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Impact of sulfentrazone, isoxaflutole and oxyfluorfen on the microorganisms of two forest soils

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Abstract

Questions regarding the application of pre-emergence herbicides for control weeds in eucalyptus are noteworthy due to the impact of this practice on the environment, particularly on soil microbial activity and beneficial microorganisms such as mycorrhizal fungi and phosphate solubilizing microorganisms. The aim of this study was to evaluate the impact of applying herbicides sulfentrazone, isoxaflutole and oxyfluorfen on mycorrhizal colonization, microbial biomass and microbial activity of two forest soils cultivated with eucalyptus. The experiment was conducted in a greenhouse using two soils (clayey and sandy loam) distributed in 12 dm^3 pots. The 4x3 factorial design was used, with three herbicides (sulfentrazone, oxyfluorfen, isoxaflutole) over the untreated control and three assessments (5, 20 and 70 days after application (DAA)), in a completely randomized design with four replications. After herbicide application, seedlings of the hybrid ***Eucalyptus grandis*** x ***Eucalyptus urophylla*** were transplanted. We evaluated the microbial biomass, respiration rate, metabolic quotient and the potential of solubilization of inorganic phosphate at 5, 20 and 70 DAA. At 70 DAA verified the percentage of roots colonized by mycorrhizal fungi and spore viability in the soil. The sulfentrazone, isoxaflutole and oxyfluorfen as well as the time of application affected the microbiological indicators differently. In the sandy loam soil sulfentrazone was more harmful to microbial biomass, mycorrhizal colonization and microorganisms solubilizing inorganic phosphate. However, in clayey soil, the application of the three herbicides did not affect microbial biomass, but the root colonization of eucalyptus by mycorrhizal fungi and the potential solubilization of inorganic phosphate were reduced. The sulfentrazone caused an increase in the number of non-viable spores of mycorrhizal fungi in the soil.

Key words: herbicides, ***Eucalyptus grandis*** x ***Eucalyptus urophylla,*** mycorrhiza, phosphate solubilization, microbial biomass.

Impacto de sulfentrazona, isoxaflutol e oxyfluorfem sobre a microbiota de dois solos florestais

Resumo

Questões referentes à aplicação de herbicidas em pré-emergência das plantas daninhas na cultura do eucalipto merecem destaque devido ao impacto dessa prática no ambiente, principalmente sobre a atividade microbiana do solo e micro-organismos benéficos, como os fungos micorrízicos e os solubilizadores de fosfatos. Objetivou-se estudar o impacto da aplicação dos herbicidas sulfentrazona, isoxaflutol e oxyfluorfem sobre a colonização micorrízica, biomassa e atividade microbiana de dois solos florestais cultivados com eucalipto. O ensaio foi conduzido em casa de vegetação utilizando um solo argiloso e um solo franco-arenoso distribuídos em vasos de 12 dm^3. O esquema fatorial utilizado foi 4 x 3, sendo três herbicidas (sulfentrazona, isoxaflutol e oxyfluorfem) mais a testemunha e três avaliações (5, 20 e 70 dias após a aplicação – DAA), no delineamento inteiramente casualizado com quatro repetições. Após a aplicação dos herbicidas na dose recomendada para a cultura transplantaram-se as mudas do híbrido de ***Eucalyptus grandis*** x ***E. urophylla***. Avaliaram-se a biomassa microbiana, a taxa respiratória do solo, o quociente metabólico e o potencial de solubilização de fosfato inorgânico aos 5, 20 e 70 DAA. Aos 70 DAA verificou-se a colonização micorrízica e a viabilidade de esporos. Os herbicidas sulfentrazona, isoxaflutol e oxyfluorfem, bem como o tempo decorrido da aplicação afetaram os indicadores microbiológicos de maneira diferenciada. No solo franco-arenoso, o sulfentrazona foi mais prejudicial à biomassa microbiana, à colonização micorrízica e aos micro-organismos solubilizadores de fosfato inorgânico. No solo argiloso, no entanto, a aplicação dos três herbicidas não afetou a biomassa microbiana, mas reduziu a colonização radicular do eucalipto por fungos micorrízicos arbusculares e o potencial de solubilização de fosfato inorgânico. O herbicida sulfentrazona se destacou por provocar aumento do número de esporos não viáveis de fungos micorrízicos arbusculares nesse solo.

Palavras-chave: herbicida, ***Eucalyptus grandis*** x ***Eucalyptus urophylla***, micorrizas, solubilização de fosfato, biomassa microbiana.

1. INTRODUCTION

Currently, *Eucalyptus* spp. planted forests occupy 6.66 million hectares in the Brazilian territory, representing 76.6% of the total area of forest plantations. The increase in plantation areas and wood consumption in 2012 were 2.1% and 7.2% higher, respectively, compared to 2011 (ABRAF, 2013), thus indicating a growing demand for wood products and the consequent need for increased production.

The genus *Eucalyptus* contains species with rapid growth and despite good competitiveness as to the establishment in the field; it is not free from damage caused by weed interference, which can compromise the quality of the product and the production (Tuffi Santos et al., 2006). This interference occurs via competition for nutrients, water, light, allelopathy and indirectly by hosting important pathogens and pests to the culture (Silva and Silva, 2007).

In commercial eucalyptus plantations, herbicides for weed control are used on a large scale for a more efficient management due to the large areas and low availability of manpower (Toledo et al., 2003). However, herbicides can cause negative impacts to the edaphic microflora, due to their different compositions, physicochemical characteristics and behavior in soil. Products applied at pre-emergence of weeds, such as sulfentrazone, isoxaflutole and oxyfluorfen directly reach the ground and may produce greater losses by increasing the risk of exposure to microorganisms in the soil, especially beneficial microorganisms, such as mycorrhizal fungi and phosphate solubilizing microorganisms.

Numerous studies have shown, by means of microbiological indicators of soil quality, the importance of soil microbiota for the successful establishment of eucalyptus plantations in several regions of the country, especially in areas of reforestation and restoration of degraded areas (Campos et al., 2011; Caproni et al., 2005; Chaer and Tótola, 2007; Coelho et al., 1997; Cordeiro et al., 2005; Lima et al., 2013; Silva et al., 2009), where soil usually has altered physical and chemical characteristics, low fertility, high content of aluminum and low pH values. One of the reasons that contribute to this success is the symbiotic association of plant roots with arbuscular mycorrhizal (AMF) and ectomycorrhizal fungi (ECMF) in soil. This mycorrhizal association provides higher density and longevity to the roots, protection against pathogens (Sylvia and Williams, 1992), greater exploration of the soil with increasing absorption of water and nutrients as well as mobilization of unavailable forms of nutrients (Marchner and Dell, 1994), which may contribute to increased productivity and competitiveness.

Other indicators such as microbial biomass carbon (MBC), respiratory rate (RR) and metabolic quotient (qCO_2) are tools that assist in the assessment of the effects of herbicide application to microorganisms in the soil. The action of these products is also reflected on the potential of solubilization inorganic phosphate (Pi), root colonization by mycorrhizal fungi and AMF spore viability. Given the above, the objective was to study the impact of applying sulfentrazone, isoxaflutole and oxyfluorfen on mycorrhizal colonization, microbial biomass and microbial activity of two forest soils with different physical and chemical characteristics.

2. MATERIAL AND METHODS

The experiment was conducted in a greenhouse and the experimental design was completely randomized with four replications. Treatments were applied to each soil following a 4x3 factorial design, with three herbicides Boral 500 SC® (sulfentrazone), Goal BR® (oxyfluorfen) and Fordor 750 WG® (isoxaflutole) at the dose recommended by manufacturers of 0.5 kg ha^{-1}, 0.72 kg ha^{-1} and 0.113 kg ha^{-1}, respectively) and a control without herbicide, and three evaluation periods (5, 20 and 70 days after application (DAA)). The soils have distinct chemical and physical characteristics, from eucalyptus plantations in the municipalities of Viçosa and Belo Oriente, both in Minas Gerais State (Table 1), being a sandy loam soil and a clayey soil, respectively.

Pots (12 dm^3) were filled with the respective soils previously fertilized according to soil chemical analysis and recommendation for the culture, and soon after, herbicides were applied. For application, we used a backpack sprayer with constant pressure maintained by CO_2, coupled to a bar with nozzle TT11002, working pressure at 250 kPa and spray volume of 150 L ha^{-1}. After applying, we transplanted standard seedlings of the clone of eucalyptus, hybrid of *Eucalyptus grandis* x *Eucalyptus urophylla*, with approximately three months old. Seedlings were daily irrigated; so as to keep the soil at field capacity.

At 5, 20 and 70 DAA, soil samples (200 g) were taken at the 0-10 cm layer of each pot, stored in plastic bags, loosely sealed and kept at 4 °C for at maximum 3 days until processing. We evaluated the respiratory rate (RR) of the soil through C-CO_2 evolution, microbial biomass carbon (MBC) and solubilization of inorganic phosphate (Pi) by microorganisms in the soil.

After sieving through 2 mm mesh and determined the water content, the moisture content of soil samples was adjusted to 75% of field capacity for incubation for 15 days. Microbial respiration was estimated for each collection time, from the amount of CO_2 evolved from 100 g soil samples and captured in bottles containing 100 mL NaOH (0.5 mol L^{-1}) in a CO_2-free continuous flow system. After incubation, we performed the indirect titration of NaOH with HCl (0.5 mol L^{-1}), according to the methodology described by Vivian et al. (2006).

Microbial biomass carbon was estimated by the method described by Vance et al. (1987) modified by Islam and Weil (1998), using, instead of chloroform (fumigation), microwave

Table 1. Chemical and physical characteristics of soil samples from the municipalities of Viçosa and Belo Oriente (Minas Gerais State)

Chemical characterization									
Origin	pH	P	K	Al^{3+}	H + Al	Ca^{2+}	Mg^{2+}	M.O.	P-rem
		-- mg dm^{-3} --		-------- $cmol_c$ dm^{-3} --------				dag kg^{-1}	mg L^{-1}
Viçosa	5.5	2.2	40	0.0	3.47	1.2	0.6	3.5	33.4
Belo Oriente	4.1	15.9	46	1.7	8.08	0.3	0.1	2.7	22.6

Physical characterization				
	Clay	Silt	Sand	Textural class
	--------- dag kg^{-1} ---------			
Viçosa	13	11	76	Sandy loam
Belo Oriente	50	18	32	Clayey

oven (irradiation). With RR and MBC data, we calculated the metabolic quotient (qCO_2- μg CO_2 μg^{-1} MBC d^{-1}), using the relationship between the C-CO_2 evolution daily (μg CO_2 g^{-1} d^{-1}) and microbial biomass carbon (μg MBC g^{-1} soil).

To determine the potential solubilization of inorganic phosphate, we transferred 1 g soil from each experimental unit, in triplicate, to the test tube with 10 mL liquid medium NBRI, pH 6.8-7.0, containing (g L^{-1}): glucose, 10; $Ca_3(PO_4)_2$, 5; $MgCl_2.6H_2O$, 0.5; $MgSO_4.7H_2O$, 0.25; KCl, 0.2; and $(NH_4)_2SO_4$, 0.1 (Nautiyal, 1999). Tubes were capped and incubatedfor15 days at 25 °C. After the incubation period, 1.0 mL of the liquid phase was subjected to centrifugation (8,000 rpm, 25 °C for 20 minutes). Then, we determined the amount of inorganic P of the supernatant by the modified colorimetric method of vitamin C in spectrophotometer at 725 nm (Braga and DeFelipo, 1974).

At 70 DAA, 50 g soil samples were collected in each plot to evaluate the viability of arbuscular mycorrhizal fungi spores. We used the wet sieving method (Gerdemann and Nicolson, 1963) and centrifugation in sucrose solution (Jenkins, 1964). The spore suspension (10 mL) was placed in lidded containers, and added with 2 mL iodonitrotetrazolium chloride (INT)(1g L^{-1}) (Walley and Germida, 1995). The containers were left at room temperature for 72 hours protected from light. Subsequently, we quantified the viable and non-viable spores. In this study, we considered as viable, the spores that reacted with INT, becoming red dish, and as non-viable, the spores with cytoplasmic content that does not react with INT, maintaining its natural color, in addition to spores without cell content.

At the end of the experiment, at 70 DAA, fine roots were collected for assessing AMF colonization, washed in tap water and stored in vials with lid containing FAA (formaldehyde: acetic acid: ethanol 96°) 1:1:18 (v/v/v) for further evaluation. In the laboratory, roots were initially discolored with 10% KOH in a water bath at 70 °C and then washed with distilled water. Then, we added a solution of 1% HCl for 5 minutes, drained, and stained with trypan blue dye (0.05% in lactoglycerol). The percentage of roots colonized by AMF was evaluated by the intersection method in gridded plate under binocular microscope (Giovannetti and Mosse, 1980). The segments with vesicles, arbuscules or hyphae, sometimes linked to typical spores were considered colonized by AMF. Data were subjected to analysis of variance. When F-value was significant, Tukey's test was applied to compare mean values of the treatments ($p<0.05$).

3. RESULTS AND DISCUSSION

Analyzing the microbial biomass carbon (MBC), according to the breakdown of the interaction evaluation period x herbicide effect, in the sandy loam soil, for the three application times, there was statistical difference only for soils treated with sulfentrazone at 20 and 70 DAA, which had, on average, 98.32 μg MBC g^{-1}soil lower than in the first evaluation (Table 2). Moreover, at 70 DAA, only the herbicide sulfentrazone has differed from the control causing a 56% reduction in MBC (Table 2).Vivian et al. (2006) also observed a negative effect of sulfentrazone on soil microorganisms, which was attributed to the high persistence in the Red-Yellow Argisol examined. Although the main degradation mechanism of sulfentrazone is microbial (FMC CROP., 1995), other studies have also demonstrated its toxic action on soil microorganisms (Chang et al., 2001; Reddy and Locke, 1998) and therefore, this herbicide should be used with caution. Also, knowing the processes of suppression, reduction of microbial activity and microbiological selection of the soil by this herbicide can assist in reducing its residual effect (Vivian et al., 2006).

In the clayey soil treated with isoxaflutole and oxyfluorfen there was an increase in MBC at 20 DAA, and a decrease at 70 DAA. The same behavior was observed for the control, with a sharper reduction between the evaluation periods (Table 2). In the evaluation performed at 5 DAA, the result possibly arises from the application of the herbicide in the soil, since the seedling had been recently transplanted and probably concentrated all energy in its establishment and development of the root system, while in the evaluation performed at 20 DAA, microbiological indicators are already being influenced by the combined action of the plant and

Table 2. Microbial biomass carbon (MBC) from sandy loam and clayey soils subjected to herbicide application and evaluated in three periods

Microbial biomass carbon (MBC – µg MBC g^{-1} soil)			
Sandy loam soil			
Herbicide	Evaluation period		
	5 DAA(1)	20 DAA	70 DAA
Control	227.72 Aa(2)	214.21 Aa	301.05 Aa
Sulfentrazone	256.57 Aa	185.27 Ba	131.23 Bb
Isoxaflutole	191.05 Aa	119.65 Aa	208.42 Aab
Oxyfluorfen	129.30 Aa	200.77 Aa	165.97 Aab
CV		36.15%	
Clayey soil			
Herbicide	5 DAA	20 DAA	70 DAA
Control	250.88 Ba	378.25 Aa	127.37 Ca
Sulfentrazone	260.53 Aa	287.54 Aa	158.25 Ba
Isoxaflutole	169.83 Ba	291.40 Aa	185.26 Ba
Oxyfluorfen	169.82 Ba	301.05 Aa	165.94 Ba
CV		22.98%	

(1)DAA: days after application of herbicides. (2)Means followed by the same uppercase in the row and lowercase in the column, for each soil, are not significantly different by Tukey's test at 5%.

Table 3. Respiration rate (RR) of sandy loam and clayey soils subjected to herbicide application and evaluated in three periods

Respiration rate (RR - µg C-CO_2 g^{-1}soil d^{-1})			
Sandy loam soil			
Herbicide	Evaluation period		
	5 DAA(1)	20 DAA	70 DAA
Control	59.87 Cb(2)	116.11 Aa	82.50 Ba
Sulfentrazone	75.17 Bb	108.17 Aa	50.72 Cb
Isoxaflutole	70.28 Bb	111.83 Aa	26.89 Cc
Oxyfluorfen	96.56 Aa	103.28 Aa	59.87 Bb
CV		11.81%	
Clayey soil			
Herbicide	5 DAA	20 DAA	70 DAA
Control	51.33 Ba	100.87 Aa	52.38 Aa
Sulfentrazone	50.11 Ba	97.78 Aa	54.35 Ba
Isoxaflutole	65.39 Ba	113.06 Aa	28.15 Cab
Oxyfluorfen	51.33 Ba	89.83 Aa	20.93 Cb
CV		19.17%	

(1)DAA: days after application of herbicides. (2)Means followed by the same uppercase in the row and lowercase in the column, for each soil, are not significantly different by Tukey's test at 5%.

the presence of the herbicide. Soils with the presence of sulfentrazone presented, in the first two periods, MBC 42% higher than that at 70 DAA. Tironi et al. (2009) studied herbicides applied at pre-emergence in sugar cane and verified an increased MBC caused by some of them, which was associated with phytotoxic effects caused by herbicides, which may increase root exudation, favoring the microbiota in the rhizosphere of the plant. Highest synthesis and exudation of amino acids and sugars root are usually influenced by stress (Bertin et al. 2003). It is inferred, therefore, that the stress caused by herbicides can influence the composition and quantity of root exudates in eucalyptus.

Respiration rate (RR) of both soils was affected by the interaction between herbicides and evaluation periods (Table 3). In the sandy loam soil, RR increased at 20 DAA, except for the treatment with oxyfluorfen, and microbial activity reduced at 70 DAA (Table 3). This may reflect the attempt of microbial biomass to metabolize the products; biomass which has not changed over time (Table 2), close to its application (20 DAA) and prevalence of toxic effects caused by these or byproducts generated, at 70 DAA. As for effect of herbicides in each period, at 5 DAA there was a greater biological activity in the sandy loam soil treated with oxyfluorfen (Table 3), which may be due to an immediate toxic effect of the product and increased activity with the purpose of detoxification, for its use as a source of carbon and energy by edaphic microorganisms. At 70 DAA, the application of the three herbicides caused reduction in RR compared to the control (Table 3), probably indicating the deleterious effects of these products within creasing application time, when it might have occurred leaching of the same to subsurface layers of the sandy loam soil.

Soil microorganisms' activity is a positive attribute for soil quality, and respiration rate is a sensitive indicator of waste decomposition, metabolic turnover of organic carbon in soil and ecosystem disturbances (Paul et al., 1999), but the interpretation should be performed carefully. A high respiration rate, indicating high biological activity, may be a desirable feature if considered as a sign of rapid decomposition of organic waste into nutrient available to plants. However, the decomposition of stable organic matter, that is, the humic fraction of the soil, is unfavorable for many chemical and physical processes, such as aggregation, cation exchange capacity and water retention capacity (Tótola and Chaer, 2000).

The clayey soil that received sulfentrazone, isoxaflutole and oxyfluorfen showed the same pattern evidenced for RR in the sandy loam soil, with an increase at 20 DAA and a decrease at 70 DAA. For the effect of herbicides, a statistical difference was only detected at 70 DAA, with a reduction above 50% in respiration activity of the soil treated with the oxyfluorfen compared with control (Table 3).

In relation to the variable qCO_2, there was no significant effect of the interaction herbicide x evaluation period in the sandy loam soil. Only the factor period was significant. At 20 DAA, the specific metabolic activity of microbial biomass was 46% greater than in other period (Table 4), which indicates a low energy efficiency of soil microorganisms in that period, i.e., a high respiration was being required to maintain a similar microbial biomass.

Metabolic quotientis a system balance indicator proposed by Anderson and Domsch (1993), which predicts that the closer to balance the system is, less energy is required to maintain microbial cell, and the farther from balance the higher specific activity is needed. Thus, a low qCO_2indicates

Table 4. Metabolic quotient (qCO_2) of sandy loam and clayey soils subjected to herbicide application and evaluated in three periods

Metabolic quotient (qCO_2 - µg C-CO_2 µg^{-1} MBC d^{-1})					
Sandy loam soil		**Clayey soil**			
Period	**qCO_2**	**Herbicide**	**Evaluation period**		
			5 DAA[(1)]	**20 DAA**	**70 DAA**
5 DAA	0.35 b	Control	0.25 Aa[(2)]	0.29 Aa	0.43 Aa
20 DAA	0.59 a	Sulfentrazone	0.18 Ba	0.34 Aa	0.34 Aab
70 DAA	0.28 b	Isoxaflutole	0.38 Aa	0.40 Aa	0.18 Bb
		Oxyfluorfen	0.30 Aa	0.30 Aa	0.16 Ab
CV	39.74%	CV		32.10%	

[(1)]DAA: days after application of herbicides. [(2)]Means followed by the same uppercase in the row and lowercase in the column, for each soil, are not significantly different by Tukey's test at 5%.

Table 5. Potential of solubilization of inorganic phosphate evaluated in three periods in sandy loam and clayey soils subjected to herbicide application

Potential of solubilization of inorganic phosphate (mg P)			
Sandy loam soil			
Herbicide	**Evaluation period**		
	5 DAA[(1)]	**20 DAA**	**70 DAA**
Control	0.60 Ba[(2)]	0.62 Bab	0.87 Aa
Sulfentrazone	0.52 Ba	0.39 Bc	0.95 Aa
Isoxaflutole	0.53 Ba	0.44 Bbc	0.96 Aa
Oxyfluorfen	0.54 Ba	0.61 Ba	1.00 Aa
CV		14.06%	
Clayey soil			
Herbicide	**5 DAA**	**20 DAA**	**70 DAA**
Control	0.65 Ca	1.35 Aa	1.05 Ba
Sulfentrazone	0.47 Ba	0.80 Ab	0.78 Aab
Isoxaflutole	0.53 Aa	0.77 Ab	0.62 Ab
Oxyfluorfen	0.59 Aa	0.74 Ab	0.71 Ab
CV		21.61%	

[(1)]DAA: days after application of herbicides. [(2)]Means followed by the same uppercase in the row and lowercase in the column, for each soil, are not significantly different by Tukey's test at 5%.

economy in the use of energy and presumably reflects a more stable environment or closer to its equilibrium state; on the contrary, high values indicate ecosystems subjected to any stress or disorder (Tótola and Chaer, 2000), designating less conservationist systems of the soil organic matter.

As an effect of the interaction between factors in the clayey soil, with the application of sulfentrazone at 20 DAA and 70 DAA, there was an increase in the metabolic quotient, probably generated by a disturbance in the soil-plant-microorganism system. Moreover, with increasing distance from the application of isoxaflutole, the metabolic quotient decreased (Table 4), also prominent among herbicides activity at the time of 70 DAA along with oxyfluorfen, indicating a more balanced environment (Table 4). These results allow to affirm that although the application of these herbicides cause changes in respiration rate and soil microbial biomass, they did not affect the stability of the system, compared with the control, also emphasizing the increased energy efficiency of soil microbiota provided by the application of oxyfluorfen and isoxaflutole in the 70 DAA evaluation.

The potential of solubilization of inorganic phosphate (Pi) for the sandy loam soil treated with sulfentrazone, isoxaflutole and oxyfluorfen was reduced at 5 DAA and at 20 DAA compared with 70 DAA (Table 5), following the same trend of the control. In this soil, the products may have shown greater mobility in soil with irrigation through the time, exerting toxic effect to a larger population of phosphate solubilizing microorganisms, occurring harmful action and selection of some of these microorganisms, subsequently increasing the potential of solubilization of Pi at 70 DAA. In the clayey soil, an effect of sampling periods was verified by applying the herbicide sulfentrazone with reduced concentration of Pi at 5 DAA (Table 5).

Rossi et al. (2005) observed that sulfentrazone showed uniform distribution up to 22.5 cm in Chernosol (sandy), proportional to the increase in rainfall, but in Red Latosol (clayey), the herbicide remained in the surface layer of 2.5 cm, showing no mobility, independent of rainfall used. Melo et al. (2010) found a descending movement of sulfentrazone to 27.5 cm in sandy loam and clayey soil up to 25 and 17.5 cm in soils with low and high organic matter content, respectively. These authors also observed the presence of isoxaflutole up to 25 cm in sandy loam soil, showing a decrease of concentration with increasing depth in the soil profile. Inoue et al. (2010) analyzed herbicide leaching in sandy and clayey soils, and verified low mobility of oxyfluorfen molecules in the 5-10 cm layer of sandy soil samples, with the use of more than 80 mm water depth. Also, these aforementioned authors observed that small movements of molecules may occur under intense rainfall, but no leaching of oxyfluorfen molecules below the surface layer (0-5 cm) was detected in tests with clay soil.

Some soil properties such as pH, clay content, total iron, organic matter, crystallinity of iron oxides (Upchurch, 1966) and moisture can reduce the effectiveness of herbicides, influencing leaching losses and consequently the impact on a wide range of microorganisms in the soil.

Regarding the factor herbicide, a variation was noted only at 20 DAA for the sandy loam soil, treatments with application of sulfentrazone had a lower potential to solubilize Pi (Table 5). For that same period, in clayey soil, the application of three herbicides reduced the potential of solubilization of Pi compared with the control (Table 5). At 70 DAA, soils with isoxaflutole and oxyfluorfen adversely affected the availability of this source of phosphorus in the soil for plants. Massenssini et al. (2008) reported that glyphosate in different commercial formulations was detrimental to the in vitro activity of phosphate solubilizing bacteria isolated from the rhizosphere of eucalyptus. Through the results of this study, it can be inferred that these herbicides applied directly to the soil adversely affect the activity of inorganic

Table 6. Percentage of eucalyptus roots colonized by arbuscular mycorrhizal fungi (AMF) in sandy loam and clayey soils subjected to herbicide application

Percentage of colonization by AMF (%)		
Herbicide	**Sandy loam soil**	**Clayey soil**
Control	22.67 a[(1)]	22.39 a
Sulfentrazone	11.80 a	9.60 b
Isoxaflutole	13.30 ab	14.33 b
Oxyfluorfen	18.41 ab	14.23 b
CV	29.44%	21.67%

[(1)]Means followed by the same lowercase in the column, for each soil, are not significantly different by Tukey's test at 10%.

Table 7. Viability of mycorrhizal fungi spores in clayey soil with eucalyptus subjected to herbicide application

Percentage of viable and non-viable spores (%)		
Herbicide	**Clayey soil**	
	Viable spores	**Non-viable spores**
Control	14.67 ab[(1)]	22.00 bc
Sulfentrazone	4.67 b	33.67 a
Isoxaflutole	12.00 ab	31.00 ab
Oxyfluorfen	20.00 a	17.00 c
CV	47.02%	19.89%

[(1)]Means followed by the same lowercase in the column, for each soil, are not significantly different by Tukey's test at 10%.

phosphate solubilizing microorganisms, with a harmful and variable action, according to soil texture and the elapsed time of the application of products.

Colonization of roots by arbuscular mycorrhizal fungi (AMF) in clayey soil was reduced by the three herbicides (Table 6). Nevertheless, in sandy loam soil, only sulfentrazone affected eucalyptus root colonization, reducing it by about 50% (Table 6).

When establishing a forest plantation, low mycorrhizal colonization of forest seedlings may be a limiting factor for field establishment (Araújo et al., 2004). Mycorrhizal colonization is a feature that can be affected by many factors, such as plant species, plant age, density of roots, fungal propagules in soil and type of management employed in soil at planting time (Campos et al., 2011). Smith and Read (1997) reported that, in eucalyptus plantations, there is a mycorrhizal succession with predominance of AMF in younger plantations and ectomycorrhizal fungi (ECMF) in plantations with advanced ages. Given the importance of this symbiosis to the culture, especially in the initial phase of growth, and the negative effects resulting from application of herbicides with residual activity and high potential for leaching, chemical weed management in forest plantations plants should prioritize the rational use of herbicides showing, among other characteristics, low risk of negative impact on arbuscular mycorrhizal fungi.

The viability of spores in the sandy loam soil was not affected by herbicide application, but, it was found that the treatment of the clayey soil with sulfentrazone resulted in low percentage of viable spores and an increased number of non-viable spores (Table 7). This result complements the information obtained in the analysis of mycorrhizal colonization, confirming its toxicity to microorganisms in the soil, affecting symbiosis and viability of spores present in the soil, thus decreasing the possibilities of plant-fungus interaction.

According Brundrett et al. (1996), density of AMF spores in the rhizosphere is quite variable and is frequently related to the distribution, morphology and physiological age of the roots, and depends on other factors influencing sporulation, such as rainfall, temperature, period of sunlight and AMF species. Steffen et al. (2010) worked with mycorrhization of *E. grandis* and noted that in some cases the presence of spores does not correspond to the degree of colonization of the roots.

The abundance and viability of propagules, like spores of mycorrhizal fungi, determine the persistence of AMF in the soil under adverse conditions, such as those arising from changes in land use or application of pesticides (Brundrett et al., 1996). There is still little known about the impact of herbicides on root colonization and density of total, viable or non-viable spores of arbuscular mycorrhizal fungi, and given the relevance of the presence of these propagules in the soil for the establishment of symbiosis with the roots of eucalyptus, other researches should be developed to evaluate the action of these products in different soils, management practices and cultures.

In the light of the above exposed the choice of herbicides for weed control in eucalyptus cultivation should prioritize not only the selectivity and efficacy as well as the low adverse impact on biomass and activity of soil microbiota. Knowledge about the behavior of each herbicide in different soils is critical for safe recommendation of such products in tropical conditions.

4. CONCLUSION

Herbicides sulfentrazone, isoxaflutole and oxyfluorfen affect the microbiological indicators in different manners, in the soils and periods evaluated.

In the sandy loam soil, sulfentrazone is the most detrimental to microbial biomass, mycorrhizal colonization and inorganic phosphate solubilizing microorganisms. In the clayey soil, the application of the three herbicide does not affect the microbial biomass compared with the control soil (without herbicide), but reduces root colonization by arbuscular mycorrhizal fungi and the potential of solubilization of inorganic phosphate. Sulfentrazone causes an increase in the number of non-viable spores of arbuscular mycorrhizal fungi in the soil.

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