PLANTA DANINHA

SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS

SBCPD

<http://www.sbcpd.org>

ISSN 0100-8358 (print)
1806-9681 (online)

Article

Action of Imazethapyr and Lactofen on the Nodulation of Conventional and Transgenic Soybean under Drought Stress Conditions

Ação do Imazethapyr e Lactofen na Nodulação da Soja Convencional e Transgênica sob Condições de Déficit Hídrico

GONÇALVES, C.G.[1]*
SILVA JUNIOR, A.C.[2]
SCARANO, M.[2]
PEREIRA, M.R.R.[3]
MARTINS, D.[2]

ABSTRACT - This research aimed at studying the action of the herbicides imazethapyr and lactofen on the root system and rhizobium/plant interaction, when they are sprayed at two phenological development stages of soybean cultivars under different soil water management. The soybean cultivars MG/BR 46 Conquista (conventional) and BRS Valiosa (RR) were submitted to three minimum soil water potential (Ψs): -0.03; -0.07 and -0.5 MPa at the V2 and V4 phenological stages. The herbicides imazethapyr at the rate (100 g a.i. ha^{-1}) and lactofen at the rate (180 g a.i. ha^{-1}) were sprayed 10 days after the start of water restrictions. Twenty-one days after the application of the herbicides, root dry matter and root nodulation were evaluated. The BRS Valiosa (RR) soybean cultivar presented better nodule formation and a more efficient and more adapted root system to support moderate drought stress conditions (-0.07 MPa). The MG/BR 46 Conquista (conventional) soybean cultivar presented lower the root system growth when submitted to the spraying of imazethapyr and lactofen. The activity of N_2-fixating bacteria was affected by the spraying of the herbicides imazethapyr and lactofen.

Keywords: ALS, *Glycine max*, phytotoxification, PROTOX, symbiotic fixation of N_2.

RESUMO - O presente estudo objetivou estudar a ação dos herbicidas imazethapyr e lactofen no sistema radicular e a interação rizóbio/planta, pulverizados em dois estádios fenológicos de desenvolvimento de cultivares de soja sob diferentes manejos de água no solo. Os cultivares de soja MG/BR 46 Conquista (convencional) e BRS Valiosa (RR) foram submetidos a três potenciais mínimos de água no solo (Ψs): -0,03, -0,07 e -0,5 MPa, nos estádios fenológicos V2 e V4. A pulverização dos herbicidas imazethapyr (100 g i.a. ha^{-1}) e lactofen (180 g i.a. ha^{-1}) foi realizada 10 dias após o início das restrições hídricas. Aos 21 dias após a aplicação dos herbicidas, foi avaliada a massa seca das raízes e a nodulação do sistema radicular das plantas. O cultivar BRS valiosa (RR) apresentou maior formação de nódulos e um sistema radicular mais eficiente e adaptado para superar uma condição de escassez hídrica moderada (-0,07 MPa). O cultivar MG/BR 46 Conquista (convencional) mostrou menor crescimento do sistema radicular quando submetido à pulverização dos herbicidas imazethapyr e lactofen. A atividade das bactérias fixadoras de nitrogênio foi reduzida, impactada pela pulverização dos herbicidas imazethapyr e lactofen.

Palavras-chave: ALS, *Glycine max*, fitointoxicação, PROTOX, fixação simbiótica de N_2.

*** Corresponding author:**
<goncalvescg.agro@hotmail.com>

Received: March 2, 2017
Approved: April 20, 2017

Planta Daninha 2018; v36:e018176280

[1] Auburn University, Auburn-AL, United States; [2] Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP, Jaboticabal-SP, Brasil; [3] Faculdade de Tecnologia de Capão Bonito, Capão Bonito-SP, Brazil.

Doi: 10.1590/S0100-83582018360100061

INTRODUCTION

Among the biotic and abiotic stress conditions affecting plant development, water deficit is the most important constraint on plant growth and productivity worldwide (Manavalan et al., 2009; Sadeghipour and Abbasi, 2012).

The effects of water deficit on shoot growth are well known (Pereira et al., 2010; Mak et al., 2014), since it causes a reduction in the turgescence pressure of the plant tissue, which reduces the expansion and growth of cells and has a direct action on cellular division and differentiation (Chaitanya et al., 2003; Karthikeyan et al., 2007), as well as morpho-anatomical, physiological and biochemical changes (Ashraf 2010; Galmés et al., 2013).

Studies have also indicated a decrease in the dry matter of the root system of soybean cultivars (Thu et al., 2014; He et al., 2017), and a better tolerance to the water scarcity condition may be related to a better root architecture, such as: greater depth and ramification, xylem diameter, roots with more root hairs, density and root angle (Fenta et al., 2014; Vadez et al., 2014; Ali et al., 2016). These characteristics increase the root surface area, and this helps the water uptake from the soil solution.

Among the agricultural crops of interest, soybean (*Glycine max*) is the most important legume in the world, and has a symbiotic interaction with soil bacteria from the genus *Rhizobium*. This is one of the main biotic factors that contribute to increase its productivity through nitrogen demand, without the increase of this nutrient in the soil by external sources (Hungria et al., 2006; Hungria and Mendes, 2014). However, the efficiency of the biological nitrogen fixation (BNF) depends on a good rhizobium/plant interaction and root system efficiency, since the exposure of soybean plants to water scarcity leads to an inhibition of rhizobia infection, with immediate losses in its activity (Ladrera et al., 2007; Mastrodomenico et al., 2013).

The use of herbicides is also a common and very important practice for the cultivation of soybean, in order to guarantee high yields through the effective control of weeds. However, the toxicity of these herbicides to the microbial soil inhabitants that make symbiotic interactions can be harmful to nodulation and nitrogen fixation (Parsa et al., 2013, Zhang et al., 2013, Wu et al., 2014). For example, there is imazethapyr (ALS inhibitor), which produces deleterious effects on *Bradyrhizobium japonicum* strains, reducing the number and diameter of nodules (Parsa et al., 2013).

It is worth mentioning that most herbicides applied in the post-emergence period are sprayed at the initial stage of plant growth, coinciding with the beginning of the rhizobia/plant infection and nodule formation (Ryle et al., 1979), thus increasing toxicological risks.

Although the water deficit (Ladrera et al., 2007, Mastrodomenico et al., 2013) and the use of herbicides (Zhang et al., 2013, Wu et al., 2014) may affect the development of soybean and impact nitrogen fixing bacteria, when analyzing the current scenario, where soybean is cultivated mainly in rainfed areas without the use of irrigation, the risks of having water restriction simultaneously to herbicide spraying are high; this could cause greater losses in the productive process. The objective of this research was to study the action of the herbicides imazethapyr and lactofen on the root system and on the rhizobia/plant interaction, when they are sprayed at two phenological stages of the soybean cultivar development under different soil water management.

MATERIAL AND METHODS

Two experiments were conducted under greenhouse conditions in the Departamento de Produção Vegetal of the Faculdade de Ciências Agrárias e Veterinárias – FCAV/UNESP, Jaboticabal - São Paulo state campus, at the geographic coordinates: latitude 21°14'43.42" S and longitude 48°17'32.80" W, in the 2014/15 agricultural year.

In this study, the soybean cultivars MG/BR 46 Conquista (conventional) and BRS Valiosa (RR) were used. The experimental plots consisted of plastic pots containing 3 kg of soil. The chemical characterization of the soil was carried out through a composite sample of the soil, which was removed before the installation of the experiments. The soil chemical analysis showed:

pH ($CaCl_2$) = 5.2; MO = 17 g dm^{-3}; P = 21 mg dm^{-3}; S = 8 mg dm^{-3}; Ca = 16 $mmol_c$ dm^{-3}; Mg = 5 $mmol_c$ dm^{-3}; K = 2.3 $mmol_c$ dm^{-3}; H + Al = 24 $mmol_c$ dm^{-3}; SB = 23.3 $mmol_c$ dm^{-3}; CTC = 47.3; and V = 49. Soil fertility was corrected according to crop recommendations (Raij et al., 1996), except for nitrogen fertilization, which was not performed. Before sowing, seeds were inoculated with *Bradyrhizobium japonicum* SEMIA 5079 and *Bradyrhizobium elkanii* SEMIA 5019 strains (9 x 10^9 viable cells kg^{-1} seeds).

In order to obtain the curve on water retention in the soil, the Richards pressure plate (Klar, 1984) was used. From the results of the retention curve, three potential soil water minimums (Ψs) were established: -0.03, -0.07 and -0.5 MPa. Before sowing, the soil was air-dried and turned daily until reaching constant moisture of 3%, in order to reduce it to a minimum, so as to perform water replenishment calculations, according to the retention curve. Through daily weighing of each pot, when observing that the pot weight reached the defined water potential for each treatment, soil water replacement was performed to the maximum potential (-0.01 MPa).

For the first study, the water conditions of soil water management started when plants reached the V2 development stage (first open trefoil), and for the second study, they started when plants reached the V4 development stage (third trefoil open), described by Fehr and Caviness (1977).

Imazethapyr (100 g a.i. ha^{-1}) and lactofen (180 g a.i. ha^{-1}) were sprayed 10 days after the beginning of water restrictions, at each studied phenological stage, through a backpack sprayer with constant CO_2 pressure and calibrated for a consumption of 200 L ha^{-1}. The spray bar was equipped with two Teejet XR 11002VS flat jet tips, spaced 50 cm apart.

Twenty-one days after the application of the herbicides, plants were collected from each plot to determine their root dry matter and root nodulation. In order to evaluate the nodules, the roots were carefully removed from the soil and washed under running water; later, nodules were removed from each plant and were then counted. Roots and nodules were dried in a forced air circulation oven at 60 °C until reaching constant weight and then measured on a precision scale.

The experiments were conducted in a completely randomized design with four replications, in a 3 x 2 x 2 factorial arrangement, consisting of three water managements (-0.03, -0.07 and -0.5 MPa), two soybean cultivars (MG/BR 46 Conquista and BRS Valiosa RR) and two doses of herbicides (with and without herbicide).

The results were submitted to analysis of variance; significance was tested for sources of significant variation by F test, and the means were compared by Tukey's test ($p>0.05$).

RESULTS AND DISCUSSION

No significant differences were observed in terms of root dry matter between conventional and transgenic cultivars at the V2 stage (Table 1). However, at the V4 stage, the root dry matter was higher for the MG/BR 46 Conquista (conventional).

The water deficit reduced the dry matter of the roots. Even under conditions of moderate water scarcity (-0.07 MPa), a 33.3% and 37.3% reduction in root dry matter accumulation was observed for treatments with imazethapyr and lactofen at the V2 stage, respectively (Table 1). This effect was even more pronounced for plants submitted to water restriction at the V4 stage, reducing by 60.9% and 56.4% on an average the root dry matter accumulation for treatments with imazethapyr and lactofen, respectively.

Bañon et al. (2004), when assessing water deficit impacts on *Lotus creticus* plants under nursery conditions, reported a substantial reduction of the root system dry weight and the root length. Researchers reported a reduction in the number of stem xylem vessels and roots. Makbul et al. (2011) observed morphological changes of the root system in soybean plants under water deficit. They reported that the exoderm in stressed plants was formed by three layers of cells with thickened walls; on the other hand, for non-stressed plants, the exoderm presented only two cell layers. It was also possible to observe that the width of the cortex and the vascular bundles of the roots were smaller in plants maintained under water deficit.

Table 1 - Mean values verified for the root dry matter of soybean plants, submitted to the application of imazethapyr and lactofen at different phenological stages, obtained in the unfolding of degrees of freedom of the variables cultivar, water management and herbicide

Condition	Root dry matter (g)			
	V2		V4	
	imazethapyr	lactofen	imazethapyr	lactofen
	Cultivar			
Conventional	0.38 a	0.44 a	1.22 a	1.32 a
RR	0.40 a	0.42 a	1.04 b	1.05 b
	Water Management (MPa)			
-0.03	0.60 a	0.67 a	2.20 a	2.25 a
-0.07	0.40 b	0.42 b	0.86 b	0.98 b
-0.50	0.17 c	0.19 c	0.34 c	0.33 c
	Herbicide			
With	0.35 b	0.42 a	0.87 b	0.98 b
Without	0.43 a	0.44 a	1.39 a	1.39 a
F Cultivar (C)	0.81^{ns}	0.48^{ns}	6.23*	15.30**
Water management (M)	99.58**	167.52**	240.67**	278.29**
Herbicide (H)	12.03**	0.30^{ns}	52.72**	37.30**
(C) x (M)	1.05^{ns}	1.48^{ns}	11.85**	12.80**
(C) x (H)	4.35*	0.07^{ns}	26.44**	17.32**
(M) x (H)	2.39^{ns}	0.15^{ns}	24.23**	25.77**
(C) x (M) x (H)	0.03^{ns}	0.03^{ns}	15.32**	16.71**
VC (%)	21.8	17.3	21.8	19.7
s.m.d. (C)/(H)	0.05	0.04	0.14	0.14
s.m.d. (M)	0.07	0.06	0.21	0.2

Means followed by the same letter in the column do not differ statistically from each other by Tukey's test (P<0.05). ** Significant at 1% probability; * Significant at 5% probability; ^{ns} Non-significant. RR = transgenic soybean.

Spraying imazethapyr and lactofen resulted in dry matter reductions of roots at the V4 stage (Table 1), except for the herbicide lactofen at the V2 stage, which was similar to the control treatment. Parsa et al. (2013) also reported a dry matter reduction in the root system of different soybean cultivars submitted to imazethapyr spraying at the recommended dose, which supports the results found in this work.

As for the interaction between cultivar and herbicide at the V2 stage, imazethapyr reduced the root dry matter of the MG/BR 46 Conquista (conventional) cultivar (Table 2). However, for the other interactions, spraying imazethapyr and lactofen was not significant.

At the V4 stage, for the cultivar x water management interaction (Table 2), it was possible to observe that under ideal conditions of water in the soil (-0.03 MPA), MG/BR 46 Conquista (conventional) had the highest root dry matter compared to BRS Valiosa (RR) (Figure 1). However, under moderate water scarcity (-0.07 MPa), these differences were not observed, since it was more harmful to MG/BR 46 Conquista (conventional).

It was highlighted that under moderate water scarcity (-0.07 MPa) cultivar MG/BR 46 Conquista presented a 69.4% and 63% reduction in its root dry matter accumulation for treatments with imazethapyr and lactofen, respectively (Table 2). As for cultivar BRS Valiosa (RR), the reduction was 48.9% and 46.8% in treatments with imazethapyr and lactofen, respectively.

In this study, there was higher tolerance from the BRS Valiosa (RR) root system to moderate water deficit conditions compared to MG/BR 46 Conquista (conventional). Fenta et al. (2014), when assessing different characteristics of the root system in different soybean cultivars, report that under ideal soil water conditions, there was no difference between them. On the other hand, under water deficit, cultivar A5409RG presented a strong sensitivity in relation to other cultivars, since a root length, surface and root volume reduction was observed.

Table 2 - Mean values verified for the root dry matter of soybean plants, submitted to the application of imazethapyr and lactofen at different phenological stages, obtained in the unfolding of freedom degrees of the interactions between: cultivar and water management, cultivar and herbicide, and water management and herbicide

Interaction	Root dry matter (g)							
	V2				V4			
	Imazethapyr		Lactofen		Imazethapyr		Lactofen	
Water management (MPa)	Cultivar							
	Conv.	RR	Conv.	RR	Conv.	RR	Conv.	RR
-0.03	-	-	-	-	2.52 aA	1.88 aB	2.62 aA	1.88 aB
-0.07	-	-	-	-	0.77 bA	0.96 bA	0.97 bA	1.00 bA
-0.50	-	-	-	-	0.38 cA	0.29 cA	0.37 cA	0.28 cA
s.m.d. (Line)	-		-		0.30		0.29	
s.m.d. (Column)	-		-		0.25		0.24	
Cultivar	Herbicide							
	With	Without	With	Without	With	Without	With	Without
Conventional	0.31 bB	0.45 aA	-	-	0.78 aB	1.66 aA	0.97 aB	1.66 aA
RR	0.38 aA	0.42 aA	-	-	0.97 aA	1.12 bA	0.99 aA	1.12 bA
d.m.s. (Line)/(Column)	0.07		-		0.20		0.19	
Water Management	Herbicide							
	With	Without	With	Without	With	Without	With	Without
-0.03	-	-	-	-	1.60 aB	2.80 aA	1.69 aB	2.80 aA
-0.07	-	-	-	-	0.69 bB	1.04 bA	0.93 bA	1.04 bA
-0.50	-	-	-	-	0.33 cA	0.35 cA	0.31 cA	0.35 cA
s.m.d. (Line)	-		-		0.25		0.24	
s.m.d. (Column)	-		-		0.30		0.29	

Averages followed by the same letter, lowercase in the column and upper case in the line, do not differ statistically from each other by Tukey's test (P <0.05). Conv. = conventional soybean; RR = transgenic soybean.

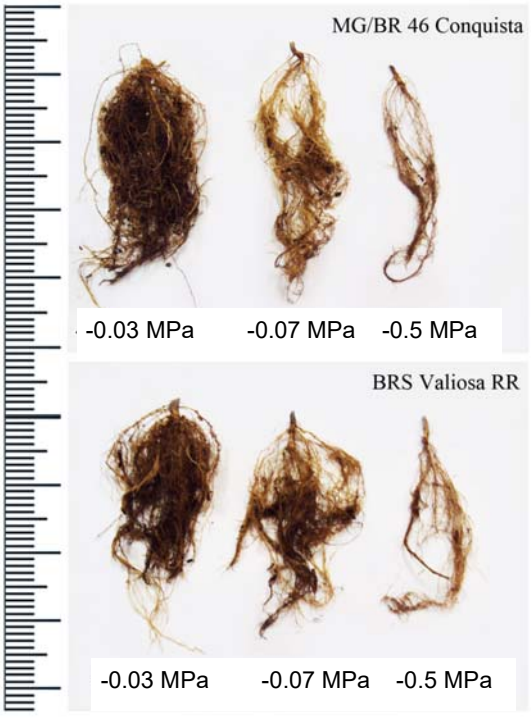

Figure 1 - Root system of conventional and transgenic soybean cultivars submitted to different water potentials at the vegetative development stage V4.

Spraying imazethapyr and lactofen at the V4 stage reduced the root dry matter of MG/BR 46 Conquista (conventional) (Table 2). In turn, spraying imazethapyr and lactofen did not affect the root dry matter of BRS Valiosa (RR). Therefore, it was possible to show greater selectivity of these herbicides for the transgenic cultivar.

As for the interaction between water management and imazethapyr and lactofen spraying, it was observed that imazethapyr reduced the root system of soybean plants conducted without water stress (-0.03 MPa), as well as for plants kept under moderate water scarcity (-0.07 MPa) (Table 2). However, for treatments sprayed with lactofen, only plants conducted without water stress (-0.03 MPa) showed a reduction in the root system.

It was observed that the number of nodules was reduced by the water restriction imposed on soybean plants at both studied phenological stages (Table 3). It is worth mentioning that, at stage V2, even a moderate water scarcity condition (-0.07 MPa) was sufficient to inhibit 100% nodule formation. Thus, a water deficit condition occurred at the V2 stage was harmful to the nodulation of

Table 3 - Mean values verified for the number of nodules of soybean plants, submitted to the application of imazethapyr and lactofen at different phenological stages, obtained in the unfolding of freedom degrees of the variables: cultivar, water management and herbicide

Condition	N. Nodules			
	V2[(1)]		V4	
	Imazethapyr	Lactofen	Imazethapyr	Lactofen
	Cultivar			
Conventional	0.90 a	1.24 a	36.25 a	46.08 a
RR	0.72 a	0.47 b	42.33 a	45.04 a
	Water Management (MPa)			
-0.03	2.44 a	2.57 a	95.94 a	109.50 a
-0.07	0.00 b	0.00 b	19.13 b	24.50 b
-0.5	0.00 b	0.00 b	2.81 c	2.69 c
	Herbicide			
With	0.38 b	0.48 b	28.75 b	41.71 a
Without	1.24 a	1.24 a	49.83 a	49.42 a
F Cultivar (C)	1.90^{ns}	59.27**	3.60^{ns}	0.71^{ns}
Water Management (M)	154.40**	294.43**	321.19**	648.87**
Herbicide (H)	42.96**	58.46**	43.30**	28.25**
(C) x (M)	1.90^{ns}	59.27**	1.97^{ns}	5.03*
(C) x (H)	9.76**	3.29^{ns}	7.18*	7.46**
(M) x (H)	42.96**	58.46**	15.87**	22.96**
(C) x (M) x (H)	9.76**	3.29*	11.83**	8.11**
VC (%)	55.80	40.38	28.30	23.31
s.m.d. (C)/(H)	0.27	0.20	6.50	8.49
s.m.d. (M)	0.39	0.30	9.58	12.51

Means followed by the same letter in the column do not differ statistically from each other by Tukey's test (P<0.05). ** Significant at 1% probability; * Significant at 5% probability; [ns] Non-significant. RR = transgenic soybean. N. Nodules = number of nodules; RR = transgenic soybean. [(1)] Data from the phenological stage V2 were transformed according to equation " $y = \sqrt{x}$ ".

plants by rhizobia, because it occurred at the same time as the beginning of the rhizobia/plant infection process (Ryle et al., 1979), which prevented nodule formation.

Spraying imazethapyr at the V2 and V4 stages of soybean damaged nitrogen-fixing bacteria, since it reduced the number of nodules in the roots of this plant (Table 3). As for lactofen, only sprayings performed at the V2 stage reduced the number of nodules; at the V4 stage, spraying this herbicide did not affect the formation of nodules.

It was also observed that, in the interaction between cultivar and water management, at V2 and V4 stages for treatments sprayed with lactofen, under ideal water conditions (-0.03 MPa), cultivar MG/BR 46 Conquista (conventional) presented a higher number of nodules, compared to BRS Valiosa (RR) (Table 4).

Although the water deficit affected the formation of nodules in both studied cultivars, a moderate water scarcity condition (-0.07 MPa) was more harmful to cultivar MG/BR 46 Conquista (conventional), since a 84.9% reduction was observed in the number of nodules, which was 69.1% for BRS Valiosa (RR) (Table 4).

Corroborating the results found here, Fenta et al. (2014), when evaluating the characteristics of the soybean root system as for water deficit tolerance, observed that cultivar A-5409RG presented, under ideal conditions of water in the soil, a greater abundance of nodules among the studied cultivars; however, under water deficit conditions, this cultivar was severely affected compared to others, indicating its low tolerance in sustaining the biological fixation of nitrogen when under water deficit conditions.

As for the cultivar x herbicide interaction, it was verified that, regardless of the cultivar and the phenological stage, spraying imazethapyr affected the number of nodules at stage V2 (Table 4).

Table 4 - Mean values verified for the number of soybean plants submitted to the application of imazethapyr and lactofen at different phenological stages, obtained in the unfolding of freedom degrees of the interactions between: cultivar and water management, cultivar and herbicide, and water management and herbicide

Interaction	N. Nodules							
	V2[(1)]				V4			
	Imazethapyr		Lactofen		Imazethapyr		Lactofen	
W. Manag. (MPa)	Cultivar							
	Conv.	RR	Conv.	RR	Conv.	RR	Conv.	RR
-0.03	-	-	3.73 aA	1.42 Ab	-	-	117.38 aA	101.63 aB
-0.07	-	-	0.00 bA	0.00 bA	-	-	17.63 bA	31.38 bA
-0.50	-	-	0.00 bA	0.00 bA	-	-	3.25 bA	2.13 cA
s.m.d. (Line)	-		0.42		-		17.69	
s.m.d. (Column)	-		0.35		-		14.7	
Cultivar	Herbicide							
	With	Without	With	Without	With	Without	With	Without
Conventional	0.27 aB	1.54 aA	-	-	21.42 bB	51.08 aA	-	-
RR	0.50 aB	0.95 bA	-	-	36.08 aB	48.59 aA	-	-
s.m.d. (Line)/(Column)	0.38		-		9.2		-	
Water Management	Herbicide							
	With	Without	With	Without	With	Without	With	Without
-0.03	1.15 aB	3.72 aA	1.43 aB	3.72 aA	73.38 aB	118.50 aA	-	-
-0.07	0.00 bA	0.00 bA	0.00 bA	0.00 bA	10.88 bB	27.38 bA	-	-
-0.50	0.00 bA	0.00 bA	0.00 bA	0.00 bA	2.00 bA	3.63 cA	-	-
s.m.d. (Line)	0.46		0.35		11.26		-	
s.m.d. (Column)	0.55		0.42		13.55		-	

Means followed by the same letter, lowercase in the column and upper case in the line, do not differ statistically from each other by Tukey's test (P <0.05). N. Nodules = number of nodules; Conv. = Conventional soybean; RR = transgenic soybean; W. Manag. = water management. [(1)] Data from the phenological stage V2 were transformed according to equation " $y = \sqrt{x}$ ".

As for the interaction between water management and herbicide at the V2 stage, only plants under a suitable water regime (-0.03 MPa) reduced the number of nodules for being sprayed with imazethapyr and lactofen (Table 4). At the V4 stage, imazethapyr also reduced nodule formation for plants under moderate water scarcity conditions (-0.07 MPa).

Table 5 shows that the cultivar MG/BR 46 Conquista (conventional) presented higher nodule dry matter compared to cultivar BRS Valiosa (RR), unlike treatments at the phenological stage V4, where these differences were no longer observed.

Thus, as for the evaluations about the number of nodules (Table 4), it was possible to confirm that the biological nitrogen fixation activity is very sensitive to water restriction conditions imposed on soybean plants (Table 5), since the nodule dry matter was severely reduced when soybean plants were maintained in a soil with a water management of -0.07 and -0.5 MPa.

It was observed that spraying imazethapyr had a negative effect on rhizobia infection (Table 5), since at both phenological stages, spraying this herbicide reduced the dry matter of nodules.

Although spraying lactofen at the V4 stage did not affect the number of nodules (Table 3), it was observed that the nodule dry matter was reduced at both phenological stages studied when plants were sprayed with this herbicide (Table 5). Therefore, these results indicate that the nodule dry matter evaluation would be more efficient than its count to evaluate rhizobia activity. Souza et al. (2008) also defined the dry matter of nodules as an efficient parameter used to evaluate the efficiency of the biological nitrogen fixation in soybean crops; this supports the results of this study.

As for the interaction between cultivar and water management at the V2 stage, under ideal water conditions in the soil (-0.03 MPa), cultivar MG/BR 46 Conquista (conventional) had higher mean of nodule dry matter compared to cultivar BRS (RR) (Table 6). However, at stage V4, these

Table 5 - Mean values verified for the dry matter of soybean plant nodules, submitted to the application of imazethapyr and lactofen at different phenological stages, obtained in the unfolding of freedom degrees of the cultivar, water and herbicide management variables

Condition	Nodule DM			
	V2[(1)]		V4	
	Imazethapyr	Lactofen	Imazethapyr	Lactofen
	Cultivar			
Conventional	0.02 a	0.027 a	0.11 a	0.13 a
RR	0.01 b	0.007 b	0.12 a	0.14 a
	Water Management (MPa)			
-0.03	0.05 a	0.51 a	0.33 a	0.37 a
-0.07	0.00 b	0.00 b	0.03 b	0.039 b
-0.5	0.00 b	0.00 c	0.01 b	0.001 c
	Herbicide			
With	0.009 b	0.009 b	0.08 b	0.11 b
Without	0.025 a	0.025 a	0.16 a	0.16 a
F Cultivar (C)	7.24*	44.40**	1.48^{ns}	0.71^{ns}
Water Management (M)	120.10**	137.40**	554.32**	648.87**
Herbicide (H)	25.35**	34.01**	78.81**	28.25**
(C) x (M)	7.24**	44.40**	0.10^{ns}	5.03*
(C) x (H)	16.69**	0.60^{ns}	9.73**	7.46**
(M) x (H)	25.35**	34.01**	48.98**	22.96**
(C) x (M) x (H)	16.69**	0.60^{ns}	18.05**	8.11**
VC (%)	63.2	59.11	26.18	23.31
s.m.d. (C)/(H)	0.006	0.006	0.02	0.02
s.m.d. (M)	0.010	0.009	0.03	0.03

Means followed by the same letter in the column did not differ statistically from each other by Tukey's test (P <0.05). ** Significant at 1% probability; * Significant at 5% probability; ^{ns} Non-significant. Nodule DM = dry matter of the nodules; RR = transgenic soybean. [(1)] Data from the phenological stage V2 were transformed according to the equation " $y = \sqrt{x}$ ".

differences were not significant. Thus, although MG/BR 46 Conquista (conventional) presented a greater amount of nodules in the root system (Table 5), BRS Valiosa (RR) had larger and heavier nodules (Table 6).

It is worth mentioning that, at the V4 stage, a moderate water scarcity (-0.07 MPa) was more harmful to cultivar MG/BR 46 Conquista (conventional), because it presented a lower nodule dry matter compared to BRS Valiosa (RR) (Table 6).

A water deficit affects the root system of plants, which in turn affects the water content of nodules, thus causing the formation of smaller nodules (King and Purcell, 2001; Fenta et al., 2014). Therefore, better adapted roots with a better architecture can maintain better nodule sustainability (Fenta et al., 2014), since roots with characteristics such as greater depth and ramification, larger xylem diameter and more root hairs have better tolerance to water scarcity conditions (Garnett et al., 2009; Vadez et al., 2014; Ali et al., 2016), and a higher capacity to maintain greater nodule turgor pressure and higher biological nitrogen fixation.

As for the interaction between cultivar and herbicide, it was observed that imazethapyr sprayed at the V4 stage affected the nodule dry matter in both studied cultivars (Table 6), unlike lactofen, which affected the dry matter of nodules only for cultivar MG/BR 46 Conquest (conventional).

In the interaction between water management and herbicide, it was observed that imazethapyr and lactofen sprayed on plants under ideal soil water conditions (-0.03 MPa) resulted in dry matter reduction of nodules (Table 6). These results corroborate those found for the number of nodules (Table 4). It was also verified that, at the V4 stage, imazethapyr also reduced the dry matter of nodules for plants conducted under conditions of moderate water scarcity (-0.07 MPa).

Table 6 - Mean values verified for the dry matter of soybean plant nodules, submitted to the application of imazethapyr and lactofen at different phenological stages, obtained in the unfolding of freedom degrees of the interactions between: cultivar and water management, cultivar and herbicide, and water management and herbicide

Interaction	Nodule DM							
	V2[(1)]				V4			
	Imazethapyr		Lactofen		Imazethapyr		Lactofen	
W. Manag. (MPa)	Cultivar							
	Conv.	RR	Conv.	RR	Conv.	RR	Conv.	RR
-0.03	0.07 aA	0.04 aB	0.08 aA	0.022 aB	-	-	0.380 aA	0.350 aA
-0.07	0.00 bA	0.00 bA	0.00 bA	0.000 bA	-	-	0.020 bB	0.060 bA
-0.5	0.00 bA	0.00 bA	0.00 bA	0.000 bA	-	-	0.001 bA	0.001 cA
s.m.d. (Line)	0.01		0.012		-		0.04	
s.m.d. (Column)	0.01		0.010		-		0.03	
Cultivar	Herbicide							
	With	Without	With	Without	With	Without	With	Without
Conventional	0.007 aB	0.036 aA	-	-	0.06 bB	0.17 aA	0.09 bB	0.17 aA
RR	0.012 aA	0.015 bA	-	-	0.10 aB	0.15 aA	0.13 aA	0.15 aA
s.m.d.(Line)/(Column)	0.01		-		0.03		0.026	
Water Management	Herbicide							
	With	Without	With	Without	With	Without	With	Without
-0.03	0.028 aB	0.07 aA	0.026 aB	0.076 aA	0.230 aB	0.434 aA	0.298 aB	0.434 aA
-0.07	0.000 bA	0.00 bA	0.000 bA	0.000 bA	0.008 bB	0.043 bA	0.034 bA	0.043 bA
-0.5	0.000 cA	0.00 cA	0.000 bA	0.000 bA	0.001 cA	0.002 cA	0.001 bA	0.002 cA
s.m.d. (Line)	0.01		0.010		0.03		0.03	
s.m.d. (Column)	0.01		0.012		0.04		0.04	

Averages followed by the same letter, lowercase in the column and upper case in the line, do not differ statistically from each other by Tukey's test (P <0.05). Nodule DM = dry matter of the nodules; Conv. = conventional soybean; RR = transgenic soybean; W. Manag. = water management. [(1)] Data from the phenological stage V2 were transformed according tothe equation " $y = \sqrt{x}$ ".

The impacts of lactofen on the activity of N_2-fixing bacteria in soybean plants were not found in literature. As for imazethapyr, Parsa et al. (2013) reported harmful effects on *Bradyrhizobium japonicum* strains, with a reduction in the number and diameter of nodules in plants treated with this herbicide.

Although the herbicides used to control weeds in soybean cultivars show good selectivity for this crop, studies have pointed out rhizobia intoxication and a reduction in nodule formation, and the activity of N_2-fixing bacteria (Dvoranen et al., 2008; Bohm et al. (Zhang et al., 2013, Wu et al., 2014). However, these effects depend on several factors, such as soil type, bacterial strain, herbicide group and used dose.

In addition, the rhizobia/plant interaction can be directly or indirectly affected by herbicides in different ways, such as: reduction of the root system, inhibition or inactivation of biochemical signaling and alteration of the indoleacetic acid balance in the host plant, hindering or compromising stability and the growth of nodules (Kremer and Means, 2009; Ahemad and Khan, 2011; Parsa et al., 2013). All these mechanisms may compromise the productive yield of soybean, since the biological nitrogen fixation is the main process responsible for the nitrogen demand and the increase in the productivity of this crop (Hungria et al., 2006; Hungria and Mendes, 2014).

Among other factors, cultivar BRS Valiosa (RR) presented a more efficient root system, adapted to overcome moderate water scarcity conditions (-0.07 MPa). It was also highlighted that the biological nitrogen fixation activity was higher for BRS Valiosa (RR) under conditions of moderate water scarcity (-0.07 MPa), evaluated by the nodule number and dry matter. In addition, cultivar MG/BR 46 Conquista (conventional) presented greater reduction of the root system when submitted to the spraying of imazethapyr and lactofen, and the activity of the nitrogen fixing bacteria was reduced by these herbicides.

REFERENCES

Ashraf M. Inducing drought tolerance in plants: recent advances. **Biotechnol Adv.** 2010;28:169-83.

Ahemad M., Khan M.S. Response of greengram [*Vigna radiata* (L.) Wilczek] grown in herbicide-amended soil to inoculation with *Bradyrhizobium* sp. (vigna) MRM6. **J Agric Sci Tech.** 2011;13:1209-22.

Ali M.L. et al. Greenhouse screening of maize genotypes for deep root mass and related root traits and their association with grain yield under water-deficit conditions in the field. **Euphytica**. 2016;207:79-94.

Bañon S. et al. Effects of water stress and night temperature preconditioning on water relations and morphological and anatomical changes of *Lotus creticus* plants. **Sci Hortic.** 2004;101:333-42.

Bohm G.M. et al. Glyphosate-and imazethapyr-induced effects on yield, nodule mass and biological nitrogen fixation in field-grown glyphosate-resistant soybean. **Soil Biol Biochem.** 2009;41:420-2.

Chaitanya K.V. et al. Water stress effects on photosynthesis in different mulberry cultivars. **Plant Growth Regul.** 2003;40:75-80.

Dvoranen E.C. et al. Nodulação e crescimento de variedades de soja RR sob aplicação de glyphosate, fluazifop-p-butyl e fomesafen. **Planta Daninha**. 2008;26:619-25.

Fehr W.R., Caviness C.E. **Stages of soybean development**. Ames: State University of Science and Technology, 1977. 11p. (Special report, 80).

Fenta B.A. et al. Field phenotyping of soybean roots for drought stress tolerance. **Agronomy**. 2014;4:418-35.

Garnett T. et al. Root based approaches to improving nitrogen use efficiency in plants. **Plant Cell Environ.** 2009;32:1272-83.

Galmés J. et al. Leaf responses to drought stress in Mediterranean accessions of *Solanum lycopersicum*: anatomical adaptations in relation to gas exchange parameters. **Plant Cell Environ.** 2013;36:920-35.

He J. et al. Conserved water use improves the yield performance of soybean (Glycine max (L.) Merr.) under drought. **Agric Water Manage.** 2017;179:236-45.

Hungria M. et al. Nitrogen nutrition of soybean in Brazil: Contributions of biological N_2 fixation and N fertilizer to grain yield. **Can J Plant Sci.** 2006;86:927-39.

Hungria M., Mendes I.C. Nitrogen fixation with soybean: the perfect symbiosis? In: De Bruijn FJ editor. **Biological nitrogen fixation**. v.2, Chapter 99. John Wiley & Sons, New Jersey. 2015. p.1005–1019.

Karthikeyan B. et al. Alterations in seedling vigour and antioxidant enzyme activities in *Catharanthus roseus* under seed priming with native diazotrophs. **J Zhejiang Univ Sci B.** 2007;8:453-7.

King C.A., Purcell L.C. Soybean nodule size and relationship to nitrogen fixation response to water deficit. **Crop Sci.** 2001;41:1099-107.

Klar A.E. Evapotranspiração. In: Klar A.E. **A água no sistema solo-planta-atmosfera**. 2ª.ed. São Paulo: Nobel, 1984. p.408.

Kremer R.J., Means N.E. Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. **Eur J Agron.** 2009;31:153-61.

Ladrera R. et al. Reduced carbon availability to bacteroids and elevated ureides in nodules, but not in shoots, are involved in the nitrogen fixation response to early drought in soybean. **Plant Physiol.** 2007;145:539-46.

Manavalan L.P. et al. Physiological and molecular approaches to improve drought resistance in soybean. **Plant Cell Physiol.** 2009;50:1260-76.

Makbul S. et al. Changes in anatomical and physiological parameters of soybean under drought stress. **Turk J Biol.** 2011;35:369-77.

Mastrodomenico A.T. et al. The response and recovery of nitrogen fixation activity in soybean to water deficit at different reproductive developmental stages. **Environ Exp. Bot.** 2013;85:16-21.

Mak M. et al. Leaf mesophyll K^+, H^+ and Ca^{2+} fluxes are involved in drought-induced decrease in photosynthesis and stomatal closure in soybean. **Environ Exp Bot.** 2014;98:1-12.

Parsa M. et al. Effect of the recommended and optimized doses of haloxyfop-P-methyl or imazethapyr on soybean-*Bradyrhizobium japonicum* symbiosis. **Ind Crop Prod.** 2013;50:197-202.

Pereira M.R.R. et al. Efeito de herbicidas sobre plantas de *Brachiaria plantaginea* submetidas a estresse hídrico. **Planta Daninha**. 2010;28:1047-58.

Raij B. van et al. **Recomendações de adubação e calagem para o Estado de São Paulo**. 2ª.ed. Campinas: IAC, (IAC. Boletim Técnico, 100). 1996. p.285.

Ryle G.J.A. et al. The respiratory costs of nitrogen fixation in soyabean, cowpea, and white clover ii. comparisons of the cost of nitrogen fixation and the utilization of combined nitrogen. **J Exp Bot.** 1979;30:145-53.

Sadeghipour O., Abbasi S. Soybean response to drought and seed inoculation. **World Appl Sci J.** 2012;17:55-60.

Souza R.A. et al. Minimal set of parameters for evaluation soil microbiota and biological nitrogen fixation in soybean. **Pesq Agropec Bras.** 2008;43:83-91.

Thu N.B.A. et al. Evaluation of drought tolerance of the Vietnamese soybean cultivars provides potential resources for soybean production and genetic engineering. **BioMed Res Int.** 2014;2014:1-9.

Wu X. et al. Responses of soil microbial community to different concentration of fomesafen. **J Hazard Mater.** 2014;273:155-64.

Vadez V. Root hydraulics: the forgotten side of roots in drought adaptation. **Field Crop Res.** 2014;165:15-24.

Zhang X. et al. Responses of soil nitrogen-fixing, ammonia-oxidizing, and denitrifying bacterial communities to long-term chlorimuron-ethyl stress in a continuously cropped soybean field in Northeast China. **Ann Microbiol.** 2013;63:1619-27.