



## Article

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## ***Urochloa brizantha* INTERFERENCE IN THE *Phaseolus vulgaris* RADICULAR SYSTEM FERTILIZED WITH PHOSPHORUS**

*Interferência de Urochloa brizantha no Sistema Radicular de Phaseolus Vulgaris Adubado com Fósforo*

**ABSTRACT** - Interference between root systems occurs below the ground surface long before the competition between plants is expressed in the shoot. Nutrients stand out for being limited in the soil, requiring supplementation, especially phosphorus. Thus, this study aimed at estimating, from sowing, visual characteristics in the root systems of bean plants from the Carioca variety (*Phaseolus vulgaris*) when in competition with brachiaria (*Urochloa brizantha*), considering also the application of phosphorus  $P_2O_5$ . A factorial design with six treatments was used resulting from the combination of three cultivation schemes (beans in isolated crops or in competition with one or two brachiaria plants), and the application or not of phosphate fertilizer ( $90 \text{ kg ha}^{-1}$  of  $P_2O_5$ ), in a completely randomized design with four replications. After the sowing of the species, the visualization results were taken at every 12 hours, by means of the photographic record of the development of the root system in rhizotrons. Greater growth and greater total area of the root system were observed in bean monocultures and when competing with one brachiaria plant in the presence of phosphate fertilization. In contrast, the bean plant in the container with two brachiaria plants invested its resources in greater length and total area of the root system when in the absence of the fertilizer. It can be stated that there is evidence of interference starting three days after the sowing of the species.

**Keywords:** competition, rhizotron, weed.

**RESUMO** - A interferência entre sistemas de raízes ocorre abaixo da superfície do solo muito antes que a competição entre plantas se expresse na parte aérea. Entre os recursos de competição, os nutrientes são destacados pela limitação nos solos, tornando necessária a suplementação, especialmente do fósforo (P). Dessa forma, objetivou-se neste estudo estimar, a partir da sementeira, características visuais nos sistemas de raízes do feijoeiro (*Phaseolus vulgaris*) de variedade Carioca, em competição com braquiária (*Urochloa brizantha*), considerando-se ainda a aplicação de fósforo  $P_2O_5$ . Utilizou-se esquema fatorial com seis tratamentos, resultantes da combinação de três esquemas de cultivo (feijoeiro em cultivo isolado ou em competição com uma ou duas plantas de braquiária) e aplicação ou não de adubação fosfatada ( $90 \text{ kg ha}^{-1}$  de  $P_2O_5$ ), conduzido em delineamento inteiramente casualizado em casa de vegetação com ambiente controlado, com quatro repetições. Após a sementeira das espécies, os resultados da visualização foram tomados a cada 12 horas, por meio do registro fotográfico do desenvolvimento do sistema radicular em rizotrons. Foi observado maior crescimento e área total do sistema radicular quando em monocultivo e em competição com uma planta de braquiária

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Received: September 26, 2017

Approved: December 11, 2017

Planta Daninha 2019; v37:e019185690

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*em presença de adubação fosfatada. Em contrapartida, o feijão em recipiente com duas plantas de braquiária investiu recursos em maior comprimento e área total do sistema radicular em ausência do fertilizante. Pode-se afirmar a existência de indícios da interferência a partir de três dias após a semeadura das espécies.*

**Palavras-chave:** competição, rizotron, plantas daninhas.

## INTRODUCTION

Understanding the competition between plant species is fundamentally important in farming systems, especially when there is an association (whether intentional and/or as a consequence of competition) between plants with different characteristics and competitive abilities. Such competition occurs both below and above the soil and has been reported as depending on the species, the climate, the soil conditions and the edaphoclimatic association (Zanine and Santos, 2004).

A considerable percentage of the plant growth reduction for the main agronomical crops, such as beans, and a consequent financial loss result from the space competition with groups of weeds that occupy the same area in a certain period of time (Raventós and Silva 1995).

In the root competition below the soil, phosphorus (P) has an important role, and it is an essential element for the plant metabolism, since it acts in the cell energy transfer, inbreathing, photosynthesis and the structural components of the nucleic acids of the chromosomes, among other processes that occur in the plant (Zucareli et al., 2006). The crops usually respond to fertilization at adequate levels, however, upon its absence or excess, its production potential is restricted, especially in tropical environments with weathered soils, in which there is a strong adsorption of P in the soil, requiring high doses for a satisfactory productivity (Novais and Smith, 1999).

Therefore, it is important to define some concepts, such as the period previous to the interference (PAI), which determines the period after sowing in which the infesting community may coexist with the crop with no damages to the production (Oliveira, 2010).

For bean plants, some authors showed that PAI may last between 7 days in the soil with 87 mg dm<sup>-3</sup> of P and clay at 27% (Oliveira et al. 2010) up to 17 after emergence in the field with Dark Red Latosol, with 26 mg dm<sup>-3</sup> of P and clay at 380 g kg<sup>-1</sup> (Salgado et al., 2007). Thus, to determine the intensity of the interference between weeds and the crop, it is necessary to evaluate the critical interference periods, which vary according to the edaphoclimatic conditions of each region and the characteristics of the weed and the crop (Pitelli and Pitelli, 2004).

Assuming the periods previous to the interference as those described in the literature is practical and ensures a more satisfactory production. However, any possibility of obtaining information as to the initial stimulation after germination – such as early changes to the architecture of the root system in the interference between plants, which would trigger the competition process – must be sought, since it will allow an adequate genetic manipulation of the plants, in the search for new strategies for the absorption and use of this nutrient, improving the exploitation of natural resources and increasing the chances of benefiting the vegetable group of interest.

The objective of this study was to characterize the initial interference of the root system of bean plants with brachiaria, in a crop with P fertilization.

## MATERIAL AND METHODS

The experiment was conducted between August and September 2015 in a greenhouse with a controlled environment, with minimal temperature of 15 °C and maximal temperature of 31 °C. The substrate used was constituted by samples of Red Latosol with sandy texture with the following chemical characteristics: pH in water at 6.60; 25 mg dm<sup>-3</sup> of P; 276.36 mg dm<sup>-3</sup> of K; 5.59 cmolc dm<sup>-3</sup>

of  $\text{Ca}^{2+}$ ; 1.71  $\text{cmolc dm}^{-3}$  of  $\text{Mg}^{2+}$ ; 8.01  $\text{cmolc dm}^{-3}$  of SB; 10.11  $\text{cmolc dm}^{-3}$  of CTC(T); 79% of V; and 2.50  $\text{dag kg}^{-1}$  of organic matter.

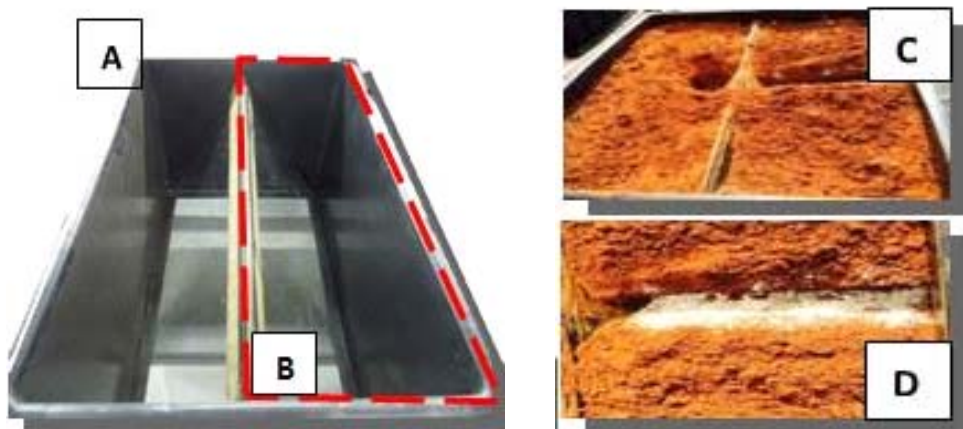
A completely randomized design was used with four replications, in a factorial with six treatments, resulting from the combination of three crop schemes: beans in isolated crops or in competition with one or two brachiaria plants (Table 1) and the application or not of phosphate fertilization ( $\text{P}_2\text{O}_5$ ).

**Table 1** - Suggested treatments to evaluate the competition of beans and brachiaria plants during the initial phase, due to the phosphate fertilization<sup>(1)</sup>

Treatment	Left size of the bean plant sowing groove <sup>(2)</sup>	Bean plant sowing groove	Right side of the bean sowing groove <sup>(2)</sup>
01	-	Bean plant without fertilizer	-
02	-	Bean plant without fertilizer	Brachiaria
03	Brachiaria	Bean plant without fertilizer	Brachiaria
04	-	Bean plant with fertilizer	-
05	-	Bean plant with fertilizer	Brachiaria
06	Brachiaria	Bean plant with fertilizer	Brachiaria

<sup>(1)</sup> Application of 5 g of simple superphosphate by groove ( $18\%\text{P}_2\text{O}_5$ ), equivalent to  $90\text{kg ha}^{-1}$ . <sup>(2)</sup> 10 cm of distance from the bean sowing groove.

The bean variety used was the Carioca variety, while *Urochloa brizantha* was used. The phosphate fertilizer in the form of simple superphosphate was applied to the central plating groove at a dose of  $90\text{kg ha}^{-1}$ , considering the rhizotron area, with  $35.5 \times 56 \times 30.5\text{ cm}$  and volume of  $60\text{ cm}^3$ . The application of P was transversal to the visualized roots and applied in the groove (5 mg) by box (Figure 1).



**Figure 1** - PVC box with high density, divided in two rhizotrons (A). Space to be filled with a soil sample to cultivate bean and brachiaria vegetable species ( $25\text{ dm}^3$ ), showing the glass window to observe the growth and development of the root system (B). Rhizotron already filled with substrate and indication of the groove for fertilization with simple superphosphate at a dose of 5 g, corresponding to  $90\text{kg ha}^{-1}$  of  $\text{P}_2\text{O}_5$ , to the right, or location where the seed will be placed with no fertilization in the rhizotron, to the left (C). Fertilized groove ready for bean sowing (D).

For the visual evaluation of the root emission and to analyze the first visible signs of competition, the substrate was placed in in rhizotron rectangular boxes, especially made, in which two rectangular glasses ( $55.5 \times 31.5\text{ cm}$ ) were arranged in an inverted V position in the center of the box that had a hole underneath, making it possible to see the spatial distribution of the roots of the plants. This was a handcrafted technique, based on the work by Zandavalli (2006), with the difference that it offered a bilateral view. The rhizotron allows the monitoring of the root growth and of the rhizosphere of the plant, in a non-destructive manner.

*Brachiaria* was sown first and, then, two days later, the bean plant was sown, that is, simulating the presence of the weed in the area. The plants had a 12 cm spacing between them, measured with a graduated scale. On the day after the crop had been sown, a photographic record was taken, at every 12 hours, of the development of the root system of bean and *brachiaria* plants. The photographic results were tabulated from the images of the viewing windows, equipped with a numeric scale (cm), avoiding any mistakes in the observed size. The measurements were taken up to 180 hours after sowing.

The irrigation system used was the dripping type, with a flow of 0.032 L min<sup>-1</sup> and irrigation twice a day, for 20 minutes.

After the processing of the images, the soil occupation by the root systems was estimated by the Image Pro Plus® software combining image contrasts.

After 30 days of coexistence, the average values of the variables evaluated, such as total area of the root system, height, number of leaves, dry mass and growth of the bean plant roots, were compared by variance analysis, after the normality and homoscedasticity presuppositions were tested, and the significant ones were tested according to Tukey's test at 5% of significance in the SISVAR statistical program. Regarding the evolution of the root length throughout the hours after sowing, the regression curves were adjusted.

## RESULTS AND DISCUSSION

No effects of the fertilization were observed on the initial length of the roots eight days after sowing regarding bean plants in monocrops (Figure 2). It is important to consider the time in which the infesting vegetation started to be controlled and the period in which it could no longer influence the productivity. In this study, emphasis was given to the root system, searching for the first visual evidence that would occasionally indicate the start of the competition, considering that the exudates released by the root have an important role in the beginning of the mediations of biological communication, therefore, the start of the competition (Chen et al., 2012).

Despite the rather homogeneous growth, when in monocrops, a significant variation in the length of the roots of plants grown in a substrate fertilized with P was observed (Figure 2).

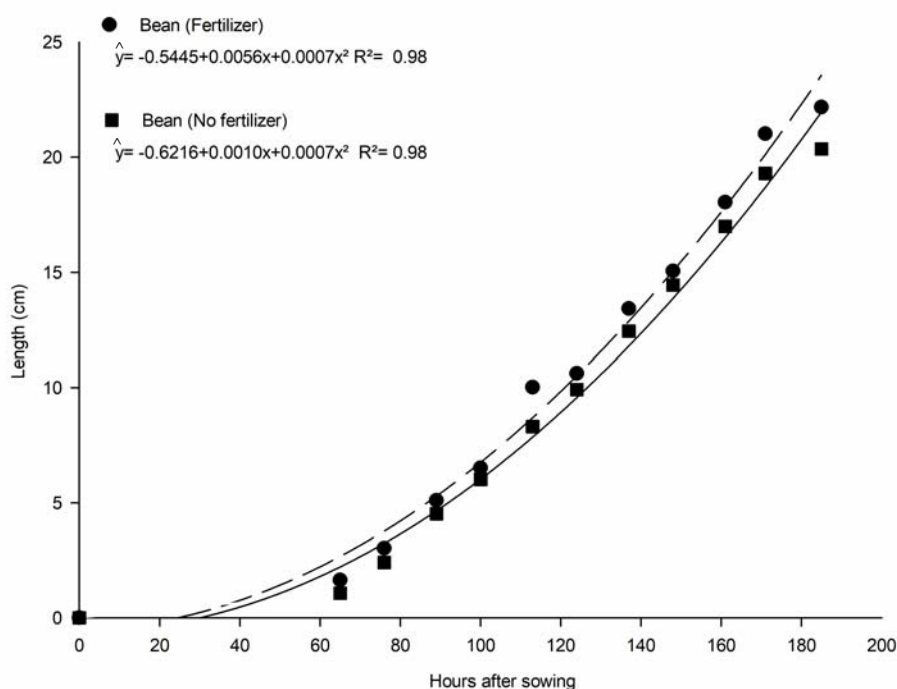


Figure 2 - Root length (cm h<sup>-1</sup>) of isolated *Phaseolus vulgaris*, due to the phosphate fertilization (equivalent to 90 kg ha<sup>-1</sup> of P<sub>2</sub>O<sub>5</sub>).

During the initial phase proposed for the research, the length measurements of the roots of the bean plant in competition with a brachiaria plant were not significant, regardless of the phosphate fertilization. The fertilizers with balanced nutrient doses according to the recommendations for each crop are usually the main responsible factor for positive effects on the growth and activity of the roots (Gregory, 1994). However, such variation may be more intense under competition conditions.

Limitations since the beginning of the vegetative cycle for bean plants may result in growth restrictions, severely limiting the production. The lack of P during a later period has less impact in the crop production than at the beginning of the cycle (Grant et al., 2001).

Such limitation does not occur in the roots of the bean plant up to 180 hours after sowing, even with the presence of a brachiaria plant (Figure 3). The limitation in the phosphorus availability may lead to serious damages to the vegetable metabolism. Usually, the low concentration of P in the soil solution, in comparison to the demand of the plant for the nutrient, induces the diffusive process, in which the nutrient is exhausted in the region near the root and through diffusion, the plant is able to meet its demand (Mundus et al., 2017).

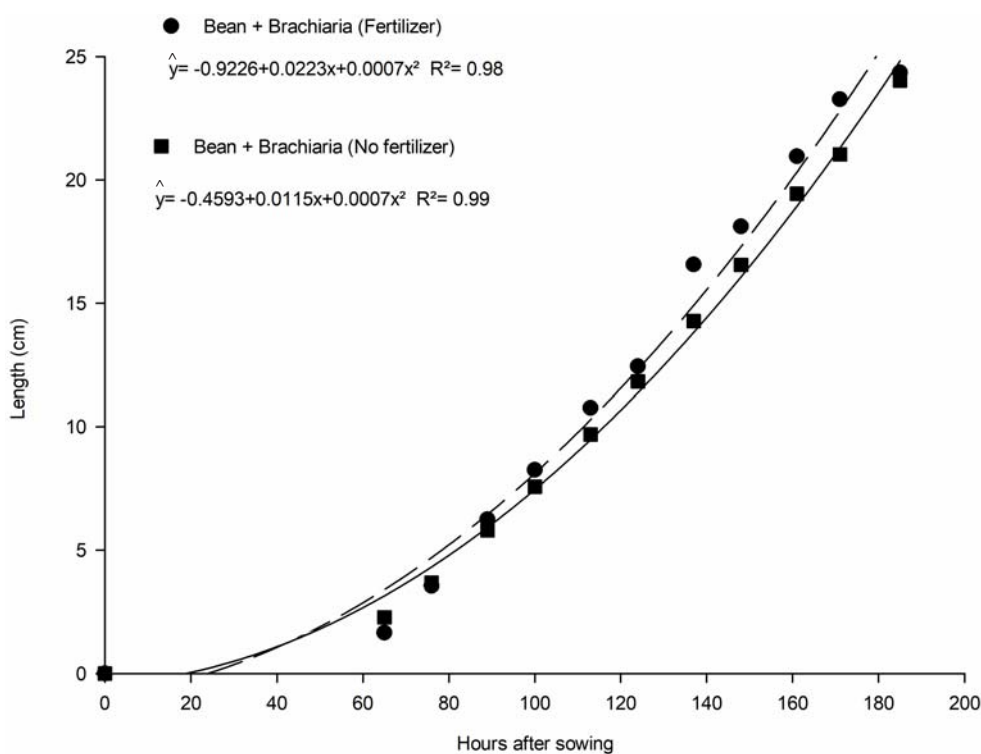


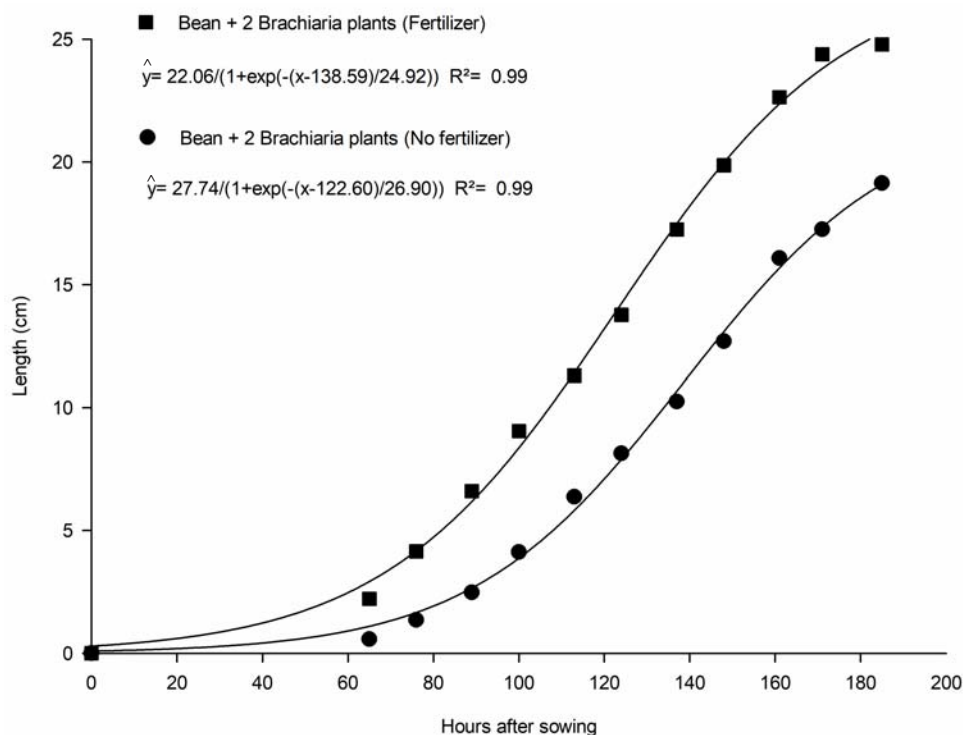
Figure 3 - Length of the roots of *Phaseolus vulgaris* (cm h<sup>-1</sup>), in competition with one *Urochloa brizantha* plant, due to the phosphate fertilization.

It is possible that, during this initial phase, the effective length of the roots is more influenced by the reserve of the seeds that ensures the viability of the seedlings. For example, seeds with greater reserve tissues produce more vigorous seedlings; therefore, larger seeds tend to emerge more rapidly (Carvalho and Nakagawa, 2000). Such assurance granted by the reserve in relation to the space explored by the roots observed in this study was not affected by the presence of a brachiaria plant up to eight days after sowing. Thus, in relation to the P fertilization or not of the substrate and the presence of another vegetable species (*Urochloa brizantha*), at low density, there are no changes to the length of the bean plant roots during the initial developmental phase.

According to Jakelaitis et al. (2006), among the factors related to competition, the density of the plants has a fundamental role. In studies associated to plant competition, the greater the density of the weeds, the larger will be the number of individuals fighting for the same resources, thus, resulting in an intense competition endured by the crop of interest.

According to Guilherme (2000), the remarkable reduction in the growth of the species, both in intra- and interspecific competition, results from the competition for space among groups of plants that occupy the same location during a certain time. Thus, when evaluating the length of the bean plant roots, due to the competition with two brachiaria plants, a different response was observed for the fertilized substrate.

A greater growth of the root system of the bean plant was observed on plants developed in a non-fertilized substrate. For example, at seven days after sowing, the root growth of the bean plant was 23 and 16 cm, with the presence and absence of P, respectively, indicating that the combined effect of the competition with *Urochloa brizantha* and the lack of P may promote a greater initial growth of the roots (Figure 4).



**Figure 4** - Length of the roots of *Phaseolus vulgaris* (cm h<sup>-1</sup>), in competition with two *Urochloa brizantha* plants, due to the phosphate fertilization.

Some studies evaluated according to the field conditions and in vases found damages due to the Competition between beans and brachiaria, usually measured at the beginning of the critical competition period (PCPI), identified as of 15 days after sowing (Salgado et al., 2007; Concenço, 2013). In all these studies, the damaging effect is explained by the reduction on the available resources, establishing the competition.

In this study, even before the emergence of the seedlings, the greater initial growth of the roots of the bean plant in rhizotron was observed, where they were sown with two other brachiaria seeds. During the initial stages in which the root length was measured, it may be stated that the stimulation for a greater growth in the case of the bean plant is not directly related to the lack of resources. It may be observed that the increase in the density on brachiaria seedlings in the absence of phosphate fertilization promotes a greater initial growth of the bean plant roots.

According to Silva et al. (2009), through the signs of the plant, it is possible to realize the lack of the nutrient and establish changes to the architecture of the root, with the purpose of increasing the absorption. López-Bucio et al. (2002) suggest that the signalization may occur due to the production and transportation of auxin. Takei et al. (2001) state that the signaling upon the absence of phosphorus and nitrogen occurs due to the production of cytokinins. This perception occurs during the initial development period of the plants, as suggested in this study,

in relation to the bean monocrop, or even with the presence of one brachiaria seedling. However, this was observed a few days after sowing, when the roots of the bean plant grew between two brachiaria seedlings, even if visually distant from them. On the other hand, assuming the low mobility of P in the soil – Novais and Smith (1999) indicate an average distance of  $0.013 \text{ mm day}^{-1}$  – the hypothesis on other mechanisms that would cause the initial stimulation to the bean plant roots preceding the competition with brachiaria at a higher density is wise.

Factors related to exudated chemical compounds or even resulting from the interactions between the roots and microorganisms may explain the fast reaction of the bean plant to the presence of the two brachiaria plants during this initial phase. The consequences of the interspecific competition of the brachiaria, as well as the intensity of the competition, are widely studied and reported for bean plants (Freitas et al., 2009; Cury et al., 2011; Manabe et al., 2015). The *Brachiaria* genus is largely influenced by the competitiveness with the bean plant at 45 days after emergence, negatively affecting the nutrition of the crop, as well as the vegetative components, number of leaves, stem and, mainly, the root system, a fact that may also be correlated to the change in the morphology and distribution of the bean plant roots (Cury et al., 2013).

It is pointed out that these studies emphasize that the reason for the Competition is the reduction of the resources in the environment (mainly water and nutrients), affecting the periods considered as “critical interference periods”. One interesting aspect about this research is the initial growth phase, in which it may be stated that the root systems have not yet occupied the space corresponding to the exhaustion of the water and nutrient resources, even if the effect has been observed on the non-fertilized substrate.

The behavioral response of the roots refers to the allelochemical substances, produced and released by vegetable species, both crops and weeds, which may be granted by the producing plant and be carried up to the receiving plant by leaching. The exudations of the roots may be responses to biotic and abiotic stress factors (Inderjit and Nielsen, 2003). The exudated compounds are: ions, free oxygen, enzymes, mucilage and several primary and secondary compounds (Bertin et al., 2003).

Under nutritional and hydric stress situations, for example, an increase in the volume of root exudates occurs, as mentioned by Liebersbach et al. (2004), changing the physical-chemical properties and the biological compositions of the rhizosphere through a series of mechanisms and the release of root exudates, directly influencing the availability of nutrients, as well as indirect effects, through interactions with soil microorganisms (Richardson et al., 2009).

Thus, in this study, the likely response of a greater length of the roots results from the rhizosphere chemical signals produced with a greater effectiveness by the bean plant under a stressful situation due to the lack of P. Under this condition, the presence of two brachiaria plants may trigger an stimulation for root increase. Such perception is only possible due to the larger amount of root exudates, both in the crop and in the two brachiaria plants.

The microorganisms in the rhizosphere are involved in several processes, such as nutrient solubilizers, as stated by Gyaneshwar et al. (2002); they also acted as antagonists to pathogens and in the production of growth-promoting substances, among others (Massenssini et al., 2014). Thus, in addition to the presence of chemical compounds indicating the presence of plants, some microorganisms may act in the communication among the root systems, for examples, through hyphae that connect vegetable species (Rodríguez and Fraga, 1999; Coelho et al., 2007).

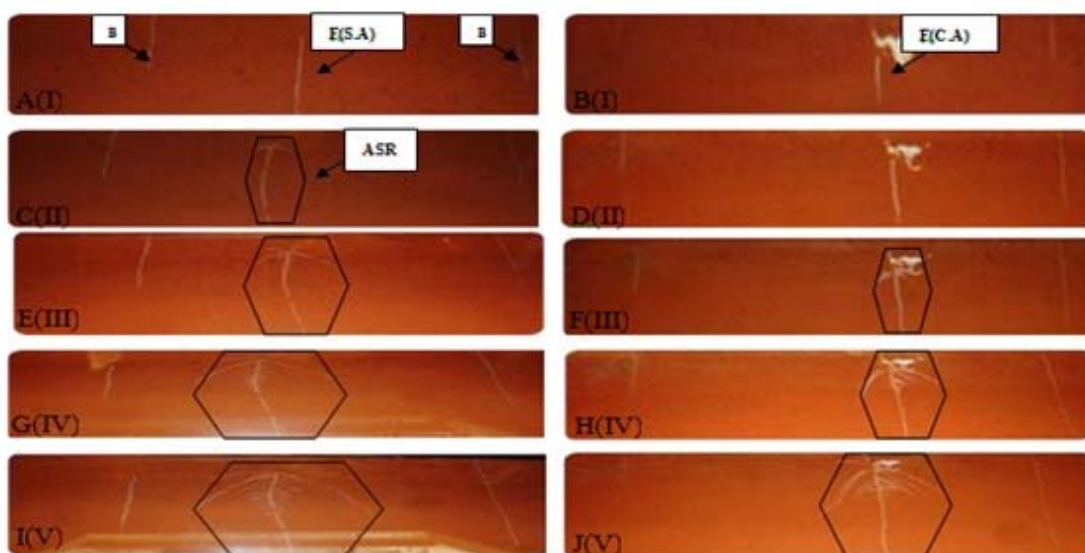
Another comparison method suggested was the total area of the root system. It is known that the optimized exploration of the rhizosphere environment depends, among other factors, on the better distribution of the roots on the soil profile. By observing the area of the root system of the plants under competition, a greater area was observed when the density of the brachiaria plants increased, with values of  $46.30 \text{ cm}^2$  and  $66.03 \text{ cm}^2$ , respectively, with the absence of fertilization (Table 2 and Figure 5).

The architectonic plasticity results from individual responses from each meristem, thus allowing that the growth of the root system reacts in the most adequate manner to the lack of uniformity of the soil, in relation to the P availability (Silva et al., 2009).

**Table 2** - Total area of the bean plant root system (cm<sup>2</sup>), 161 hours after sowing with and without phosphate fertilization, in three plating schemes (monocrop or with one or two brachiaria plants)

Fertilization	Bean planting scheme <sup>(1)</sup>		
	F	F + B	B + F + B
Presence	37.81 aA	40.33 aA	35.48 bA
Absence	39.69 aA	46.30 aA	66.03 aB
VC (%)	20.28		

<sup>(1)</sup> Planting schemes: F: bean plant; B: brachiaria (*Urochloa brizantha*). Averages followed by the same uppercase letter in the same row and lowercase letter in the same column are not statistically different from each other according to Tukey's test at 5% of error probability.



**Figure 5** - Photographic record, by hour, of the growth and area of the root system of bean plants in competition with two brachiaria plants, with the absence and presence of P, respectively, after sowing in hours: 65(I), 89(II), 113(III), 137(IV) and 161(V). Br, brachiaria; F(SA), bean with no fertilizer; F(CA), bean with fertilizer; ASR, area of the root system.

P has a slow diffusion rate; therefore, the exhaustion of this nutrient in the proximities of the roots increases the importance of the morphology and geometry of the root system in order to maximize its absorption (Raj, 1991). Under adverse conditions, such as in soils with a scarce presence of nutrients, plants with adaptative characteristics show, for example, greater root growth, allowing a better exploration of the soil (Schachtman et al., 1998). Thus, the explanation for the observed different is in agreement with that offered for the greater length of the roots, that is, it is related to the root exudates and/or an occasional microbial signalization in environments with two brachiaria plants and the absence of phosphate fertilization.

At the end of 30 days of coexistence, the height, number of leaves and the dry mass of the bean plants were measured and damaging effects were observed both for the competition and the absence of phosphate fertilization. In monocrops or in competition with one brachiaria plant, damages were observed in the height of the plants, the number of leaves and the dry mass of the shoot in the absence of phosphate fertilization (Table 3). However, no differences were observed between the monocrop or the competition with the grassy weed, in each type of substrate.

The bean plants crop with the presence of two brachiaria plants had a negative effect on the height, the number of leaves and the dry mass of the bean plant in both substrates. In the case of the shoot dry mass, the final effect was a result of the sum of the effects of the competition with two brachiaria plants and the lack of phosphate fertilization, and the lowest value was observed with 1.07 g per plant.

For the scheme with the bean plant and two brachiaria plants, the estimated effects were observed as consequence of the changes presented by the plants way before the roots got physically close. Thus, it is confirmed that the greater length of the roots and the increase in the soil



**Table 3** - Average values for height, number of leaves and dry mass of bean under the effect of fertilization and competition 30 days after sowing

Fertilization	Planting schemes <sup>(1)</sup>					
	F	F+B	B+F+B	F	F+B	B+F+B
	Height (cm)			Number of leaves (units)		
Presence	16.05 aA	15.5 5 aA	11.42 aB	6.75 aA	7.50 aA	4.00 aA
Absence	10.10 bA	10.32 bA	10.4 5aA	4.50 bA	4.50 bA	4.00 aA
VC (%)	20.70			29.56		
	Dry mass (g per plant)					
Presence	2.98 aA		2.83 aA		2.38 aA	
Absence	1.22 bA		1.34 bA		1.07 bA	
VC (%)	39.46					

<sup>(1)</sup> Planting schemes: F: bean plant; B: brachiaria (*Urochloa brizantha*). Averages followed by the same uppercase letter in the same row and lowercase letter in the same column are not statistically different from each other according to Tukey's test at 5% of error probability.

exploration area were the first visible signs of the competition that would be established before the 30 days of coexistence.

From what has been shown, it may be stated that the height, number of leaves and shoot dry mass variables for the crop were higher, which may be considered as a benefit of the application of phosphate fertilization. This fact reaffirms the importance of phosphorus to the metabolism of the plants, performing a fundamental role in the energy transfer of the cell, in breathing and photosynthesis.

Brachiaria is considered as a species with high competition capacity, since it negatively affects all vegetable components of the bean crop (Cury et al., 2011). According to Teixeira et al. (2009), the bean plant has a low competitive capacity, exposing the crop to an intense interference by weeds.

In this study, it was clear that, way before the competition was established, factors that are probably connected to root exudates or even to the interaction with microorganisms may signalize the future competition for soil resources, causing the bean plant roots to grow and better explore the region around the rhizosphere.

The results may lead to the conclusion that the first approach that starts the competition between the plants is preceded by the signalization in the soil, due to exudates of the rhizosphere that would defend the plant, promoting a greater growth and explored area of the bean plant root system during an initial phase, even before the roots got physically close. For such, the study synthesizes information from principles regarding plant interference, indicating new possibilities for new studies on which are the exudates that are part of this signalization.

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