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Mycorrhizal Association and Microbial Activity of Soil Cultivated with Cassava After Application of Mesotrione and Fluazifop-p-Butyl[1]

Associação Micorrízica e Atividade Microbiana do Solo Cultivado com Mandioca após Aplicação do Mesotrione e Fluazifop-p-Butil

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ABSTRACT - Soil is a very heterogeneous environment that allows the establishment of wide range of microorganisms populations, whose balance is affected by biotic and abiotic factors. This study has aimed to assess the effect of doses of mesotrione and fluazifop-pbutyl herbicides and two assessment periods on microbial activity and biomass of soil cultivated with cassava Cacau-UFV cultivar, besides the root colonization by arbuscular mycorrhizal fungi. Two trials were conducted in a protected environment where was realized post-emergence application of mesotrione in the doses of 72, 108, 144 and 216 g ha^{-1} and fluazifop-p-butyl in the doses of 100, 150, 200 and 300 g ha^{-1}, besides a control without application. Soil samples were collected for determination of soil respiratory rate (RR), microbial biomass carbon (MBC), metabolic quotient (qCO_2), and colonization of roots by arbuscular mycorrhizal fungi at the 30 and 60 days after applications (DAA) of the herbicides. Fluazifop-p-butyl increased the RR, MBC and the percentage of cassava roots colonized by mycorrhizal fungi in the assessment performed at 60 DAA. The larger effects of mesotrione on soil microbial indicators were up to 30 DAA, being the changes minimized at 60 DAA. It is concluded that the herbicides alter the soil microbial indicators, with effects dependent of the product, of dose applied and also of the period of assessment.

Keywords: microbial biomass, arbuscular mycorrhizal fungi, herbicide, *Manihot esculenta.*

RESUMO - O solo é um ambiente bastante heterogêneo que permite o estabelecimento de diversas populações de microrganismos, cujo equilíbrio é afetado por fatores bióticos e abióticos. Este trabalho teve como objetivo avaliar o efeito de doses dos herbicidas mesotrione e fluazifop-p-butil e duas épocas de avaliação na atividade e biomassa microbiana do solo cultivado com o cultivar de mandioca Cacau–UFV, bem como a colonização das raízes por fungos micorrízicos arbusculares. Dois ensaios foram realizados em ambiente protegido, constituindo-se da aplicação em pós-emergência do mesotrione nas doses de 72, 108, 144 e 216 g ha^{-1} *e do fluazifop-p-butil nas doses de 100, 150, 200 e 300 g* ha^{-1}*, além de uma testemunha sem aplicação. Aos 30 e 60 dias após a aplicação (DAA) dos herbicidas, amostras de solo foram coletadas para determinação da taxa respiratória do solo (TR), do carbono da biomassa microbiana (CBM) e do quociente metabólico* ($\mathbf{q}CO_2$)*, sendo também avaliada a colonização das raízes por fungos micorrízicos arbusculares. O fluazifop-p-butil aumentou a TR, o CBM e a porcentagem de raízes de mandioca colonizada por fungos micorrízicos na avaliação realizada aos 60 DAA. Já os maiores efeitos do mesotrione sobre os indicadores microbiológicos do solo foram até os 30 DAA, e aos 60 DAA as alterações foram minimizadas. Conclui-se que os herbicidas alteram os indicadores microbianos do solo, sendo os efeitos dependentes do produto, da dose aplicada e também da época de avaliação.*

Palavras-chave: biomassa microbiana, fungo micorrízico arbuscular, herbicida, *Manihot esculenta.*

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INTRODUCTION

The growth and development of plants are closely related to the activity of soil microorganisms. Usually, small changes in soil quality are associated with changes in its microbiological properties, which have a high sensitivity to disturbance resulting from the management (Pankhurst et al., 1997; Tótola & Chaer, 2002). The assessment of soil quality and quantification of changes in its attributes have received attention due to the intensification of use and management systems, enabling the monitoring of sustainable soil productivity (Neves et al., 2007) and conservation of natural resources. The maintenance of ecosystem productivity depends, in large part, of the transformation of the organic matter process and, therefore, the microbial biomass (Gama-Rodrigues & Gama-Rodrigues, 2008). The microbial biomass and its activity have been identified as good indicators because of a high sensitivity to changes in soil quality caused by use changes and management practices (Traninn et al., 2007).

Cassava is considered one of the crops most dependent on mycorrhizal association (Balota et al., 1999). Under natural conditions, there is a large number of species of arbuscular mycorrhizal fungi (AMF) associated with the crop, which is extremely important, as this presents a root system composed of thick and less abundant roots, with little absorbent hair, resulting in lower specific surface available for absorption of water and nutrients from the soil (Colozzi-Filho & Nogueira, 2007). According to Omorusi & Ayanru (2011), these characteristics can explain the valuable contribution of the AMF in the *Manihot* genus plants because the cassava plants have the ability to extract a large amount of nutrients in soils with low natural fertility, if associated with AMF. As to the mechanism of absorption and nutrient mobilization, the AMF are more effective than the roots. When phosphorus is added in a medium containing mycorrhizal fungi, it may be realized that all inorganic phosphorus is generally absorbed by hyphae (Nielsen et al., 2002).

The use of pesticides – especially herbicides – has been reported as a practice with a high potential to influence the dynamics of soil microorganisms and may have harmful, beneficial or null effects (Santos et al., 2005, 2007; Reis et al., 2008). Fluazifop-p-butyl is a herbicide that has action on inhibition of the enzyme Acetyl-CoA carboxylase (ACCase), blocking the synthesis of lipids in susceptible plants (Burke et al., 2006); it presents an average persistence of 30 days and high efficiency in the control of grasses derived from seeds. Mesotrione inhibits carotenoid biosynthesis and interferes with the activity of the enzyme HPPD (4-hydroxyphenylpyruvate-dioxygenase) in chloroplasts, causing whitening, with subsequent necrosis and death of plant tissues in about one to two weeks (Lee, 1997; Witchert et al., 1999); its persistence in soil is short (Rodrigues & Almeida, 2005). The soil microbial activity plays a critical role in the degradation of mesotrione and fluazifopp-butyl, but there are few studies in the literature on the impact of these herbicides on soil microbiota.

To assess the effects of herbicides on soil microbial activity, several indicators have been used, such as microbial biomass carbon, the activity of certain enzymes and soil basal respiration (Tótola & Chaer, 2002). Among these, the assessment of soil respiratory rate (RR), of microbial biomass carbon (MBC) and of the metabolic quotient (qCO_2) have been widely used to study the effects of the herbicides in the soil by their rapid response and sensitivity to the presence of these products, as evidenced in tropical climate soils and in bean and sugar cane crops (Santos et al., 2005; Vivian et al., 2006; Reis et al., 2008).

Some studies have reported the potential use of herbicides fluazifop-p-butyl and mesotrione to control weeds in post-emergence of cassava (Silva et al., 2011; Silveira et al., 2012). However, there is little information on the effect of such products on soil microorganisms. Therefore, the research objective of this study was to assess the carbon indicators of microbial biomass, respiratory rate and metabolic quotient of the soil cultivated with cassava after application of herbicides and the occurrence and implications on the association with arbuscular mycorrhizal fungi.

MATERIALS AND METHODS

Two experiments were conducted in a protected environment using samples of a typical distrophic Red-Yellow Latosol of a clayey texture (56% clay, 6% silt and 38% sand) with the following chemical characteristics: pH (water) of 5.4; organic matter content of 1.8 dag kg^{-1}; P = 1.4 and K = 10 mg dm^{-3}; Ca = 0.5, Mg = 0.2, Al = 0.4, H+Al = 4.4 and $CTC_{effective}$ = 1.7 $cmol_c$ dm^{-3}. As a basic fertilizer were applied the equivalent to 300 kg ha^{-1} of dolomite limestone, 220 kg ha^{-1} of simple superphosphate and 40 kg ha^{-1} of potassium chloride. Standardized maniocs of the CacauUFV cassava cultivar, measuring 20 cm and having five gems, were planted in pots with a volumetric capacity of 12 dm^3 of soil. Nitrogen fertilization on covering was done at 30 days after planting (DAP) of the crop at a dose of 40 kg ha^{-1} of urea previously dissolved in water. Irrigation by micro spray was done as needed by the crop.

The experimental design used was done in randomized blocks with three replications and each pot containing a plant, constituting one experimental unit. The treatments were arranged in a 5 x 2 factorial design for both experiments. The first factor consisted of doses of herbicides mesotrione (0, 72, 108, 144 and 216 g ha^{-1}) and fluazifop-p-butyl (0, 100, 150, 200 and 300 g ha^{-1}); in each experiment, these doses are equivalent to 0.0, 0.5, 0.75, 1.0 and 1.5 times the commercial dose recommended by the manufacturers. The second factor, for each experiment, corresponded to the assessment periods 30 and 60 days after herbicide application (DAA).

Herbicide application was performed at 30 DAP with backpack sprayer pressurized at CO_2, equipped with two TTI 110.02 nozzles, spaced 0.5 m wide, kept in pressure of 200 kPa and a spray volume of 150 L ha^{-1}.

At 30 and 60 DAA soil samples were taken from each experimental unit in the vicinity of the roots; then it was passed through a sieve with a 2 mm mesh. These samples had moisture adjusted to 60% of field capacity, and subsequently were packed in hermetically sealed containers with 100 g of soil for 15 days in order to assess the respiratory rate. The respirometric method was used to assess the C-CO_2 evolved from the soil, according to the methodology described by Vivian et al. (2006), wherein the C-CO_2 released from the soil was adduced by a continuous (CO_2 free) streaming air to a tube containing 100 mL of a solution of NaOH 0.5 mol L^{-1}. The C-CO_2 evolved from the titration of 10 mL of the NaOH solution with a solution of HCl 0.5 mol L^{-1} was estimated. As a standard for comparison, indicating the air quality, flasks without soil were used, called “white” samples in relation to the others.

After the incubation period, the microbial biomass (MBC) carbon analysis was performed following the method described by Vance et al. (1987), modified by Islam & Weil (1998). Two soil portions were collected from each treatment (18 g) and one was subjected to a microwave irradiation with a power of 900 W per time of 60 + 60 s. 80 mL of K_2SO_4 0.5 mol L^{-1} were added to the soil samples. Then the samples were shaken for 30 minutes in a horizontal shaker table and allowed to rest for 30 minutes for decantation. Subsequently, the supernatant was filtered on filter paper Whatman no. 42. 10 mL of the filtrate were added in digester pipes, then 2 mL of solution $K_2Cr_2O_7$ 0.0667 mol L^{-1} and 10 mL of concentrated H_2SO_4 were added. After cooling, the solution was made up to 100 mL with distilled water and ferroin indicator (eight drops) was added, then proceeding to titration with a solution of $(NH_4)_2Fe(SO_4)_2$ 0.0333 mol L^{-1} until the color changed to vitreous red. MBC was estimated by the difference between the irradiated and non-irradiated samples. With the relationship between the amounts of C-CO_2 and MBC the metabolic quotient (qCO_2) was determined, which represents the amount of CO_2 daily evolved per unit of microbial biomass per day.

To check the occurrence of mycorrhizal colonization in cassava roots, the separation of roots and soil was followed by later washing them in running water, 1 g of roots of approximately 1 cm was taken, which was preserved in FAE (formaldehyde: acetic acid: ethanol) 1:1:18 (v/v/v). Subsequently, they were washed and clarified with 10% KOH and subjected to trypan blue staining (0.05% by lactoglycerol), proceeding to the observation of fungal structures by the method of checkered plate, according to Giovannetti & Mosse (1980).

Data were subjected to analysis of variance and the means of the qualitative factor (assessment periods) were compared by the Tukey test, and the ones of the quantitative factor (dose) by Dunnett's test, both at 5% probability.

RESULTS AND DISCUSSION

The respiratory rate (RR) of soil cultivated with cassava at 60 DAA of fluazifop-p-butyl was higher than that observed at 30 DAA, with the exception of the highest dose (Table 1). For mesotrione there was greater RR at 60 DAA for doses of 0.50 and 1.00, while for the highest dose of 0.75 the highest RR was observed at 30 DAA (Table 1).

Comparing the application of the herbicide doses with the control, there was a higher RR in the soil at 60 DAA of fluazifop-p-butyl at doses of 0.50 and 0.75 (Table 1). The application of mesotrione in doses equivalent to 0.75 and 1.50 of the recommended dose has provided an elevation of soil microbial activity at 30 DAA, being a reduced RR observed at 60 DAA compared to the control in the treatments that received the dose of 0.75. In working with bean crops, Santos et al. (2005) have observed a higher CO_2 evolution when the soil was treated with 1.0 L ha^{-1} of fluazifopp-butyl in a no-till farming system in relation to soil where there was no application. The results reported by these authors indicate that fluazifop-p-butyl features the ability to stimulate microbial activity of the soil, which can be a response to its biodegradation. However, the application of pesticides can positively or negatively interfere in the activity of soil microorganisms, allowing the metabolism of these products or causing poisoning of soil biota, respectively (Santos et al., 2005; Vivian et al., 2006).

For the microbial biomass carbon (MBC), lower values were observed at 30 DAA in the treatment with fluazifop-p-butyl at doses of 0.5, 0.75 and 1.0 (Table 2). However, for the same doses, as there were advanced phenological stages of the cultivar (60 DAA), there was an increase in soil MBC for fluazifop-p-butyl and, at the highest dose, for mesotrione at 30 DAA, if compared to treatments without a herbicide (Table 2).

It was seen in this study that, depending on the dose, the application of herbicides can change the soil MBC; however, it is important to note that the MBC presents variable responses and depends on the applied herbicide, soil type, plant species, microbiota and their interactions, being a very sensitive indicator and responsive to use changes and soil management (Carrera et al., 2007; Ferreira et al., 2008, 2010). The herbicide-soil-microorganism interaction is demonstrated in some studies where, for example, atrazine has not caused changes in

Table 1 - Respiratory rate (RR) of soil cultivated with cassava under different doses of herbicides fluazifop-p-butyl and mesotrione, at two times after application

Dose[1/]	Fluazifop-p-butyl		Mesotrione	
	30 DAA[2/]	60 DAA	30 DAA	60 DAA
	(μg CO_2 g^{-1} soil d^{-1})			
0.00	577.50 B[3/]	1200.83 A	577.50 B	1200.83 A
0.50	889.17 B	2612.50 A*	870.83 B	1265.00 A
0.75	641.66 B	2538.33 A*	1127.50 A*	623.33 B*
1.00	755.83 B	1347.50 A	605.00 B	990.00 A
1.50	1127.50 A	1356.66 A	1210.00 A*	880.00 A

[1/] Equivalent to the recommended commercial dose of each herbicide. [2/] DAA: days after herbicide application. [3/] Means followed by the same capital letter in the row for each herbicide do not differ by Tukey test ($p > 0.05$) and followed with * in the column differ from the control of each assessment by Dunnett's test ($p > 0.05$).

CV (%)	23.33	23.14

[1/] Equivalent to the recommended commercial dose of each herbicide. [2/] DAA: days after herbicide application. [3/] Means followed by the same capital letter in the row for each herbicide do not differ by Tukey test (p > 0.05) and followed with * in the column differ from the control of each assessment by Dunnett's test (p > 0.05).

Table 2 - Microbial biomass carbon (MBC) of the soil cultivated with cassava under different doses of herbicides fluazifopp-butyl and mesotrione, at two times after application

Dose[1/]	Fluazifop-p-butyl		Mesotrione	
	30 DAA[2/]	60 DAA	30 DAA	60 DAA
	(μg MBC g^{-1} soil)			
0.00	219.99 A[3/]	304.63 A	219.99 A	304.63 A
0.50	216.10 B	689.30 A*	335.80 A	411.30 A
0.75	232.70 B	587.70 A*	219.90 B	458.90 A
1.00	279.80 B	632.90 A*	343.50 A	412.40 A
1.50	393.70 A	464.40 A	499.80 A*	216.90 B
CV (%)	29.81		28.01	

the sandy soil MBC (Ghani et al., 1996) and, on the other hand, it has favored the increase of the MBC in soil that is clayey and with a high organic matter content (Moreno et al., 2007). In both studies there was no plant cultivation.

Whereas herbicides can influence soil microbiota, leading to a reduced microbial biomass and a respiratory rate increase, or decrease or increase of both, more significant estimates of these effects can be expressed by assessing the metabolic quotient (qCO_2) (Reis et al., 2008). The metabolic quotient (qCO_2), at 30 and 60 DAA, was not influenced by fluazifop-p-butyl herbicide and by doses used (Table 3), showing that the efficiency of the microbial biomass in the use of the resources was not affected. Only for mesotrione herbicide at dose of 0.75 there was a reduction of qCO_2 at 60 DAA. Some studies have found higher qCO_2 values in soils submitted to herbicide application, indicating a disorder in the system with lower efficiency of soil microbiota in the use of carbon (Santos et al., 2007, 2010). In this study, increases in respiratory rate of the soil (Table 1) were followed, in general, by microbial biomass carbon increases (Table 2), which makes it possible to infer that there was a stimulus to the growth of the population of microorganisms in the soil in the presence of certain herbicide doses, possibly because they serve as a source of carbon and energy in the process of biodegradation.

There was an occurrence of mycorrhiza in cassava roots and an effect of application herbicides and their doses on the association with arbuscular mycorrhizal fungi (AMF) in the two assessment periods (30 and 60 DAA) (Table 4). At 30 DAA, at doses of 0.75, 1.0 and 1.5 L ha^{-1} of fluazifop-p-butyl, a reduction of the percentage of mycorrhizal colonization in comparison to the control was observed, possibly indicating deleterious effects of herbicides on the AMF or a negative influence on the establishment of the symbiosis. After the application of the herbicides, death of the sensitive microbiota and of the mycorrhizal fungi, loss of viability of AMF spores in the soil or even metabolism of these herbicides by the microorganisms may have occurred. These facts may result in higher or lower RR, because

Table 3 - Metabolic quotient (qCO_2) of soil cultivated with cassava under different doses of herbicides fluazifop-pbutyl and mesotrione, at two times after application

Dose[1/]	Fluazifop-p-butyl		Mesotrione	
	30 DAA[2/]	60 DAA	30 DAA	60 DAA
	(μg CO_2 μg MBC^{-1} d^{-1})			
0.00	0.2123^{ns}	0.2646	0.2123 A[3/]	0.2646 A
0.50	0.3050	0.2670	0.1720 A	0.2250 A
0.75	0.1800	0.2980	0.3790 A	0.0930 B
1.00	0.2040	0.1530	0.1460 A	0.1720 A
1.50	0.1920	0.2200	0.1860 A	0.2750 A
CV (%)	46.54		42.02	

[1/] Equivalent to the recommended commercial dose of each herbicide. [2/] DAA: days after herbicide application. [3/] Means followed by the same capital letter in the row for each herbicide do not differ by Tukey test (p > 0.05). ^{ns} non-significant.

Table 4 - Mycorrhizal colonization of cassava roots grown in soil under different doses of herbicides fluazifop-p-butyl and mesotrione, at two times after application

Dose[1/]	Fluazifop-p-butyl		Mesotrione	
	30 DAA[2/]	60 DAA	30 DAA	60 DAA
	(%)			
0.00	77.30 A[3/]	48.68 B	77.30 A	48.68 B
0.50	81.07 A	64.17 B*	63.36 A*	60.13 A
0.75	67.63 A*	64.21 A*	65.95 A*	59.15 A
1.00	63.13 A*	63.81 A*	61.81 A*	50.50 A
1.50	58.34 A*	56.87 A	66.21 A*	44.87 B
CV (%)	9.68		12.06	

[1/] Equivalent to the recommended commercial dose of each herbicide. [2/] DAA: days after herbicide application. [3/] Means followed by the same capital letter in the row for each herbicide do not differ by Tukey test ($p > 0.05$) and followed with * in the column differ from the control of each assessment by Dunnett's test ($p > 0.05$).

in addition to getting a source of carbon and energy, there is a tendency to establish a new equilibrium condition for the population by means of the selection process.

As an effect of the best nutrient acquisition, the mycorrhizal colonization provides increased photosynthetic rates to the plants and thus higher growth (Matos et al., 1999). At 60 DAA, there was an increase of mycorrhizal colonization due to the application of doses of 0.5, 0.75 and 1.0 of fluazifop-p-butyl. According to Santos et al. (2004), the mixture fomesafen + fluazifop-p-butyl has reduced the mycorrhizal colonization in bean plants grown in a conventional system, although this effect was observed only up to 12 days after application.

The application of mesotrione has caused a reduced mycorrhizal colonization only in the assessment at 30 DAA for all doses assessed (Table 4). Regarding the times assessed, at 60 DAA a smaller percentage of colonized roots in the control and in the dose of 1.50 was observed, compared to the assessment performed at 30 DAA. According to Silveira et al. (2012), mesotrione has shown little changes in growth characteristics and toxicity of cassava cultivars, arising mainly from its selectivity. These authors have found that the mild symptoms of poisoning caused by said herbicide were decreased as it distanced from the application period, which may also reflect on the ability of the plants to influence the rhizospheric environment and the soil microbial activity.

The herbicides have shown a high ability to change the microbiological indicators assessed. Fluazifop-p-butyl has increased the RR and the MBC in a magnitude that was higher than mesotrione in the assessment performed at 60 DAA. However, this variation was not able to change the soil qCO_2, since the variations caused in the MBC were followed by variations in the same direction in the RR. This has been reflected in the greater cassava mycorrhizal treated with fluazifop-p-butyl in the last assessment, compared to the control. As for the highest effects of mesotrione on the soil microbial indicators, they went up to 30 DAA; at 60 DAA, the changes were minimized.

Although cassava presents selectivity to these herbicides, choosing products that are less harmful to the biomass and microbial activity, in addition to the mycorrhizal association is essential given the importance of soil microorganisms for the crops. Herbicides fluazifop-p-butyl and mesotrione alter the microbial indicators of soil, the effects being dependent on the applied dose and also the time elapsed from the application.

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