ISSN: 1984-5529 Científica Jaboticabal v.46, n.1, p.17–29, 2018

http://dx.doi.org/10.15361/1984-5529.2018v46n1p17-29

Pseudomonas spp. as growth promoting agents of sunflower and jack bean in soil with sulfentrazone

Pseudomonas spp. como agentes promotores de crescimento de girassol e feijão-de-porco em solo com sulfentrazone

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Recebido em: 12-01-2017; Aceito em: 24-08-2017

Abstract

Bacteria that present potential to promote plant growth can bring direct benefits both to the remediating plants and to the soil in the sulfentrazone bioremediation process. In view of this, the objective of this study was to evaluate the inoculation response of bacteria of the genus *Pseudomonas* in soil contaminated with the herbicide sulfentrazone on the growth of the remediating species jack bean (*Canavalia ensiformis*) and sunflower (*Helianthus annuus*). The experiment was conducted in a greenhouse in a completely randomized design with four replicates. For each phytoremediating species, three factors were considered, the first being the presence or absence of inoculation with selected bacterial consortium, the second consisting of cultivation with the same species or with the other species, and the third corresponding to the bioremediation time (25, 45, 65 and 85 days after thinning). The leaf area and the dry matter of roots, stem and leaves were evaluated at the end of each time and for each of the species. In general, both remediating species showed lower growth when cultivated together with a plant of the same species. In most of the evaluations, inoculation provided greater accumulation of leaf area and dry matter of leaves, stem and roots of *Canavalia ensiformis* and *Helianthus annuus*, with increases varying from 18 to 55%. The results showed the ability of *Pseudomonas* spp. isolates to promote the growth of plants cultivated in soil with sulfentrazone.

Additional keywords: *Canavalia ensiformis*; *Helianthus annuus*; herbicide; plant growth-promoting bacteria.

Resumo

Bactérias que apresentam potencial para promover o crescimento vegetal podem trazer tanto benefícios diretos às plantas remediadoras quanto ao solo no processo de biorremediação do sulfentrazone. Diante disso, objetivou-se avaliar a resposta da inoculação de bactérias do gênero *Pseudomonas* em solo contaminado com o herbicida sulfentrazone sobre o crescimento das espécies remediadoras feijão-de-porco (*Canavalia ensiformis*) e girassol (*Helianthus annuus*). O experimento foi conduzido em casa de vegetação, no delineamento inteiramente casualizado, com quatro repetições. Para cada espécie fitorremediadora, consideraram-se três fatores, sendo o primeiro a presença ou a ausência de inoculação com consórcio bacteriano selecionado; o segundo, o cultivo com a mesma espécie ou com a outra espécie, e o terceiro, o tempo de biorremediação (25; 45; 65 e 85 dias após o desbaste). Avaliaram-se, ao final de cada tempo e para cada uma das espécies, a área foliar e a massa da matéria seca da raiz, do caule e de folhas. De modo geral, ambas as espécies remediadoras apresentaram menor crescimento quando cultivadas juntamente com uma planta da própria espécie. Na maior parte das avaliações, a inoculação proporcionou maior acúmulo de área foliar, massa da matéria seca de folhas, caule e raiz de *Canavalia ensiformis* e *Helianthus annuus*, com aumentos variando de 18 a 55%. Os resultados encontrados comprovam a capacidade de promoção de crescimento das plantas cultivadas em solo com sulfentrazone pelos isolados de *Pseudomonas* spp.

Palavras-chave adicionais: bactérias promotoras de crescimento de plantas; *Canavalia ensiformis*; *Helianthus annuus*; herbicida.

Introduction

Within bioremediation, phytoremediation is one of the most studied techniques, standing out due to the low cost and efficacy in decontaminating soils with herbicide residues. The application of this technique has been consolidated and boosted by the increasing number of studies that have shown the possibility of using plants to reduce the possible negative impacts of herbicides to susceptible crops sown in sequence (Pires et al., 2006; Procópio et al., 2007; Procópio et al., 2009).

Herbicides that have a long residual effect on the soil can cause carryover problems, making it infeasible to grow sensitive species in succession, which is the case of sulfentrazone (Artuzi & Contiero, 2006; Dan et al., 2010). Moreover, the permanence of the product in the soil for a long time (Blanco et al., 2010) increases the risks of leaching (Melo et al., 2010a) and contamination of groundwater (Santos et al., 2015). In this sense, the concern is to prevent or remedy the negative effects of the presence of this herbicide in the soil.

Sulfentrazone(N-{2,4-dichloro-5-[4-(difluoromethyl)-4,5-dihydro-3-methyl-5-oxo-1H-1,2,4-triazol-1-yl]phenyl}methanesulfonamide) is an herbicide that has residual soil activity (Melo et al., 2010b; Blanco et al., 2010) and is recommended for pre-emergence application in soybean, sugarcane, coffee, eucalyptus, citrus, tobacco and pineapple crops (AGROFIT, 2017). It presents the following physicochemical characteristics: high solubility in water (780 mg L^{-1} at pH 7.0), low vapor pressure (1 x 10^{-9} mmHg at 25 ºC), low sorption (Koc = 43), dissociation constant (PKa) of 6.56 and partition coefficient (Kow pH7) of 9.79 (IUPAC, 2017), with microbial degradation in the soil as the major degradation pathway (Martinez et al., 2010).

In Brazil, although studies on the phytoremediation of herbicide-contaminated environments have gained prominence and diffusion in the scientific community, the association between plants and bacteria in the bioremediation of contaminated soils is still little investigated.

Canavalia ensiformis, commonly known as jack bean, a legume commonly used as a green fertilizer and with a potential to remediate different herbicides, and *Helianthus annuus* (sunflower), an oilseed crop grown in several regions of Brazil, were selected as sulfentrazone-remediating plants and tested for the capacity and efficiency in reducing its toxic residues in the soil, through bioassays (Belo et al., 2011; Madalão et al., 2012).

Many species of bacteria, most associated with the rhizosphere, have demonstrated the ability to promote plant growth, being called plant growth-promoting (rhizo)bacteria. These bacteria can induce the growth and development of plants, directly or indirectly. The direct influence includes the production of phytohormones, such as indole-acetic acid, auxins and gibberellic acid (Ahemad & Khan, 2012); phosphate solubilization and production of siderophores (Jha et al., 2009a); and biological nitrogen fixation (Jha et al., 2009b). Indirectly, these bacteria can act as biological control agents of phytopathogens, releasing substances such as β-glucanases, proteases, cellulases, chitinases and hydrocyanic acid (Almaghrabi et al., 2013; Hernández-León et al., 2015).

Several genera and species of bacteria have already been reported with such ability. Genera such as *Bacillus*, *Pseudomonas*, *Rhizobium*, *Klebsiella*, among others, have demonstrated growth promoting action in perennial and annual agronomic species (Sharma et al., 2011; Zucareli et al., 2011; Almaghrabi et al., 2013). The inoculation of maize seeds with isolates of *Pseudomonas* and *Azospirillum* promoted plant growth at the field level, increasing height, seed weight, number of seeds per ear, leaf area, as well as the dry matter (Gholami et al., 2009). Greenhouse and field experiments demonstrated that the inoculation with *Pseudomonas jessenii* PS06 and *Mesorhizobium ciceri* C-2/2 increased the productivity of *Cicer arietinum* (chickpeas), being 52% higher than in the uninoculated treatments (Valverde et al., 2006).

While recognizing the importance of microorganisms in the degradation of herbicides in the soil, research involving their inoculation into contaminated soils is still scarce. The selection of sulfentrazone-degrading bacteria (Melo et al., 2017), combined with their possibility of acting as plant growth promoters, can increase the chances of success and optimization of the bioremediation process. Given the above, the objective of this study was to evaluate the inoculation response of bacteria of the genus *Pseudomonas* in sulfentrazone-contaminated soil on the growth of jack bean (*Canavalia ensiformis*) and sunflower (*Helianthus annuus*).

Material and methods

The experiment was conducted in a greenhouse from April to July 2014. Each experimental unit corresponded to pots with a capacity of 12 dm^3 (28.5 cm in diameter and 30 cm in height), filled with 10 kg of a sample of the surface horizon of a dystrophic Red-Yellow Argisol with sandy loam texture (25% clay, 12% silt and 63% sand) (EMBRAPA, 2013), with the following chemical characteristics: pH (water) 6.1; organic matter 4.12 dag kg^{-1}; P and K 6.9 and 200 mg dm^{-3}, respectively; Ca, Mg, Al, H + Al and effective CEC 3.7, 1.0, 0.0, 1.15 and 5. 21 $cmol_c$ dm^{-3}, respectively. Before filling the pots, the soil was previously fertilized with ammonium sulfate (0.20 g dm^{-3} N) and simple superphosphate (1.80 g dm^{-3} P_2O_5).

Subsequently, sulfentrazone was applied with a CO_2 constant pressure backpack sprayer, coupled with a bar with two TT110 02 fan-type tips, spaced 0.5 m, working at a pressure of 250 kPa, with syrup volume of approximately 140 L ha^{-1}, at 0.5 m from the soil, at the dose of 1000 g ha^{-1} a.i. This dose is commonly applied in the sugarcane crop for the control of tiririca

(*Cyperus rotundus*). The climatic conditions at the time of application were T = 27 ºC, RH = 72% and wind speed of 1.9 km h^{-1}.

After application of the herbicide, sowing of the sulfentrazone-remediating species jack bean (*Canavalia ensiformis*) (Madalão et al., 2012) and sunflower (*Helianthus annuus*) (cultivar Tera 860 HO) (Belo et al., 2011) was performed. Sowing was carried out at 5 cm depth using 6 seeds per pot. Thinning was done 15 days after sowing leaving two plants of the same species or one of each (mixed cultivation) in each experimental unit. Manual irrigation was performed daily as needed.

Half of the pots were inoculated with a bacterial consortium selected based on the potential degradation of the isolates, according to a previous study (Melo et al., 2017). The consortium consisted of six isolates identified as *Pseudomonas putida*, *Pseudomonas lutea*, *Pseudomonas plecoglossicida* and three isolates of *Pseudomonas* sp. These were grown separately in nutrient broth (1.0 L water; 2.0 g Na_2HPO_4; 3.0 g NaCl; 3.0 g meat extract; 5.0 g peptone; pH 6.8) for approximately 10 h at 30 °C and 150 rpm until optical density of 0.6. The medium was centrifuged (5000 rpm, 5 min, 4 °C) and the bacterial cells suspended in saline solution (0.85% NaCl). The consortium was established by pipetting solution volumes of each of the six isolates in order to guarantee equal amounts of colony forming units (CFU mL^{-1}) of each species, totaling 12 mL of solution and inoculation of 4.5 x 10^4 CFU g^{-1} soil in the corresponding treatments after thinning.

The experimental design was completely randomized, with four replications. The treatments, for each phytoremediating species, consisted of the combination of three factors. The first factor consisted of the presence or absence of inoculation with a selected bacterial consortium, the second factor consisted of the cultivation with the same species or with the other species, and the third corresponded to the bioremediation time (25, 45, 65 and 85 days after thinning (DAT)). In the aforementioned bioremediation times, the jack bean plants (*Canavalia ensiformis*) were in the vegetative stages V2 to V3, V4 to V5, V6 to V7 and V8 to V9, and the sunflower plants (*Helianthus annuus*) in the stages V16 to V18, R1 to R3, R5.1 to R5.8 and R5.10 to R6, respectively.

Jack bean and sunflower plants were cultivated for 25, 45, 65 and 85 DAT, and at the end of each period, the leaves, the stem (at ground level) and the root system were collected. Leaf area (LA - cm^2/plant) was determined using a leaf area meter (Li-Cor, model LI-3100). The plant material was conditioned separately in paper bags and placed in a forced-air oven at 65 ± 2 °C until reaching a constant mass, to obtain the dry matter of leaves (DML – g $plant^{-1}$), stem (DMS – g $plant^{-1}$) and roots (DMR – g $plant^{-1}$) in a semi-analytical precision balance.

The data were submitted to analysis of variance by the F test at 5% probability. The effects of the bioremediation times were evaluated by regression analysis and the choice of the models were based on the significance of the coefficients, the biological phenomenon and the coefficient of determination. The regression model identity test was used (Littell et al., 2006).

Results

Sunflower

As a result of the factors interaction, the leaf area accumulated by the sunflower plant was higher when it coexisted with a jack bean plant for 45 DAT in soil inoculated with bacterial consortium. The same behavior was evidenced in the plants grown for 65 and 85 DAT, regardless of soil inoculation (Table 1).

Table 1 - Leaf area accumulated by sunflower plant in cultivation with the same specie or with jack bean, in soil with sulfentrazone, in the absence and presence of bacterial consortium.

Cultivation	[(1)]Leaf area (cm^2 $plant^{-1}$)	
	With inoculation	Without inoculation
	25 DAT[(2)]	
Sunflower + Sunflower	1451.67Aa	1470.82Aa
Sunflower + Jack bean	1441.30Aa	1802.31Aa
	45 DAT	
Sunflower + Sunflower	3981.83Ab	4029.00Aa
Sunflower + Jack bean	5380.00Aa	3719.50Ba
	65 DAT	
Sunflower + Sunflower	4512.67Ab	4653.17Ab
Sunflower + Jack bean	6320.65Aa	5990.67Aa
	85 DAT	
Sunflower + Sunflower	3455.00Ab	3443.67Ab
Sunflower + Jack bean	5713.31Aa	5330.00Aa
CV (%)	10.84	

[(1)] Means followed by the same lowercase letters in the column and upper case in the row, for each time, do not differ by Test F (p> 0.05). [(2)]DAT: days after thinning.

Positive effect of soil inoculation with bacteria of the genus *Pseudomonas* on the leaf area accumulation of sunflower was verified when it coexisted with the legume for 45 DAT (Table 1).

As a result of the unfolding of the cultivations and inoculation within the bioremediation times, it was observed identity between the sunflower + sunflower models with and without inoculation, being presented in a common curve. These treatments accumulated, over time, a smaller leaf area than those consisting of the mixed cultivation of sunflower + jack bean (Figure 1). Sunflower plants, in the presence of a jack bean plant and inoculation, stood out against the other combinations and accumulated a maximum leaf area of 6550.52 cm^2 $plant^{-1}$ at 67 DAT (Figure 1). For all treatments, the leaf area data fitted to the quadratic polynomial model, increasing until reaching a maximum point (Figure 1).

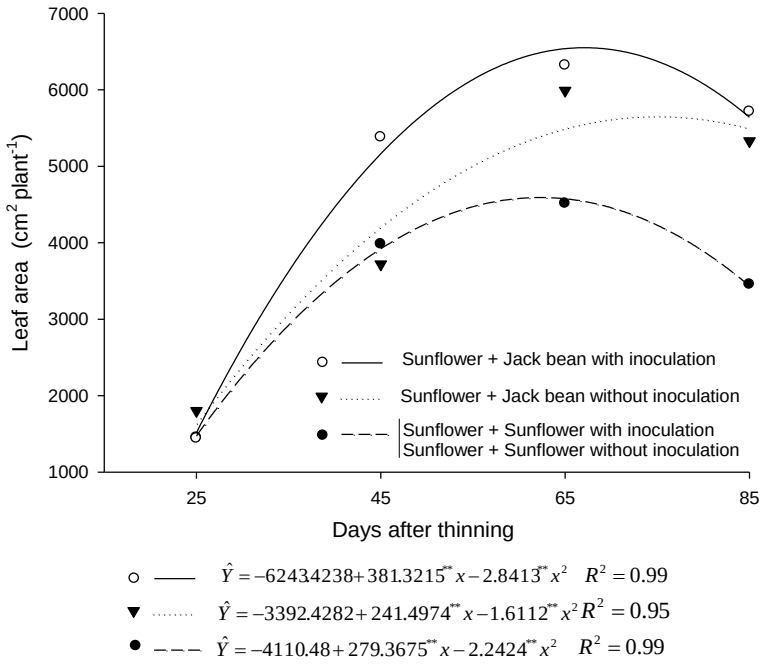

Figure 1 - Leaf area accumulated by sunflower plant in cultivation with the same specie or with jack bean, for different times, in soil with sulfentrazone, in the absence and presence of bacterial consortium.
Models that did not differ from each other by the Model Identity Test ($p \leq 0.05$) were presented in a common curve. ** significant at 5% by Test t.

The accumulation of dry matter of roots of sunflower at 65 and 85 DAT was lower in plants submitted to intraspecific competition compared to those in mixed cultivation (Table 2). Increase of more than 50% in the DMR was evidenced in the sunflower plants at 45 DAT by the presence of *Pseudomonas* bacteria in the soil (Table 2).

Table 2. Root dry matter accumulated by sunflower plant, in cultivation with the same specie or with jack bean, in soil with sulfentrazone, in the absence and presence of bacterial consortium.

	[(1)]Root dry matter (g $plant^{-1}$)			
	Days after thinning			
Cultivation	25	45	65	85
Sunflower + Sunflower	1.06 a	6.02 a	10.10 b	13.51 b
Sunflower + Jack bean	1.15 a	4.19 a	14.85 a	17.90 a
Inoculation				
With	0.97 a	6.93 a	12.18 a	16.10 a
Without	1.24 a	3.28 b	12.78 a	15.31 a
CV (%)	20.73			

(1) Means followed by the same lowercase letters in the column, for each factor, do not differ by Test F (p> 0.05).

Throughout the 85 DAT, sunflower + jack bean treatment accumulated higher DMR than sunflower + sunflower, evidenced by the greater slope of the line (Figure 2A). Regarding the effect of inoculation over bioremediation time, this was not significant, with DMR presented in a single increasing linear curve (Figure 2B).

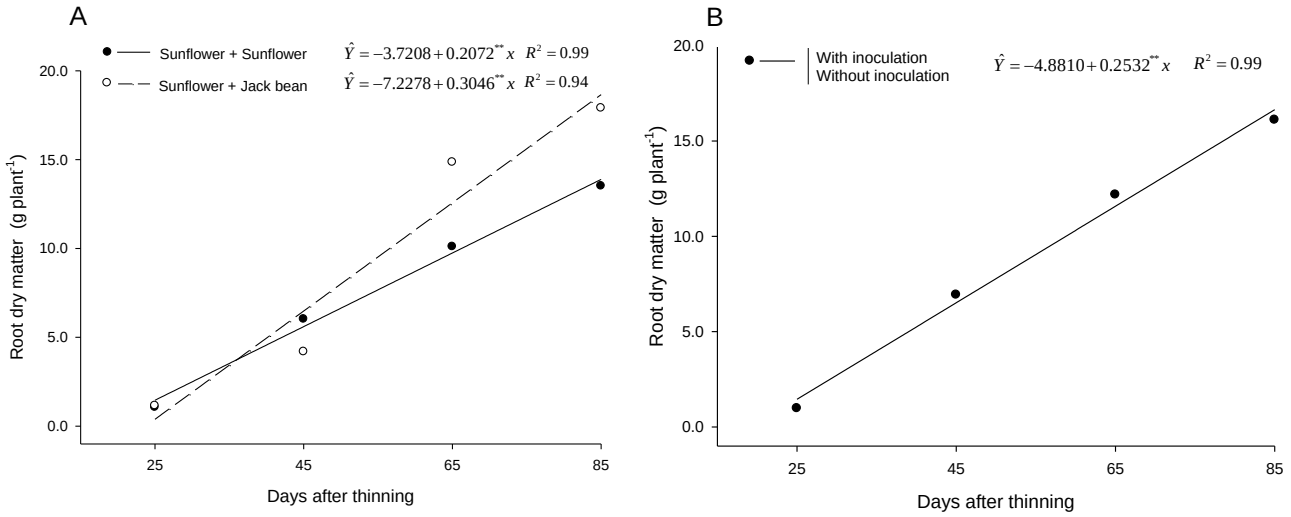

Figure 2 - Root dry matter accumulated by sunflower plant, in cultivation with the same specie or with jack bean (A), for different times, in soil with sulfentrazone, in the absence and presence of bacterial consortium (B).
Models that did not differ from each other by the Model Identity Test ($p \leq 0.05$) were presented in a common curve. ** significant at 5% by Test t.

Significance was verified for DMS in the interactions cultivation x time and cultivation x inoculation. In the unfolding of the first, greater accumulation of dry matter of stem was observed in the cultivations sunflower + jack bean by 65 and 85 DAT (Table 3). However, no difference was observed between the cultivations throughout the bioremediation times, with the maximum DMS reached at 69 DAT (Figure 3).

Also in the interaction cultivation x inoculation, sunflower + sunflower showed lower DMS in relation to mixed cultivation, regardless of inoculation with bacterial consortium. Only in the combination sunflower + jack bean, the addition of selected microorganisms to the soil provided greater accumulation of dry matter of stem (Table 3).

The DML accumulated per sunflower plant was 15, 34 and 39% lower in cultivation with the same species at 45, 65 and 85 DAT, respectively (Table 4). As for DMR (Table 2), soil inoculation provided increased DML of sunflower plants grown for 45 DAT (Table 4).

The cultivation of sunflower and jack bean provided greater accumulation of dry matter of leaves over time, as evidenced in Figure 4A. Notwithstanding, soil inoculation with selected bacteria did not lead to significant changes in the DML accumulation of sunflower plants (Figure 4B).

Table 3 - Stem dry matter accumulated by sunflower plant, in cultivation with the same specie or with jack bean, in soil with sulfentrazone, in the absence and presence of bacterial consortium.

[(1)]Stem dry matter (g plant^{-1})				
Cultivation	Days after thinning			
	25	45	65	85
Sunflower + Sunflower	2.33 a[1]	13.46 a	28.31 b	19.89 b
Sunflower + Jack bean	2.32 a	11.11 a	42.40 a	35.73 a
Cultivation	Inoculation			
	With		Without	
Sunflower + Sunflower	16.26 Ab		15.74 Ab	
Sunflower + Jack bean	24.99 Aa		20.78 Ba	
CV (%)	17.48			

[(1)] Means followed by the same letters, lowercase in the column, and upper case in the row, do not differ by Test F ($p > 0.05$).

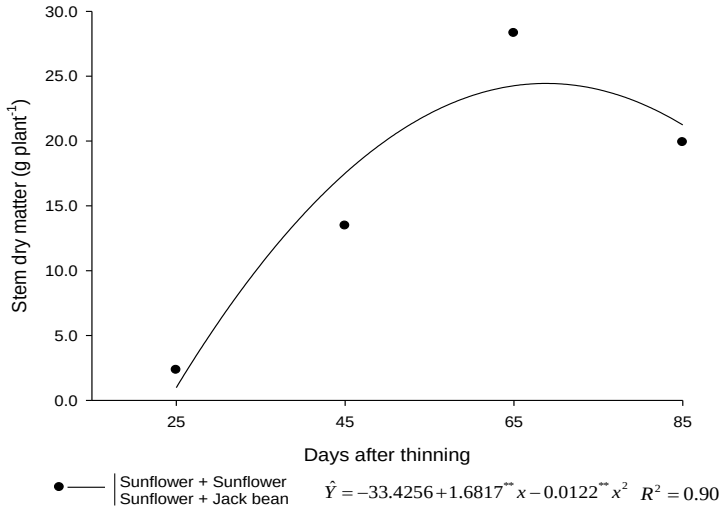

Figure 3 - Stem dry matter accumulated by sunflower plant, in cultivation with the same specie or with jack bean, at different times, in soil with sulfentrazone, in the absence and presence of bacterial consortium.
Models that did not differ from each other by the Model Identity Test (p ≤ 0.05) were presented in a common curve. ** significant at 5% by Test t.

Table 4 - Leaves dry matter accumulated by sunflower plant in cultivation with the same specie or with jack bean, in soil with sulfentrazone, in the absence and presence of bacterial consortium.

	[1]Leaves dry matter (g plant^{-1})			
	Days after thinning			
Cultivation	25	45	65	85
Sunflower + Sunflower	4.77 a	15.42 b	19.84 b	20.17 b
Sunflower + Jack bean	5.48 a	18.08 a	29.85 a	32.85 a
Inoculation				
With	5.02 a	19.08 a	24.81 a	26.38 a
Without	5.23 a	14.43 b	24.98 a	26.83 a
CV (%)	11.42			

[1] Means followed by the same lowercase letters in the column, for each factor, do not differ by Test F (p> 0.05).

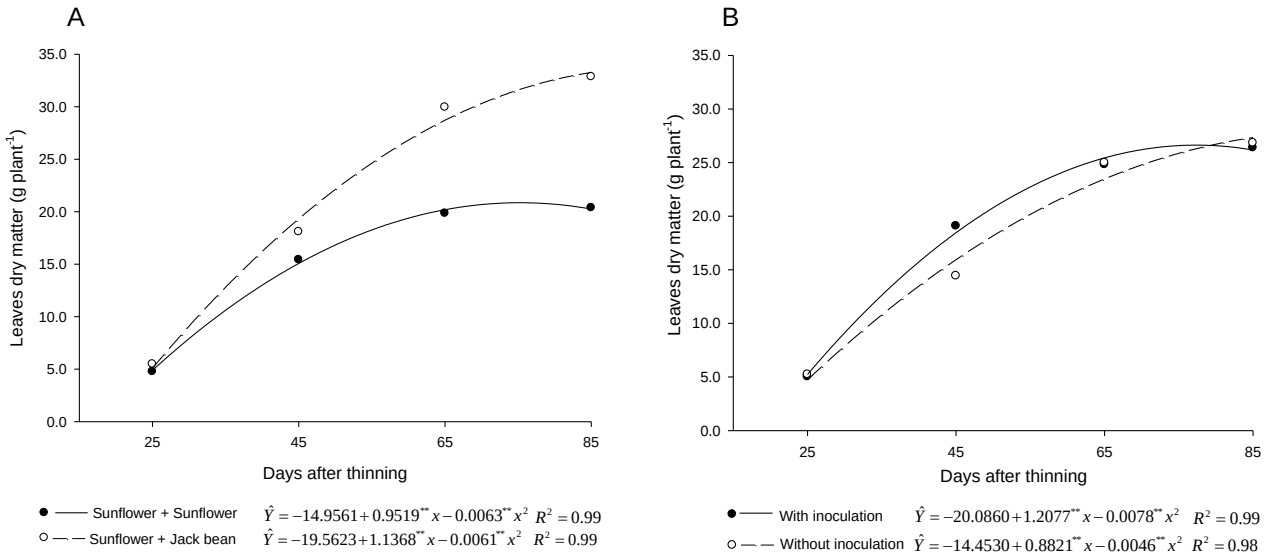

Figure 4 - Leaves dry matter accumulated by sunflower plant in cultivation with the same specie or with jack bean (A), at different times, in soil with sulfentrazone, in the absence and presence of bacterial consortium (B).
** significant at 5% by Test t.

Jack Bean

The coexistence with plants of the same species by 65 and 85 DAT caused a reduction of the leaf area accumulated by jack bean plants (Table 5). The highest leaf area was observed in jack bean plants cultivated in inoculated soil for 25 and 45 DAT, increasing by 51 and 26%, respectively, in relation to the plants cultivated in uninoculated soil (Table 5).

Table 5 - Leaf area accumulated by jack bean plant in cultivation with the same specie or with sunflower, in soil with sulfentrazone, in the absence and presence of bacterial consortium.

Cultivation	(1)Leaf area (cm^2 $plant^{-1}$)			
	Days after thinning			
	25	45	65	85
Jack bean + Jack bean	395.83 a	1405.29 a	2006.13 b	2301.83 b
Jack bean + Sunflower	537.75 a	1818.25 a	3241.00 a	3455.50 a
Inoculation				
With	627.58 a	1854.38 a	2596.00 a	2687.63 a
Without	306.00 b	1369.17 b	2651.13 a	3069.50 a
CV (%)	18.69			

(1) Means followed by the same lowercase letters in the column, for each factor, do not differ by Test F (p> 0.05).

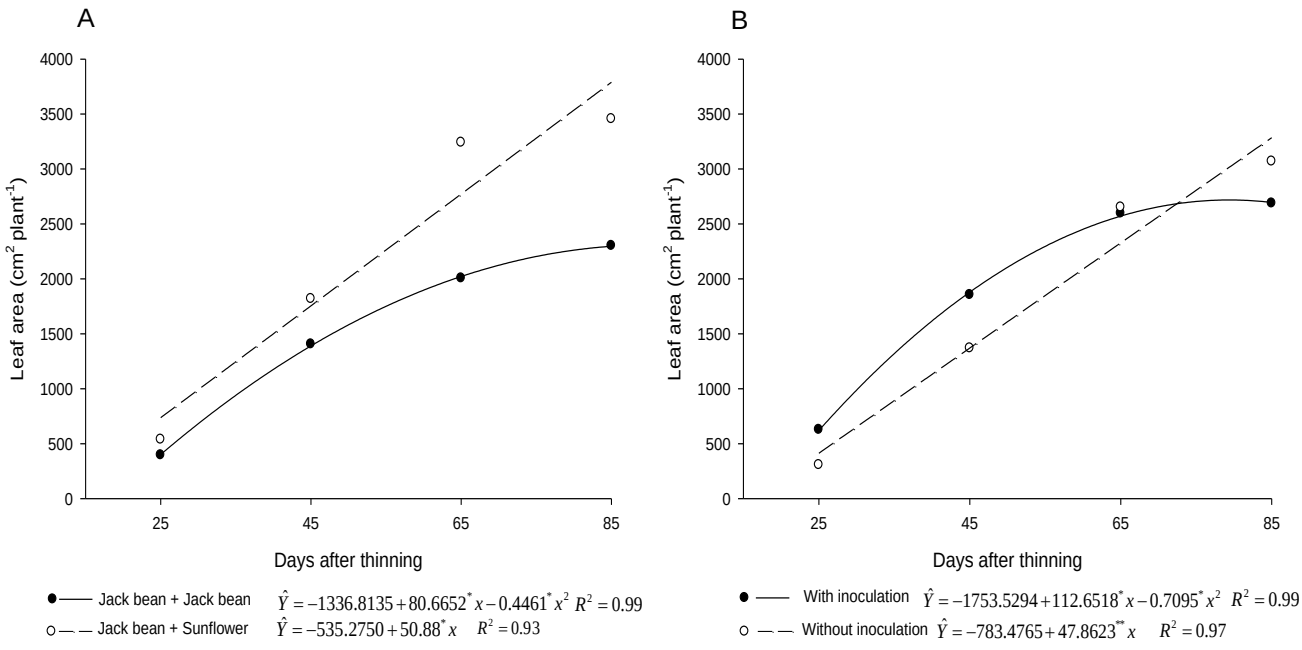

Figure 5 - Leaf area accumulated by jack bean plant in cultivation with the same specie or with sunflower (A), at different times, in soil with sulfentrazone, in the absence and presence of bacterial consortium (B).
**, * significant at 1 and 5% respectively by Test t.

Throughout the period of bioremediation, the treatment jack bean + sunflower showed an increasing linear trend in leaf area, differently than in the presence of another plant of the same species, whose increases were decreasing from 45 DAT (Figure 5A). Plants grown on inoculated soils accumulated higher leaf area than the jack bean plants cultivated in soil uninoculated with bacterial consortium, for almost all the study time (Figure 5B).

The only significant interaction found for DMR was inoculation x time. Greater DMR was observed in jack bean plants grown by 65 and 85 DAT in soil with inoculation (Table 6). Over time, inoculation led to a higher accumulation of dry matter of roots in these plants than in soil uninoculated with bacterial isolates (Figure 6A).

Table 6 - Root dry matter accumulated by jack bean plant, cultivated with the same specie or with sunflower, in soil with sulfentrazone, in the absence and presence of bacterial consortium.

Inoculation	(1)Root dry matter (g plant^{-1})			
	Days after thynning			
	25	45	65	85
With	0.45 a	0.93 a	2.39 a	3.29 a
Without	0.50 a	0.67 a	1.96 b	2.17 b
CV (%)	19.82			

(1) Means followed by the same lowercase letters in the column do not differ by Test F (p> 0.05).

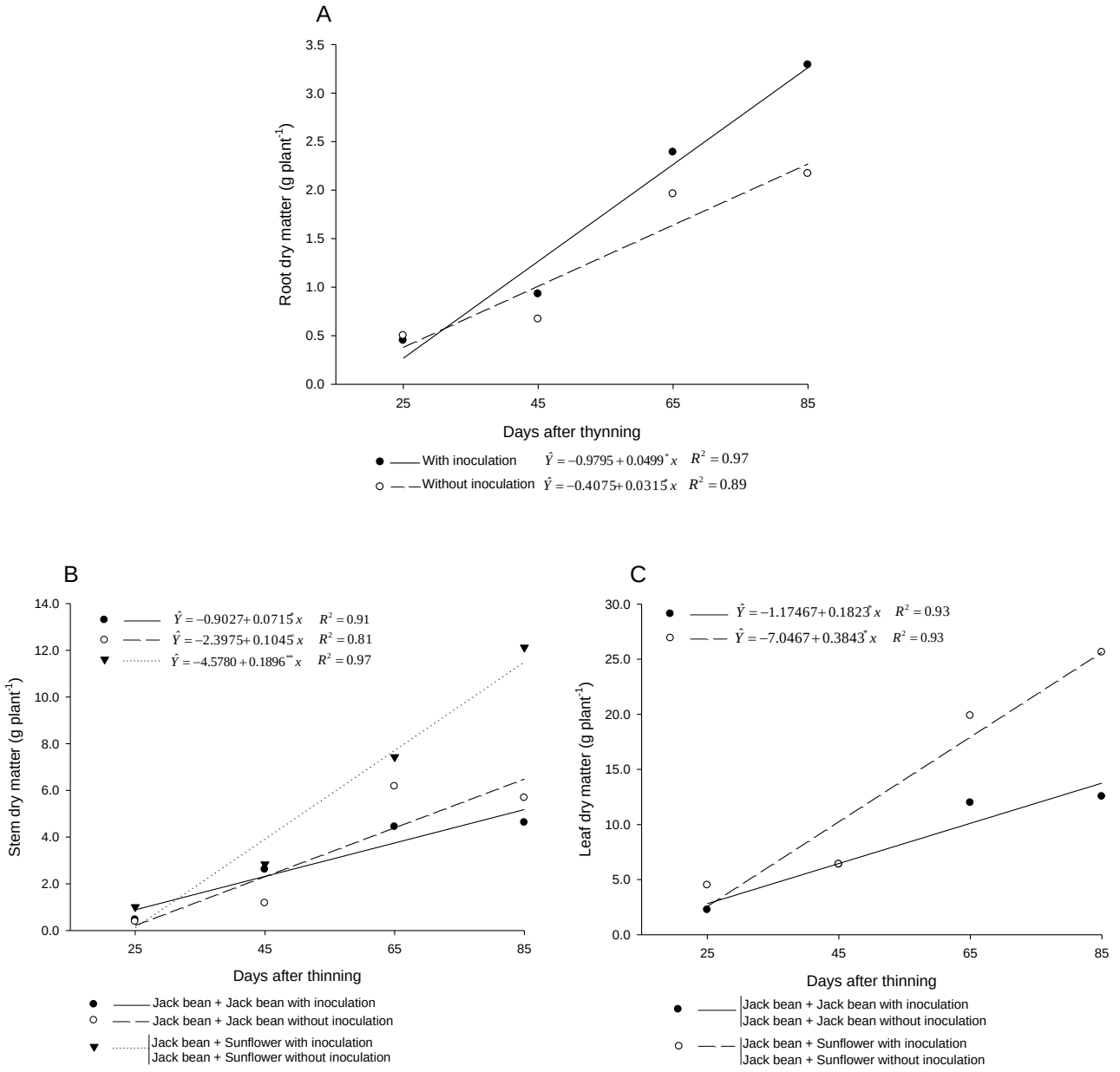

Figure 6 - Dry matter of root (A), stem (B) and leaves (C) of jack bean in cultivation with the same specie or with sunflower, at different times, in soil with sulfentrazone, in the absence and presence of a bacterial consortium.
Models that did not differ from each other were presented in a common curve by the Model Identity Test ($p \leq 0.05$).
**, * and ^{o} significant at 1, 5 and 10%, respectively, by Test t.

Regardless of the inoculation of the soil with the bacterial consortium, jack bean plants grown in the presence of another plant of the same species by 65 and 85 DAT accumulated lower DMS than in the presence of a sunflower plant (Table 7). As effect of the inoculation, there was increase and reduction of the DMS at 45 and 65 DAT, respectively, in the treatments jack bean + jack bean. At 85 DAT, jack bean plants cultivated with sunflower showed an increase of 18% in the DMS in inoculated soil (Table 7).

Table 7 - Stem dry matter accumulated by jack bean plant, cultivated with the same specie or with sunflower, in soil with sulfentrazone, in the absence and presence of bacterial consortium.

Cultivation	[1]Stem dry matter (g plant^{-1})	
	With inoculation	Without inoculation
	25 DAT[2]	
Jack bean + Jack bean	0.46Aa	0.38Aa
Jack bean + Sunflower	1.01Aa	0.74Aa
	45 DAT	
Jack bean + Jack bean	2.61Aa	1.17Ba
Jack bean + Sunflower	2.84Aa	1.57Aa
	65 DAT	
Jack bean + Jack bean	4.44Bb	6.17Ab
Jack bean + Sunflower	7.43Aa	7.78Aa
	85 DAT	
Jack bean + Jack bean	4.62Ab	5.68Ab
Jack bean + Sunflower	12.12Aa	9.96Ba
CV (%)	18.82	

[1] Means followed by the same letters, lowercase in the column and upper case in the row, for each time, do not differ by Test F (p> 0.05). [2] Days after thinning

The treatment jack bean + sunflower, regardless of inoculation, presented higher DMS throughout the period of bioremediation, followed by the treatments jack bean + jack bean with and without inoculation, all with positive linear behavior (Figure 6B).

In the absence of soil inoculation, jack bean plants cultivated with a sunflower plant, for 45 DAT, presented higher DML than in the presence of another jack bean plant. The same behavior was observed at 65 and 85 DAT, in inoculated and uninoculated soils (Table 8). The inoculation resulted in a reduction in the DML of plants of the treatment jack bean + jack bean at 65 DAT, while at 85 DAT an increase was observed in the DML of jack bean + sunflower plants (Table 8).

Table 8 - Leaf dry matter accumulated by jack bean plant, cultivated with the same specie or with sunflower, in soil with sulfentrazone, in the absence and presence of bacterial consortium.

Cultivation	[1]Leaf dry matter (g plant^{-1})	
	With inoculation	Without inoculation
	25 DAT[2]	
Jack bean + Jack bean	2.25Aa	2.34Aa
Jack bean + Sunflower	4.49Aa	2.99Aa
	45 DAT	
Jack bean + Jack bean	6.37Aa	3.56Ab
Jack bean + Sunflower	6.39Aa	7.15Aa
	65 DAT	
Jack bean + Jack bean	11.97Bb	15.78Ab
Jack bean + Sunflower	19.87Aa	21.13Aa
	85 DAT	
Jack bean + Jack bean	12.54Ab	13.75Ab
Jack bean + Sunflower	25.62Aa	20.89Ba
CV (%)	16.58	

[1] Means followed by the same letters, lowercase in the column and upper case in the row, for each time, do not differ by Test F (p> 0.05). [2] Days after thinning

During the bioremediation time, the same cultivations with and without inoculation were represented in a single curve, evidencing a greater accumulation of dry matter of leaves by jack bean plants cultivated with a sunflower plant (Figure 6C).

Discussion

Both remediating species presented lower growth when cultivated together with a plant of the same species. The cultivation of two sunflower plants in the same pot by 65 and 85 DAT caused reduction of all growth variables. In general, the same behavior was observed for jack bean plants. Possibly, in these periods, growth resources were already limiting in the soil, establishing intraspecific competition more strongly.

The use of two plants per pot, equivalent to the density of 31 plants m^{-2}, also contributed to the establishment of competition and detrimental effect to the growth of the species. In a study on the effect of sowing density (10, 20, 40, 80 and 160 plants m^{-2}) on the biomass accumulation of green manure species, Fernandes et al. (1999) verified that jack bean was the only species in which a reduction was observed in the dry matter of shoots, as a consequence of the sowing density. According to the authors, the limitation imposed on growth may be of nutritional or hydrological nature, as well as attributed to light competition.

Species that have similar morphophysiological characteristics usually present the same requirements, making competition more intense and yield reductions higher (Silva & Durigan, 2006). Thus, in the present study, the two plants of the same species concentrated their demands at the same time, further increasing the dispute for the resources of the environment. The cultivation of two species with different growth habits, such as sunflower and jack bean, reduced the intensity of competition, allowing a better use of resources and greater individual growth and development of the species.

Mixed cultivation, in addition to being less harmful in the context of competition, may be a good alternative for the bioremediation of herbicide-contaminated soils, by exploring the remediating potential of each, contributing multiple benefits. In addition to the reduction of herbicide residues in the soil, which can be increased in mixed cultivations in relation to single ones (Yang et al., 2013), the nitrogen input to the soil by legumes such as jack bean, and the use of sunflower as a raw material for the production of biofuel, the cultivation of two species increases the microbial activity and the diversity of microorganisms in the soil, due to the diversity of exudates released therein (Moreira & Siqueira, 2006; Melo et al., 2014).

The inoculation provided greater accumulation of leaf area and dry matter of leaves, stem and roots of *Canavalia ensiformis* and *Helianthus annuus*, at certain times. The results showed the ability of *Pseudomonas* spp. isolates to promote plant growth in soil with sulfentrazone.

Several studies report the ability of various bacterial genera to promote plant growth, including the genus *Pseudomonas*, which appears frequently in the literature. *Pseudomonas* spp. are common and abundant inhabitants of different geographic regions and produce a series of enzymes and metabolites of biotechnological importance involved in plant growth (Mishra et al., 2010; Deshwal & Kumar, 2013).

Pseudomonas strains (*P. aeruginosa*, *P. putida*, *P. cepacia* and *P. fluorecens*), inoculated alone in rice seeds, provided an increase in the dry weight of shoots, roots and whole plant of rice, ranging from 52.80 to 157.72, 172.04 to 408.06 and 93.15 to 233.84%, respectively, in relation to the control (Deshwal & Kumar, 2013). Similarly, Mishra et al. (2010) reported growth promotion by *Pseudomonas* strains with a 27.6% increase in the productivity of *Pelargonium graveolens* L. Herit. In a study with *Mucuna pruriens*, *Pseudomonas* MR-18 increased the dry matter and plant height by 84 and 24%, respectively (Deshwal et al., 2011). Zucareli et al. (2011) verified a mean increase in the diameter of corn ears due to seed inoculation based on *P. fluorescens*. In the present study, increases of 31, 53 and 24% in the accumulation of leaf area, DMR and DML were observed, respectively, in sunflower cultivated for 45 DAT. In the case of jack bean, the positive response of the inoculation was evidenced in all growth variables, both in the presence of another plant of the same species and in the presence of a sunflower plant, being also punctual at certain times.

For the sunflower, expressive effect of the inoculation only at 45 DAT (Tables 1, 3 and 4) may reflect the stage of development of the plant, which was at the beginning of the reproductive stage with development of the floral bud. In this stage, there may have been a greater interaction between plant and microorganisms in the soil, allowing growth promotion. In the plant growth promotion, several mechanisms are involved that can be activated by bacteria in response to stimuli from plants (Bais et al., 2006). Therefore, it is possible that, at a given time, a synergism occurs between the production and release of growth-promoting chemicals in the soil and the ability of plants to absorb and use them.

Effect of the inoculation of the bacterial consortium on jack bean plants was already observed in plants cultivated for 25 and 45 DAT, with a 51 and 26% increase in leaf area accumulation (Table 5). Such behavior may be related to the greater dependence of microorganisms by the plant or to the great capacity of this species to stimulate the associated microbiota in herbicide-contaminated soils (Pires et al., 2005).

Studies on isolation and selection of isolates in different plant species have demonstrated that the ability of some bacteria to produce plant growth-promoting substances may be highly specific to certain plant species or even cultivars, as well as to the different environments where they are inserted, or even affected by the stress imposed on the microbial community due to environmental and anthropogenic alterations (Mendes et al., 2007; Oliveira et al., 2009; Jha et al., 2009b; Prakamhang et al., 2009). In this work, it was further verified that such ability may be related to the cultivation time and the combination of plants. Such evidence reinforces the complexity of interactions between plants and soil microorganisms.

Symbiotic (*Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*) and non-symbiotic bacteria (*Pseudomonas*, *Bacillus*, *Klebsiella*, *Azotobacter*, *Azospirillum*, *Azomonas*) are being used as bioinoculants to promote the growth and development of plants under various stress conditions caused by the presence of heavy metals (Ma et al., 2011; Oves et al., 2013), salinity (Ali et al., 2014), and herbicides (Ahemad & Khan, 2010; 2011). Some bacterial isolates with multiple properties, such as tolerance to the herbicide in question and ability to promote plant growth, were used to allow the growth of *Vigna radiata* and *Lens esculenta*, sensitive species, in soils contaminated with quizalafop-p-ethyl and clodinafop in India (Ahemad & Khan, 2010; 2011). However, the herbicide degradation capacity of the isolates in these works has not been studied.

Bano and Mussarat (2004) isolated and selected a bacterium identified as *Pseudomonas* sp., which has the capacity to perform multiple biological activities. In that study, *Pseudomonas* sp. showed efficacy in the degradation of carbofuran, besides an antagonistic action to phytopathogens by the production of hydrocyanic acid and siderophores, in addition to the potential to promote plant growth by the production of indoleacetic acid and solubilization of inorganic phosphates. The possibility of isolating and selecting bacteria capable of degrading herbicides in the soil and still exerting some effect on the growth and development of remediating species represents a new perspective to be explored in bioremediation programs.

Conclusions

The addition of a consortium formed by *Pseudomonas* spp. to the soil contaminated with sulfentrazone promoted the growth of the remediating species sunflower and jack bean, with the response of jack bean to the inoculation being more effective and lasting. Growth promotion is related to the cultivation time and the combination of plant species. Such ability presented by the bacterial consortium and the plants may contribute to the bioremediation process of sulfentrazone-contaminated soils.

Acknowledgements

To the National Council for Scientific and Technological Development (CNPq) and the Foundation for Research Support of the State of Minas Gerais (FAPEMIG) for the scholarship and support granted.

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