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EFFECT OF EUCALYPTUS BARK COMPOST AND PHOSPHORUS ON THE ECTOMYCORRHIZAL DEVELOPMENT OF *Eucalyptus* PLANTLETS INOCULATED *in vitro* WITH *Pisolithus tinctorius*.

Efeito da matéria orgânica e do fósforo no desenvolvimento de ectomicorrizas em "plantlets" de Eucalyptus inoculadas "in vitro" com Pisolithus tinctorius

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RESUMO: "Plantlets" de *Eucalyptus urophylla* e de um híbrido de *E. urophylla x E. grandis* foram inoculadas *in vitro* com discos de agar com micélio de *Pisolithus tinctorius* em substrato de tecido não tramado (Coursel) umidecido com meio de enraizamento. Após 28 dias de inoculação em câmara de crescimento, 70 e 76% dos tubos inoculados apresentaram colonização ectomicorrízica *in vitro*, respectivamente para cada clone. Testes de crescimento micelial de *P. tinctorius in vitro* permitiram reduzir a concentração de açúcares no meio de cultura de 28g/l para até 4g/l sem reduzir significativamente o desenvolvimento micelial. Também não houve diferença entre a utilização de glucose ou de sacarose como fonte de carbono para o fungo. Dois experimentos foram conduzidos com outro clone (híbrido de *E. urophylla x E. grandis*) inoculado in vitro em meio líquido com 4g de sacarose/l como fonte de carbono em substrato de feltro. Após 28 dias de incubação em câmara de crescimento, 69,6 e 93,9% dos "plantlets" apresentaram formação de ectomicorrizas respectivamente em cada experimento. "Plantlets" micorrizados do segundo experimento foram transferidos para casa de vegetação com temperatura e umidade controladas, em tubetes contendo solo arenoso misturado com 4 doses de composto orgânico na forma de casca de eucalipto (0; 20; 40 e 60% V/V), combinadas com 4 níveis de fósforo aplicados (0); 30; 60 e 90 ppm). O transplante em casa de vegetação permitiu 100% de pegamento das "plantlets". A avaliação foi aos noventa dias após a transferência dos tubetes para as condições de viveiro. Nos níveis de 0 e 30 ppm de fósforo aplicado, a adição de composto orgânico aumentou o crescimento das mudas. A matéria orgânica reduziu drasticamente o desenvolvimento ectomicorrízico no nível de 30 ppm de fósforo. Nos níveis de 60 e 90 ppm não houve efeito da adição de composto orgânico. O desenvolvimento ectomicorrízico no geral foi baixo. Na ausência de composto orgânico, houve maior desenvolvimento de ectomicorriza com 30 ppm de fósforo aplicado e maior crescimento das mudas com 60 ppm de fósforo aplicado. Na presença de composto orgânico não houve efeito do fósforo aplicado, tanto no crescimento das mudas como no desenvolvimento micorrízico.

PALAVRAS-CHAVE: *Eucalyptus*, Micorriza, Viveiro, Fósforo, Matéria Orgânica.

SUMMARY: Plantlets of an *Eucalyptus urophylla x E.grandis* hybrid that were inoculated *in vitro* with *Pisolithus tinctorius* were transplanted to polyethylene tubes containing a

sandy soil to which 4 doses of *Eucalyptus* bark compost were added (0%; 20%; 40% and 60% V/V), combined with 4 levels of applied phosphorus (0; 30; 60 and 90 ppm). After a 90 days growth period under nursery conditions, the addition of organic matter reduced the ectomycorrhizal development. Ectomycorrhizal development, however, was in general low. At the 0 and 30 ppm of applied phosphorus levels, the addition of organic matter increased plantlet growth significantly, whereas no effect was observed of organic matter at 60 and 90 ppm levels of phosphorous. In the absence of organic matter, the best mycorrhizal development was observed at the 30 ppm level of applied phosphorus, whereas the highest plant growth was observed at the 60 ppm level of phosphorus. In the presence of applied organic matter, application of phosphorous did not affect either plantlet growth or ectomycorrhizal development.

KEY WORDS: *Eucalyptus*, Mycorrhiza, Forest nursery, Phosphorus, Organic Matter.

INTRODUCTION

Research on *in vitro* inoculation with ectomycorrhizal fungi has demonstrated that *Eucalyptus* has great capability to form ectomycorrhizae under axenic conditions (Malajczuck & Hartney, 1986; Poissonier, 1990; Tonkin *et al.*, 1989; Vida *et al.*, 1991). However, this phenomenon is not expressed in the post-transplant phase, when a significant reduction of the mycorrhizal formation or even its disappearance occurs (Vida *et al.*,1991).

The residual sugars and other organic compounds added to the rooting medium may affect the association after nursery transplant through their effect on the rhizosphere microbiota. Furthermore, physical and chemical factors in the nursery substratum may condition the mycorrhizal development after plantlet transplant to the nursery, such as type and levels of organic matter (Bettiol & Krügner, 1986a ; Bettiol & Krügner, 1986b; Reddel & Malajczuck, 1984) and the amount of phosphorus (Soares *et al.*,1990; Vieira & Peres, 1988a; Vieira & Peres, 1988b).

The objective of this paper was to evaluate the effects of *Eucalyptus* bark compost and phosphorus levels on mycorrhizal development under nursery conditions.

MATERIAL AND METHODS

Plantlets of an *E.urophylla* x *E. grandis* hybrid were produced based on the methodology developed by Gonçalves (1975) for *Eucalyptus* micropropagation. A felt substratum and a 4g sucrose/l liquid medium were used. Inoculation with *Pisolithus tinctorius* (Pers) Coker & Couch (isolate ESALQ 1603) was made with two agar disks (5mm in diameter) when rooting began. After 4 weeks, plantlets with 80% to 100% mycorrhization were selected to set up the experiments with organic matter and phosphorus levels applied to the nursery substrate to which they are transplanted. Polyethylene tubes were used as containers. The basic fertilization employed in the experiment constituted of 113 ppm of K in the potassium chloride form and 100 ppm of N in the form of ammonium nitrate.

Four levels of phosphorus were tested (0; 30; 60; and 90 ppm), added in the mo-

Table 1

Chemical analysis of the soil used in the study, at the different levels of phosphorus (P) and organic compost (O.C.) applied

Análise química do solo usado no experimento, em diferentes níveis de fósforo (P) e composto orgânico (C.O) aplicados.

TREATMENT				**meq/100 cm³**				
ppm P	% O.C.	pH	P (ppm)	K	Ca	Mg	H + Al	C.E.C.
0	**0**	4.3	2	0.30	0.0	0.1	2.0	2.4
0	**20**	5.9	6	0.54	5.1	1.7	2.2	9.7
0	**40**	6.1	12	0.72	9.3	2.9	2.2	15.2
0	**60**	6.2	23	0.84	14.2	4.1	2.5	21.6
30	**0**	4.5	15	0.34	0.0	0.1	2.0	2.4
30	**20**	6.0	26	0.40	6.2	1.8	2.2	10.6
30	**40**	6.1	38	0.54	8.2	2.5	2.2	13.4
30	**60**	6.2	45	0.66	12.6	3.3	2.5	19.1
60	**0**	4.5	36	0.30	0.0	0.0	2.0	2.3
60	**20**	5.9	49	0.38	5.5	1.6	2.2	9.7
60	**40**	6.1	65	0.45	8.5	2.3	2.5	13.8
60	**60**	6.2	81	0.69	14.7	3.9	2.5	21.8
90	**0**	4.5	55	0.27	0.0	0.1	2.0	2.3
90	**20**	5.9	88	0.34	6.2	1.7	2.2	10.4
90	**40**	6.1	121	0.56	8.7	2.4	2.2	13.9
90	**60**	6.2	123	0.54	11.6	3.3	2.5	17.9
0	**100**	6.5	76	0.96	42.2	7.0	2.5	52.7

nobasic potassium phosphate (KH_2PO_4) form, combined with four doses of Eucalyptus bark compost (0; 20; 40; and 60% V/V).

Based on the soil analysis results (Table 1) a correction was made for calcium and magnesium levels in treatments without *Eucalyptus* bark compost, since these elements were practically absent in the soil. A 4 : 1 calcium sulfate:magnesium sulfate was added to equate these element levels in all treatments.

The experiment constituted of a factorial with 16 treatments (4 x 4) in a completely randomized experimental design, replicated 10 times, each plantlet consisting of an experimental plot.

After transplanted, the plantlets were kept in the greenhouse at 90% relative humidity and 26°C for 7 days. After this period, the plantlets were kept another 7 days under a screen roofing, under 50% shade. The plantlets were thereafter placed directly in the sun.

After a period of 30 days, N applications were done in 10 day intervals, using a 25 ppm ammonium nitrate dose per tube with syringe. Results were evaluated after 90 days. Besides the mycorrhizal percentage observed visuallly, the following plantlet growth parameters were also considered: root collar diameter, height and shoot dry

matter weight. Microtome root sections 20 *u*m thick were also observed under light microscope with 50 observations per treatment, for observation of mantle and Hartig net formation.

RESULTS AND DISCUSSION

Ectomycorrhizal development after 90 days under nursery conditions

The substrate compost of eucalyptus bark compost and soil allowed for mycorrhyzal development in several treatments (Table 2; Figure 1). Phosphorus and organic matter addition to the soil had significant effects on mycorrhyzal development.

Organic matter addition reduced ectomycorrhizal formation but improved seedling growth. The largest amount of ectomycorrhizae observed was at 30 and 60 ppm of phosphorus. This interval of variation is in agreement with results found by other researchers. Soares *et al.* (1990), studying the effect of phosphorus levels on ectomycorrhizal formation with *Eucalyptus grandis x Pisolithus tinctorius* in a dark red latosol, observed that mycorrhizal formation was inhibited at 75 ppm of phosphorus. Vieira & Peres (1988a) conducted an experiment in a greenhouse with *E. grandis x P. tinctorius* and found that the highest infection rate was at the 47 ppm level of phosphorus, and that the symbiotic effect only occurred at the 23 ppm level of phosphorus.

Table 2

Percentage of mycorrhizal root sections , 90 days after plantlets being transplanted to tubes under nursery conditions for different levels of Eucalyptus bark compost and phosphorus applied.*

Porcentagem de secções de raízes micorrizadas*, 90 dias após o transplante das "plantlets" para tubetes sob condições de viveiro, para diferentes níveis de composto de casca de eucalipto e fósforo aplicados.

Phosphorus levels (ppm)	Organic compost (%V/V) 0	20	40	60	Means
0	22	20	6	0	12.0
30	72	70	20	0	40.5
60	34	24	6	0	16.0
90	22	4	0	0	6.5
Means	37.5	29.5	8.0	0.0	

* Based on observations of 50 microtome sections per treatment

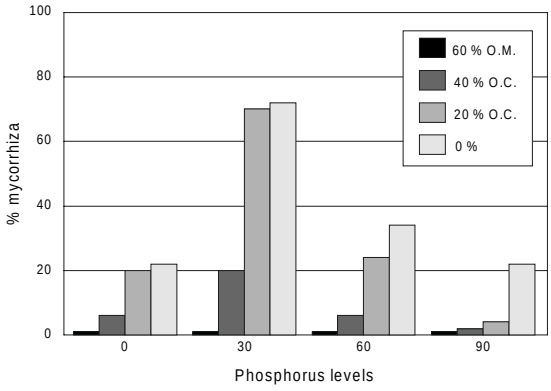

Figure 1

Percentage of mycorrhiza infection visually estimed, for different levels of Eucalyptus bark compost (O.C.) and phosphorus applied.

Porcentagem de infecção micorrízica visualmente estimada, para diferentes níveis de composto de casca de eucalipto e fósforo aplicados

Table 3

Analysis of variance for percentage of mycorrhyzal root sections, 90 days after plantlets being transplanted to tubes under nursery conditions for different levels of Eucalyptus bark compost and phosphorus applied.

Análise de variância para porcentagem de secções de raízes micorrizadas, 90 dias após o transplante das "plantlets" para tubetes sob condições de viveiro, para diferentes níveis de composto.

SOURCE of VARIATION	D.F.	Sum of Square	Mean Square	F	Prob. > F
Organic compost (O.C.)	3	83.93031	27.97677	22.07	0.00001
Phosphorus (P)	3	14.11951	4.70651	3.71	0.01303
O.C. x P	9	50.38071	5.59785	4.42	0.00012
Error	144	182.53067	1.26757		
Total	159	330.96121			

Variation coefficient = 68.9 %

Plant development in the nursery

Organic matter and phosphorus doses which promoted the best plant development were not the same which allowed for the best mycorrhizal development. Plant growth increased with the addition of organic matter and phosphorus to the soil. The greatest growth was with 60% organic matter and phosphorus applied levels at 30 and 60 ppm (Tables 4 to 9).

The optimal level for mycorrhizal association was below the optimal level for seedling growth. Several authors (Malajczuk *et al.*,1975; Poissonier, 1986) suggest that the beneficial effect of inoculating mycorrhizal fungi must manifest itself under field conditions, in adverse locations, even if during their short period in the nursery they present equal or smaller sizes than seedlings without mycorrhizae in more fertile substrates.

Table 4

Plantlet height (cm) , after 90 days under nursery conditions, for different levels of Eucalyptus bark compost and phosphorus applied.*

Altura das "plantlets" (cm)*, após 90 dias sob condições de viveiro, para diferentes níveis de composto de casca de eucalipto e fósforo aplicados.

Phosphorus levels (ppm)	Organic compost (%V/V) 0	20	40	60	Means*
0	13.3	18.8	21.3	22.0	18.9
30	20.0	21.6	23.3	25.6	22.6
60	23.2	24.8	24.8	25.0	24.5
90	22.0	22.0	22.6	23.0	22.4
Means	19.6	21.8	23.0	23.9	

* Means of 10 replicates per treatment.

Table 5

Analysis of variance for plantlet height, after 90 days in tubes under nursery conditions, at different levels of Eucalyptus *bark compost and phosphorus applied.*

Análise da variância para altura das "plantlets", após 90 dias sob condições de viveiro, para diferentes níveis de composto de casca de eucalipto e fósforo aplicados.

SOURCE of VARIATION	D.F.	Sum of Square	Mean Square	F	Prob. > F
Organic compost (O.C.)	3	411.48112	137.16041	21.10	0.00001
Phosphorus (P)	3	654.20625	218.06875	35.55	0.00001
O.C. x P	9	258.35625	28.70625	4.42	0.00012
Error	144	936.05000	6.50035		
Total	159	2260.09375			

Variation coefficient = 11.5%

Table 6

Plantlet root collar diameters (mm), 90 days after transplant to tubes under nursery conditions at different levels of Eucalyptus bark and phosphorus applied.*

Diâmetro do colo das mudas (mm)*, 90 dias após o transplante para tubetes sob condições de viveiro para diferentes níveis de composto de casca de eucalipto e fósforo aplicados.

Phosphorus levels (ppm)	Organic compost (%V/V) 0	20	40	60	Means*
0	2.4	2.8	3.2	3.2	2.92
30	2.8	3.0	3.1	3.0	3.04
60	3.0	3.3	3.3	3.4	3.23
Means	2.18	3.14	3.19	3.25	

* Means of 10 replicates per treatment.

Table 7

Analysis of variance for plantlet root collar diameters, after 90 days in tubes under nursery conditions, at different levels of Eucalyptus *bark and phosphorus applied.*

Análise de variância para diâmetro do colo, após 90 dias em tubetes sob condições de viveiro, para diferentes níveis de composto de casca de eucalipto e fósforo aplicados.

SOURCE of VARIATION	D.F.	Sum of Square	Mean Square	F	Prob. > F
Organic compost (O.C.)	3	4.70815	1.56938	23.45	0.00001
Phosphorus (P)	3	2.44715	0.81572	12.19	0.00001
O.C. x P	9	1.27959	0.14217	2.13	0.03011
Error	144	9.63504	0.06691		
Total	159	18.06994			

Variation coefficient = 8.3%

Table 8

Plantlet shoot dry matter weight (g), after 90 days in tubes under nursery conditions, at different levels of* Eucalyptus *bark compost and phosphorus applied.*

Peso seco das "plantlets" (g)*, após 90 dias em tubetes sob condições de viveiro, para diferentes níveis de composto de casca de eucalipto e fósforo aplicados.

Phosphorus levels (ppm)	Organic compost (%V/V) 0	20	40	60	Means*
0	0.81	1.32	1.62	1.93	1.42
30	1.03	1.51	1.74	1.97	1.56
60	1.07	1.62	1.70	1.88	1.57
90	0.87	1.95	1.78	2.14	1.68
Means	0.95	1.60	1.71	1.98	

* Means of 10 replicates per treatment.

Table 9

Analysis of variance for plantlet dry matter weight, after 90 days in tubes under nursery conditions, at different levels of Eucalyptus *bark compost and phosphorus applied.*

Análise de variância para peso seco, após 90 dias em tubetes sob condições de viveiro, para diferentes níveis de composto de casca de eucalipto e fósforo aplicados.

SOURCE of VARIATION	D.F.	Sum of Square	Mean Square	F	Prob. > F
Organic compost (O.C.)	3	13.84886	4.61629	43.65	0.00001
Phosphorus (P)	3	0.82811	0.27604	2.61	0.05607
O.C. x P	9	1.00353	0.11150	1.05	0.40566
Error	80	8.46049	0.10575		
Total	95	24.14101			

Variation coefficient = 20.8%

CONCLUSIONS

The results obtained in this study allow the following conclusions:

1) Adding composted *Eucalyptus* bark reduced ectomycorrhizal formation, totally inhibiting the association when added at 40% or more;

2) The optimal phosphorus level applied to the soil for mycorrhizal formation lies between 30 and 60 ppm whereas the 0 and 90 ppm levels limited ectomycorrhizal development.

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