungus that reduce the diffusion path for P and other immobile ions in the soil, and as extensions of the host's root system, hyphae prove superior for absorption and assimilation of these nutrients (Harley & Smith, 1983). Indeed, the relationship between the growth of the internal and the extramatricial mycelia has been related to mutualistic associations, when their growth is in balance (Graham et al., 1982; Abbott et al., 1984), or parasitic associations, when their growth is not in balance (Bethlenfalvay et al., 1982).

Apparently, extramatricial hyphae are also primarily responsible for water uptake when the soil water potential is low (Sylvia, 1988) and may play a role in altering the populations of bacteria responsible for stabilizing aggregate structure in soil (Bethlenfalvay & Ames, 1987). Colonization of a host root by an endomycorrhizal fungus has a dramatic effect on the amount of soil fungi present in soil (Graham et al., 1982; Hamel et al., 1990; Sylvia, 1992). While it appears that the largest proportion of fungal hyphae derives from the VAM fungi that were inoculated into a fumigated soil (Abbott et al., 1984, Bethlenfalvay & Ames, 1987, Sylvia, 1988, Nagahashi et al., 1996) or the AM fungi found in native soils (Kabir et al., 1996), AM fungi may also stimulate the growth of other soil fungi indirectly.

The uptake and translocation process requires a system of metabolically active extramatrical hyphae (Harley & Smith, 1983). For this reason, knowledge of the proportion of metabolically active extramatrical mycelium (AEM), involved in nutrient uptake, compared to total extramatrical mycelium (TEM), involved in nutrient transport (Schubert et al., 1987), is important in the study and appreciation of the processes involved in nutrient transport. Additionally, the physiological interaction between host and fungus (Pacovsky, 1989) depends on exchange between the active cytoplasm of the macro- and micro-symbionts.

The objective of this work was to quantify the total and active extramatrical mycelium of the AM fungus *Glomus etunicatum* grown in association with maize (*Zea mays* L.) plants which received phosphate fertilizer. Furthermore, it was determined whether there is a relationship between the amounts of active extramatrical mycelium and host plant growth.

MATERIALS AND METHODS

The experiment was arranged in a (4 x 2 x 3) randomized block design with four levels of added phosphorus (0, 25, 50 or 75 mg kg⁻¹), two levels of inoculation [without *Glomus* (-G) or with *Glomus* (+G)], and three harvests (30, 60 and 90 days). There were four replicates per treatment for a total of 96 plants.

The AM fungus used, *Glomus etunicatum* (Becker & Gerdemann), was multiplied in pot cultures of *Brachiaria decumbens*, and the inoculum consisted of 50.0 g of soil from these pots, which contained approximately 1250 spores and 200 infected root segments between 1.0 cm and 2.0 cm length. The inoculum was placed in a band approximately 5.0 cm below the soil surface. About 50.0 g of soil from pots where *Brachiaria decumbens* had grown without spores of any AM fungus, were similarly placed 5.0 cm below the soil surface, in control pots that had received no mycorrhizal inoculum.

Maize (*Zea mays* L. cv. Piranão) seeds were surface sterilized (Lambais & Cardoso, 1988), and planted into 3.0 kg of a mixture of soil (Paredão Vermelho series, a Typic Hapludox) and washed sand (1:1 mixture). This soil:sand mixture was autoclaved (120°C) for 2 h, and dolomitic lime (44% CaO and 25% MgO) was added at a rate of 1.13 g per kg of substrate based on the method of Raji (1991), where 70% base saturation is reached following liming. The chemical composition of the substrate following autoclaving and liming was pH 6.0, 1.4% organic matter, 0.2 mmol_c P dm⁻³, 0.5 mmol_c K dm⁻³, 1.4 mmol_c Ca dm⁻³, 1.0 mmol_c Mg dm⁻³ and 47.0 mmol_c H and Al dm⁻³ soil.

Phosphorus fertilizer was added as "simple superphosphate" (18.5% P₂O₅, 26% CaO, 11.6% S) at four different rates at the beginning of the experiment. To the 3.0 kg of substrate in each pot, either no P fertilizer was added (0.0 mg kg⁻¹), 0.90 g of P fertilizer was added (25 mg kg⁻¹ of P), 1.82 g of P fertilizer was added (50 mg kg⁻¹), or 2.73 g of P fertilizer was added (75 mg kg⁻¹). A total of exactly 24 pots received each of the various fertilizer additions.

Additional fertilizer was applied to soil in all the treatments based on Malavolta (1980) and Freire et al. (1980). Fertilizer addition, per kg of substrate, amounted to: 80 mg N (in the form of NH₄NO₃), 150 mg K (as K₂SO₄), 2.0 mg Zn (as ZnSO₄ 7H₂O), 0.5 mg B (as H₃BO₃) and 0.1 mg Mo (as H₂MoO₄). Nitrogen was applied four times, 20 mg each time for a total of 80 mg, at planting, and then 26, 46 and 66 days after planting (DAP).

After each harvest, shoots were collected, dried to a constant weight at 70°C, and the nutrient content was determined (Sarruge & Haag, 1974). Roots were collected for determination of mycorrhizal colonization (Phillips & Hayman, 1970; Giovanetti & Mosse, 1980). Growth differences for any characteristic between mycorrhizal and non-mycorrhizal plants at the same rate of P addition was calculated according to the following formula:

Percent difference = [(mycorrhizal plant - non-mycorrhizal plant)/non-mycorrhizal plant] 100%, according to the usage of Bethlenfalvay et al. (1982). Subsamples of the rhizosphere soil (10.0 g) were collected to extract and measure the total extramatrical mycelium (TEM) and active extramatrical mycelium (AEM) of the soil fungi by methods (Melloni & Cardoso, 1999) that were modified from those of Sylvia (1988) and Schubert et al. (1987).

An analysis of variance was performed on the data (SAS methods) followed by regression analysis using either the increasing levels of added P or harvest times as independent variables. Statistically significant separations (P < 0.05) between means were determined by using the confidence intervals for the generated regression curves. Correlations between variables were also performed according to regression analysis, and the correlation coefficient and the significance levels were stated for any of the derived correlations.

RESULTS AND DISCUSSION

Shoot dry weight

At the first harvest (30 days), there were no statistically significant (P < 0.05) differences between the shoot dry weights for maize plants, either for those P-fertilized non-inoculated plants

or for those plants colonized by *G. etunicatum* (Figure 1). However, by the second harvest (60 days), mycorrhizal maize (+G plants) at the lowest levels of added P were significantly larger than the -G plants at the same P level, whereas at 50 and 75 mg kg⁻¹ added P, no significant differences existed between plants with or without the fungal endophyte (Figure 1). At the rate of 0 and 25 mg kg⁻¹, these growth differences between +G and -G plants amounted to 205 and 78%, respectively.

At both 60 and 90 days, maize fertilized with different P levels had shoot dry weights that were statistically significantly different from one another, whether these plants were +G plants or -G plants (Figure 1). At the third harvest, the growth differences between plants with and without *Glomus* at the 0 and 25 mg kg⁻¹ P rate of P application was equivalent to 212 and 60%, respectively.

Such growth differences between -G and +G plants at the lowest, but not at the highest levels of P addition indicated that the positive, beneficial effects due to the fungal endophyte occurred when P was limiting, but not when P was abundant. Such results with fungal inoculation have been commonplace in the literature (Antunes & Cardoso, 1991). Relative (Bethlenfalvay et al., 1982) and absolute (Cardoso et al., 1986) growth depressions in AM-inoculated plants at very high rates of P fertilization have been observed before, but not at the low P levels used in this study.

Colonized root length

Intramaterial colonization by *G. etunicatum* was only slightly affected by the rates of P addition at either 30, 60 or 90 DAP (Figure 2). At the highest level of fertilizer addition (75 mg kg⁻¹), mycorrhizal colonization was reduced about 18%, the common situation with AM fungal colonization (Graham et al., 1981). However, % colonization by the endophyte was statistically significant between the various harvests. On the average, % root length colonized by *G. etunicatum* was 24% at 30 DAP, 45% at 60 DAP and 55% at 90 DAP (Figure 2), indicating that the fungus continued to spread as the root grew and developed, a fact in line with the observations of Sanders & Sheik (1983), who measured the spread of secondary infections in AM roots as new root growth occurred.

The larger relative amounts of shoot dry weight in +G plants compared to -G maize at the lowest levels of soil available P during 60 and 90 DAP (Figure 1), indicated that the endophyte continued to be beneficial to the host plant even as fungal colonization (Figure 2) and biomass (Figure 3) increased. These increases in fungal colonization and biomass did not adversely affect mycorrhizal corn plants even at the highest P level or the final harvest (Figure 1), indicating P was not in excess (Cardoso et al., 1986).

Total extramatrical mycelium

At the first harvest, TEM increased for inoculated soils, and decreased for non-inoculated soils (Figure 3), with the mean TEM values at the 0 and 75 mg kg⁻¹ levels displaying significant differences (58% increase for +G soil and 65% decrease for -G soil). Although the soil was previously sterilized by autoclaving, the presence of soil fungi in soils not inoculated with *G. etunicatum* was because plants were kept in a greenhouse under non-aseptic conditions. Fungal spores carried to the soil by water and air quickly reestablished fungal growth in all pots.

The effect of inoculation with *G. etunicatum* was considered to be the difference in TEM between the +G and -G soils, although it is unlikely to be a simple

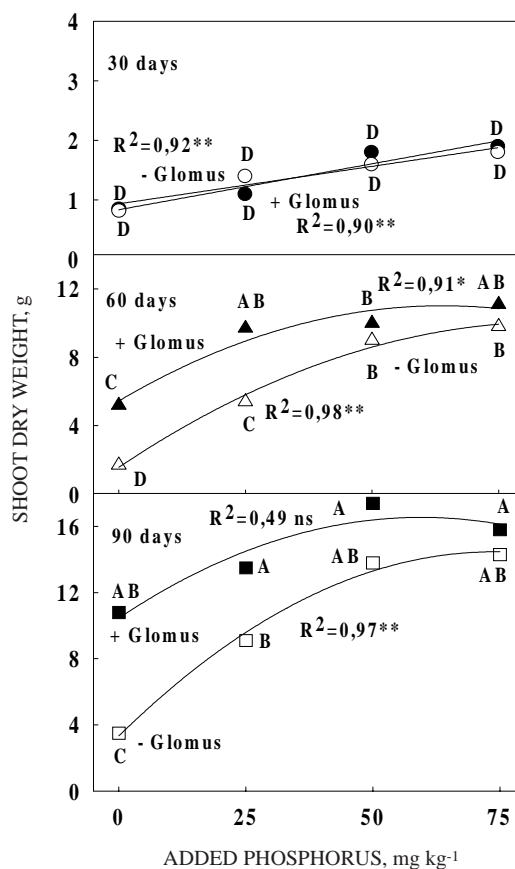


Figure 1. Shoot dry weight for maize (*Zea mays* L. var. Piranão) plants grown in a sterilized soil either left non-inoculated (○) or inoculated with the arbuscular mycorrhizal fungus *Glomus etunicatum* (●). The plants were fertilized with either 0, 25, 50 or 75 mg kg⁻¹ of P (in the form of simple superphosphate) and were harvested at either 30, 60 or 90 days post-emergence. * or ** stand for significance at the P < 0.05 and P < 0.01 levels of probability; ns stands for not significant.

subtraction between the two treatments, due to the influence of the fungus in the mycorrhizosphere (Ames et al., 1984). However, a simple subtraction as a first approximation of this effect was performed and a function for the increase of TEM due to *Glomus* alone was derived. One wonders whether this decline in TEM for general fungi in the -G soil occurred with a concomitant increase in microbial biomass or a decline in the soil biomass occurred in -G soils.

At 60 DAP, TEM increased in +G soils (Figure 3) significantly more than in +G soils at 30 days, and the differences in TEM between the two harvests were statistically significant at every rate of P application. The decrease in TEM at 60 DAP in -G soils was indistinguishable from the decline in TEM in non-inoculated soils at 30 DAP. At the second harvest, increase in TEM due to *G. etunicatum* alone was over 3-fold from 0 to 75 mg kg⁻¹.

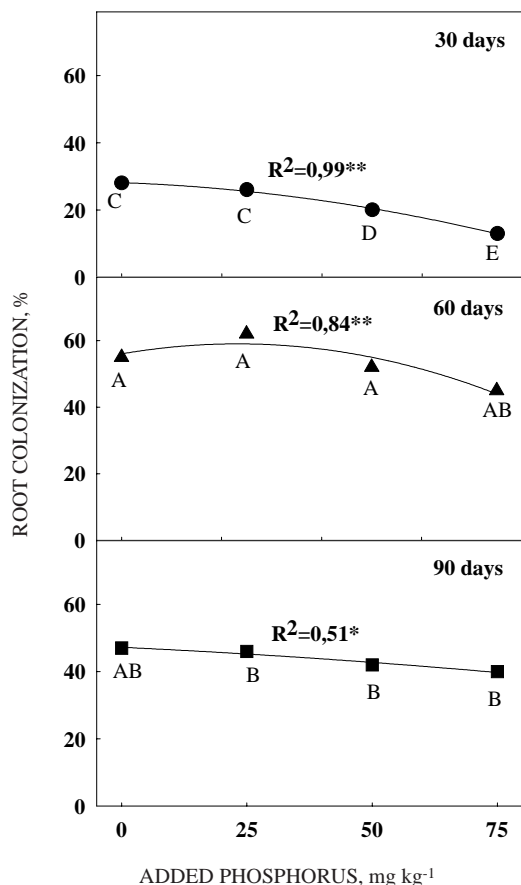


Figure 2. Percent root colonization (% colonization) of corn roots by the arbuscular mycorrhizal fungus *Glomus etunicatum*. Plants were fertilized with either 0, 25, 50 or 75 mg kg⁻¹ of P and were harvested at either 30, 60 or 90 days post-emergence. * or ** stand for significance at the P < 0.05 and P < 0.01 levels of probability; ns stands for not significant.

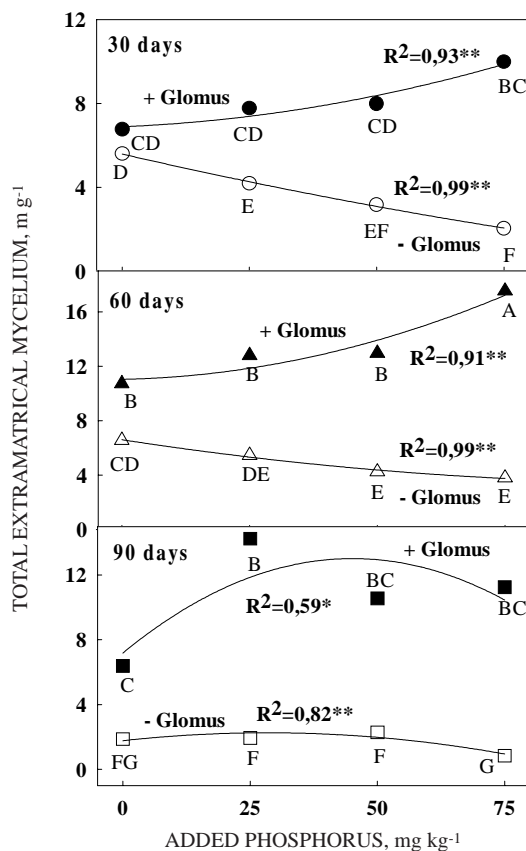


Figure 3. Total extramatrical mycelium (TEM, in m g⁻¹) for soils which supported maize (*Zea mays* L. var. Piranão) plants that were either left non-inoculated (○) or were inoculated with the arbuscular mycorrhizal fungus *Glomus etunicatum* (●). Plants were fertilized with either 0, 25, 50 or 75 mg kg⁻¹ of P (in the form of simple superphosphate) and were harvested at either 30, 60 or 90 days post-emergence. * or ** stand for significance at the P < 0.05 and P < 0.01 levels of probability; ns stands for not significant.

Control soil TEM was less (50 to 70%) at 90 DAP, than at the first two harvests (Figure 3). For the +G soil, TEM began to taper off at the highest levels of P, which likely indicated that, at this time, there was a decline in the extramatrical growth of the mycorrhizal endophyte relative to the harvests at one and two months. The same situation was noted previously by other researchers (Schubert et al., 1987; Sylvia, 1988; Hamel et al., 1990). Values for TEM between +G and -G soils were significantly different at all levels of added P. Such a continuing decline in total fungal hyphae, noted at 30 days, suggests that this may be due to a general decline in total soil biomass.

At the last harvest, TEM due to *G. etunicatum* alone more than doubled with P application, but only at the lowest P level was the value for TEM

significantly different from the other treatment means. Although fungal hyphae are rather abundant in soil, there are no reliable estimates on the proportion of fungal hyphae that is mycorrhizal. Estimates that do exist for total soil hyphae range from less than one to over 26 m g⁻¹ of soil (Abbott et al., 1984; Schubert et al., 1987; Sylvia, 1988). These values are similar to those reported here, ranging from 1 to 6 m g⁻¹ for -G and from 2 to 18 m g⁻¹ for +G soils. Obviously, natural soils will contain fungal populations more diverse and variable (Crush, 1976), and perhaps at higher densities, than those found in this greenhouse study using a sterilized soil. However, our results here would suggest that, in a previously fumigated AM-inoculated soil, at least 35 and up to 70% of all fungal hyphae will arise from the inoculated AM fungus. Therefore, it must be emphasized that a large fraction of TEM found in soil represents hyphae from *G. etunicatum*.

These values could be improved by excluding hyphae with external diameters of less than 5 µm (Bethlenfalvay & Ames, 1987). These estimates are actually quite conservative when compared to the work of Kabir et al. (1996), who reported that up to 83% of all fungal hyphae in soil under corn or barley was from AM fungi. It is likely that our estimates of AM fungal biomass represent an overestimation since the complex fungal populations of the soil could not be taken into account (Ahmadsad, 1984). In the future, a more definitive measure of AM external hyphae will be needed (Kabir et al., 1996) to determine the extramatrical mycelium and P inflow rates for AM plants, especially for work performed in agricultural settings or in native ecosystems.

Here we have noted first a rapid increase in *G. etunicatum* internal mycelium (Figure 2), followed by a relatively slower increase in TEM (Figure 3), which confirms the work of Hamel et al. (1990). Increasing P addition rates can dramatically affect this growth pattern, and this may be tied to the growth depressions seen at the higher rates of P addition. Certainly, the carbon cost of the fungus relative to the P acquired by the host is an important parameter in this balance (Douds et al., 1988; Jakobsen & Rosendahl, 1990), but it is not likely the only factor involved in this growth depression. Although it has been reported that high P inhibits AM fungal proliferation (Miranda et al., 1989), our results here indicate that total soil fungi were inhibited as P increased under the conditions of our experiment, but early in the symbiosis (30 and 60 days) AM hyphae proliferated as available P increased. At 90 days, AM fungal proliferation was inhibited by high available P suggesting that single harvest experiments must be interpreted with great care. Similarly, suggestions that the P effect is a salt effect (Nagahashi et al., 1996) must be reconsidered since the inhibition of *G. etunicatum* hyphal spread did not occur during the first and second month at 50 and 75 mg kg⁻¹ of P.

Active extramatrical mycelium

At the first harvest, active extramatrical mycelium (AEM) in the non-inoculated soil started below 1 m g⁻¹ soil at 0 mg kg⁻¹ of added P and then declined to approximately 0 m g⁻¹ at 75 mg kg⁻¹ of fertilizer P amendment (Figure 4). For the +G soil, AEM was high at the lowest soil available P level, and AEM increased slightly as P increased. We continue to estimate, as for TEM, that the difference in the level of AEM between the +G and -G soils was due solely to *G. etunicatum*. At 30 DAP, approximately all of the AEM was due to *G. etunicatum*.

After 60 days, AEM in the -G soil maintained itself at ca. 1.0 m g⁻¹ at all P levels (Figure 4). The values for AEM from +G soils were statistically different from those of non-inoculated soils at all rates of P addition (Figure 4). Besides, AEM values for the +G soils were different at 50 and 75 mg kg⁻¹ levels of added P and were also different from the AEM values at the lower levels of P amendment.

At 90 DAP, AEM for the non-inoculated soil declined slightly, from 0.7 to 0.3 m g⁻¹, but the values for AEM were not statistically significantly different from one another at any level of P addition (Figure 4). As the P amendment increased from 0 to 25 mg kg⁻¹, values for AEM in the soil inoculated with *G. etunicatum* increased. However, as added P continued to increase from 25 to 75 mg kg⁻¹, there was a general decline in AEM. AEM values for +G soils were statistically different from the AEM levels found in -G soils at all levels of fertilizer P input. Except for the 50 and 75 mg kg⁻¹ level of P addition, all AEM values were statistically different from one another in the soil inoculated with the AM fungus. The AEM attributable to *G. etunicatum* showed a slight rise followed by a general decline in a pattern similar to the AEM for inoculated soils.

The degree of viable, metabolically active extramatrical mycelium is a physiologically more important characteristic than is total extramatrical mycelium since it is the active mycelium that will influence nutrient uptake and translocation in the plant root (Schubert et al., 1987).

Non-viable hyphae may influence bacterial populations in the soil (Ames et al., 1984) and mycorrhizosphere (Sylvia, 1988); therefore, certain indirect effects, such as soil aggregation may not necessarily require living hyphae (Bethlenfalvay & Ames, 1987). In this sense, TEM mycelium may play other important roles in the soil (Ahmadsad, 1984), even after it became no longer physiologically important to the AM fungus.

Percent AEM/TEM

The proportion of active extramatrical mycelium compared to total extramatrical mycelium (that is, % AEM/TEM) could be used as a measure of the metabolic competence of the AM fungus. At 30 days after planting, 98% of the total mycelium was active at the P level of 0 mg kg⁻¹ and from there, it declined

to 36% AEM/TEM at 75 mg kg⁻¹ P amendment (Figure 5). Mean values for % AEM/TEM were statistically significantly different from one another at all levels of added P at all three harvests. At 60 DAP, values for the % AEM/TEM were not significantly different from the levels of % AEM/TEM at 30 days, i.e., the general increases seen in TEM (Figure 3) and AEM (Figure 4) at the second harvest were more or less in balance. At 90 DAP, where declines in TEM (Figure 3) and in AEM (Figure 4) have already been noticed, declines in AEM exceeded those for TEM, and the values for % AEM/TEM were statistically different from those values at 30 and 60 days at all levels of P amendment, except at 0 mg kg⁻¹ (Figure 5). Values for % AEM/TEM at 0 mg kg⁻¹ were between 97 and 99% indicating that metabolic activity in the fungus was maintained at

low levels of P. Under these conditions, P acquisition demanded participation of both partners in the host-endophyte association. At this last harvest, % AEM/TEM fell to only 10% at 75 mg kg⁻¹ added P, the lowest level recorded, suggesting that, when P was plentiful, fungal metabolism in the soil was limited. This result is in agreement with Abbott et al. (1984), Miranda & Harris (1994) and Nagahashi et al. (1996).

There was a general decline in the proportion of AEM compared to TEM in the soil as P increased or as the host-endophyte association aged (Figure 5). Schubert et al. (1987) and Hamel et al. (1990) also found that AEM declined with time. As shown here, the process of hyphal decline was also very sensitive to P. Therefore, the initial levels of P in the soil, the ability of the soil to sorb P, as well as the placement, form, and solubility of P amendments will have an

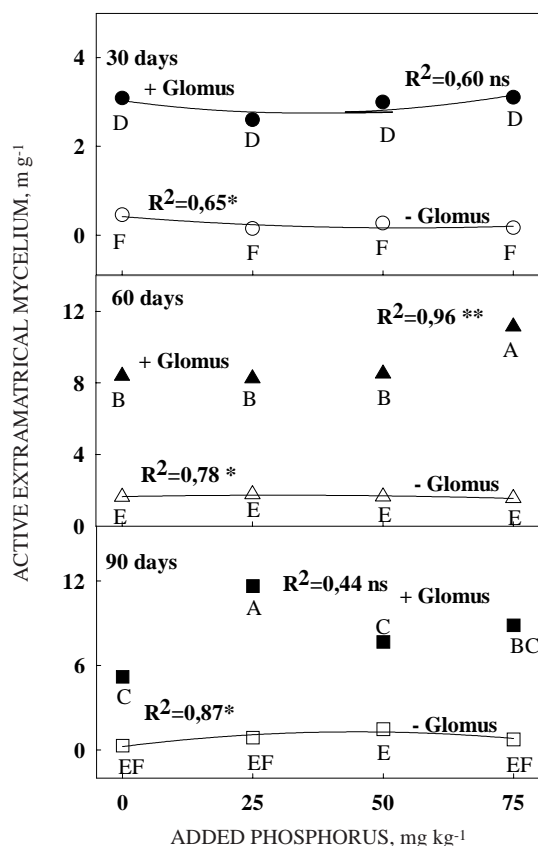


Figure 4. Active extramatrical mycelium (AEM, in m g⁻¹) for soils which supported maize (*Zea mays* L. var. Piranão) plants that were either left non-inoculated (○) or were inoculated with the arbuscular mycorrhizal fungus *Glomus etunicatum* (●). Plants were fertilized with either 0, 25, 50 or 75 mg kg⁻¹ of P (in the form of simple superphosphate) and were harvested at either 30, 60 or 90 days post-emergence. * or ** stand for significance at the P < 0.05 and P < 0.01 levels of probability; ns stands for not significant.

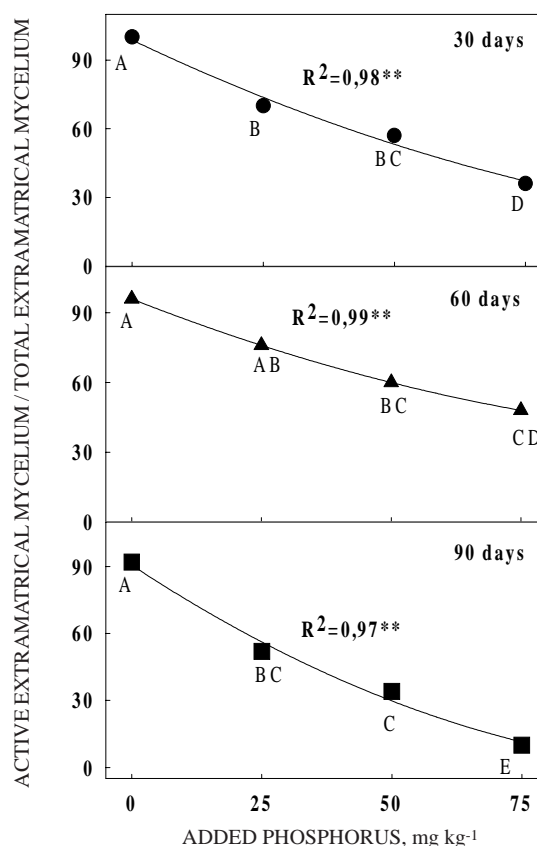


Figure 5. The percentage of active extramatrical mycelium relative to total extramatrical mycelium (% AEM/TEM) for the arbuscular mycorrhizal fungus *G. etunicatum*. Plants supporting *G. etunicatum* were fertilized with either 0, 25, 50 or 75 mg kg⁻¹ of P (in the form of simple superphosphate) and were harvested at either 30, 60 or 90 days post-emergence. * or ** stand for significance at the P < 0.05 and P < 0.01 levels of probability; ns stands for not significant.

effect on the growth and viability of AM hyphae in the soil.

The soil used in this experiment is a natural soil from an agricultural area, and therefore it will reflect the processes of P sorption and the natural equilibrium between P in the soil solution and P release by the fertilizer added. However, this soil was sterilized prior to use, and so the results concerning the proportion of total hyphae that came from the inoculated arbuscular mycorrhizal fungus should not be generalized to field or natural conditions. Additional approaches to specifically label the AM mycelium under natural conditions to quantify the proportion of AM hyphae in a native, non-fumigated or non-sterilized soil are underway in this laboratory.

CONCLUSIONS

1. The modified methods for the extraction, visualization and quantification of both total and active extramatrical mycelium, based on the work by Melloni & Cardoso (1999), proved to be an improvement over the original methods. Not only were the modifications easier to perform in the laboratory, but the results were more uniform between replicates of a given treatment.

2. The alterations in the proportion of total or active mycelium with changes in the P status of the soil indicated that *G. etunicatum* made a large contribution to fungal soil biomass. While total extramatrical mycelium in +G stayed constant at all P levels at 30 DAP, at 60 DAP there was an increase, and at 90 DAP a decrease at higher P levels. The ratio of active extramatrical mycelium to total extramatrical mycelium, however, decreased steeply with time and with increasing P levels. Therefore, this proportion may allow for an evaluation of the effectiveness of an AM fungus for a given host plant.

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