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Inoculation of *Bradyrhizobium* with cellular additives and micronutrients in soybean seeds cultivated in Oxisol under no-tillage system

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In the bid to get increased yield of soybean, several studies on biological fixation of nitrogen have been done in order to enhance the results of this technology with cellular additives and micronutrients. The aim of this study was to evaluate the use of inoculant (*Bradyrhizobium japonicum* SEMIA and 5019) associated with cellular additives, cobalt and molybdenum (Fertiactyl® leg) in soybeans seeds in Oxisol cultivated under no-tillage system. The experiment was conducted in a randomized block with two treatments (with and without commercial product) and 10 repetitions, totaling 20 experimental plots in the cultivation of soybean. The experiment was done in 2011/2012 and 2012/2013. We evaluated the N content in grain yield and weight of 100 grain in the 2011/2012 and grain yield, weight of 100 grain and foliar content of N, Ca, Mg, K, P and S in the 2012/2013 crop. Inoculation of the *B. japonicum* with cellular additives, cobalt and molybdenum shows greater accumulation of N in soybean grain, but does not influence the weight of 100 grains and yield in the culture of soybean in 2011/2012 and 2012/2013. There was an increase of Cu and reduction of K, P and S in the leaf tissue of soybeans with the presence of the *B. japonicum* with cellular additives, cobalt and molybdenum (Fertiactyl® leg). It is recommended that farmers should not sow soybean with seed treated with fungicide and insecticide for 12 h together with *B. japonicum* inoculation, cellular additives, Co and Mo; they cause nutritional changes without interfering in the yield under no-tillage in Oxisoil.

Key words: *Bradyrhizobium japonicum*, *Glycine max*, seed treatment, glifosate, fungicide, insecticide.

INTRODUCTION

Soybean is one of the most economically important crops in the world, as the area under cultivation is the expressiveness of production (USDA, 2013). Indeed it is one of the most cultivated crops in the world, used primarily as a source of protein for human and animal (Graham and Vance, 2003; USDA, 2013).

The interest in increasing production is no longer focused on increasing crop areas, but the increase in production capacity in the same area. This is favored by technologies such as fertilization with biofertilizer (Moreira and Zibetti, 2011), soluble, reactive and natural phosphates (Luchini et al., 2012), poultry litter (Piano and Seidel, 2012).

Nevertheless, the increasing potential of production in the world also requires adopting effective and economically viable ways to availability of nitrogen (N) for grain production (Zilli et al., 2006, 2010b). This is because approximately 40% of soybeans are composed of proteins (Embrapa, 2011; Rodrigues and Silva, 2011), which explains the high demand of this nutrient.

In Brazil, nitrogen fertilizer in soybean is unusual, being the biological nitrogen fixation (BNF) responsible for the provision of most of the nutrients needed to produce and achieve high yield (Embrapa, 2003; Zilli et al., 2006; Embrapa, 2011). In the 1980s, Vargas et al. (1982) stated that BNF could sustain grain yield of up to 4 t ha^{-1}. Since most soils cultivated with soybeans in Brazil belong to the class of Oxisols with low levels of soil organic matter (OM), consequetely low N, appropiate agronomic practices can increase the efficiency of BNF and increase yield (Pauferro et al., 2010).

Soil management can also interfere with temperature, moisture, nutrients, organic matter and crop management to increase the efficiency of N-fixing bacteria (Campos and Gnatta, 2006; Lucca and Hungria, 2014) and provide increased yield in soybean. Consolidated no-tillage systems have soils with lower rate of oxidation of organic matter and consequently a higher content of organic matter, mainly by the presence of soil cover. The result is the maintenance of higher moisture and lower thermal variation in the soil, and the availability of nutrients, reducing the need for fertilizers. It also favors the increase and maintenance of the populations of microorganisms in the soil, especially nitrogen-fixing bacteria (Zilli et al., 2006).

The process of BNF in Brazil is responsible for nitrogen accumulated by plants; it represents about 200 kg ha^{-1} N (Zilli et al., 2010b), which is no longer applied via mineral fertilizers. This reduces the cost of production (Albareda et al., 2009). Thus the application reduces too much nitrogen fertilizer, which can contaminate water tables (Jadoski et al., 2010; Macdonald et al., 2011). The overuse of mineral fertilizers, such as nitrogen has caused environmental problems in some parts of the planet. In Europe, environmental costs including all nitrogen losses were recently estimated at 70-320 billion euros per year, which exceeds the direct economic benefits of N in agriculture (Foley et al., 2011).

The efficiency of BNF, in turn, is dependent not only bacterial strains used and symbiosis with culture, but on adequate availability of certain chemical elements. Cobalt (Co) and molybdenum (Mo) are essential for BNF (Taiz and Zeiger, 2013). The first B12 vitamin is essential for the processing of BNF and other parts of the molibdo-enzymes, used in absorption and metabolism of nitrogen (Novais et al., 2007).

The processing of BNF can reduce the pH of the soil, especially next to the root system of the plants. This reduction is responsible for the increased absorption of some nutrients in the soil, like iron (Fe) (Souza et al., 2010). These authors mention that by the time it becomes effective symbiosis, soybean plants may exhibit Fe deficiency, making it not observed later with the low pH in the rhizosphere. According to Silva et al. (2011a), the survival of bacteria can vary depending on the pH, salinity, and bactericidal action of some products, to achieve the application of Mo and Co with *Bradyrhizobium* sp. in soybean seeds.

However, studies have shown the incompatibility between *Bradyrhizobium* sp. and practices of seed treatment with fungicides and insecticides in soybean (Campo and Hungria, 1999; Bueno et al., 2003; Zilli et al., 2010a; Pereira et al., 2010; Embrapa, 2011; Marks et al., 2013; Favero and Lana, 2014). In this sense, Tedesco and Campos (2000), citing inoculants 2000s, recommended that inoculated soybean seeds should not be stored for more than a day, so the suggested level of viable cells per seed *B. japonicum* is reached. In the search for new technologies and product formulations to enhance seed treatment with *Bradyrhizobium* sp., cellular additives were tested to enable greater concentration of live microorganisms in seeds during inoculation process (Marks et al., 2013). This enables performing advance inoculation before sowing.

Thus, the objective of this study was to evaluate the use of inoculant (*B. japonicum*) associated with cellular additives, cobalt and molybdenum in soybean seeds in Oxisoil grown in no-tillage system.

MATERIALS AND METHODS

The work was executed in the West of the State of Paraná, Brazil, in soil classified as Oxisoil very clayey (Embrapa, 2013) with the following coordinates: 24º15'S and 54°10'W and altitude of 338 m (Figure 1). The farm has cultivated soybeans for 30 years and used no-tillage for 20 years in succession of crops, using soybean in summer and wheat/corn in the winter. Before the experiment, the area was occupied by wheat crop.

The soil granulometric characteristics of the property are 650 g kg^{-1} clay, 130 g kg^{-1} sand and 220 g kg^{-1} silt, and the result of the

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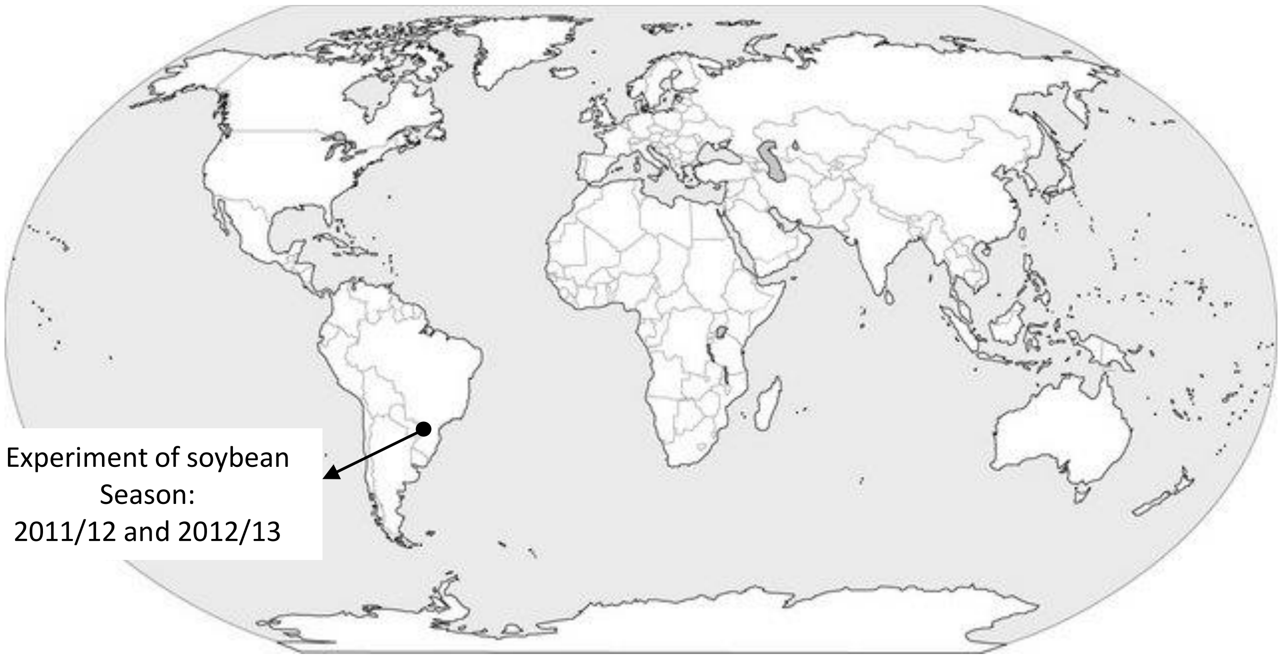

Figure 1. West of Paraná state, Brazil.

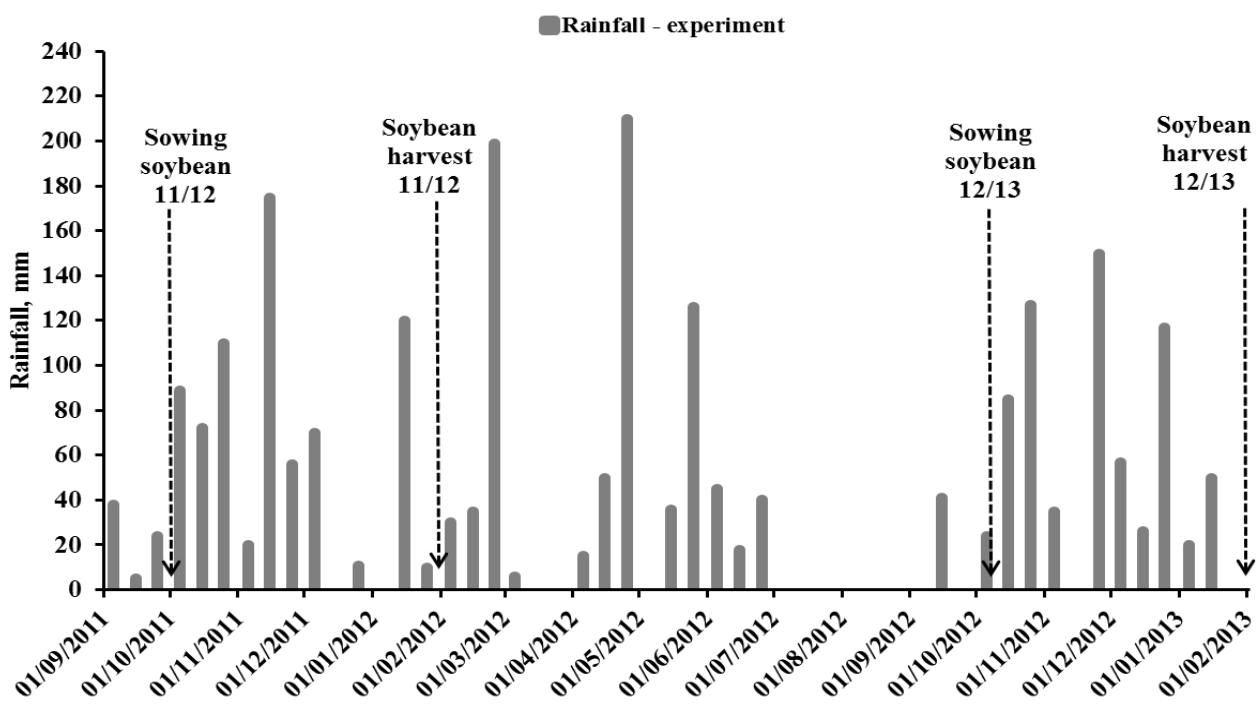

Figure 2. Rainfall accumulated (mm) every ten days in the experimental area during the driving period between 09/01/2011 to 02/01/2013.

chemical analysis gives the following values: pH in $CaCl_2$ = 4.80;C = 16.70 g dm^{-3}; P = 12.40 mg dm^{-3}; K^+ = 0.20 $cmol_c$ dm^{-3}; Ca^{+2} = 4.67 $cmol_c$ dm^{-3}; Mg^{+2} = 1.64 $cmol_c$ dm^{-3}; H+Al = 5.76 $cmol_c$ dm^{-3}; Al^{+3} = 0 $cmol_c$ dm^{-3}; SB = 6.51 $cmol_c$ dm^{-3}; CEC = 12.27 $cmol_c$ dm^{-3}; e V% = 53.06. At this location, the accumulated rainfall every ten days recorded during the experiment is shown in Figure 2 and second Koppen, the climate is Cfa, subtropical with rains well distributed throughout the year and hot summers (Caviglione et al., 2000). The rainfall for the first season of soybean in 2011/2012 at sowing to harvest was 733 mm and in 2012/201313, it was 621 mm.

The experiment was arranged in randomized complete block design with two treatments and 10 replications, totaling 20 experimental plots in two seasons (2011/12 and 2012/13). The treatments consisted of control with no inoculation and cobalt (Co) and molybdenum (Mo) and treatment Fertiactyl leg ® [*B. japonicum* SEMIA 587 and 5019 inoculant with Extender (additive cellular), to keep the bacteria viable for long, with cobalt (Co) and molybdenum (Mo) and natural sources of amino acids, humic and fulvic acids] (Timac Agro Brazil, 2013) with double dose due to pH = 4.80 $CaCl_2$ soil used. In both seasons, the soybean seed treatment was performed 12 h before sowing. In the same manner, two cultivations were carried out with seed treated with fungicides fludioxonil (25 g L^{-1}) + metalaxyl – M (10 g L^{-1}), in the recommendation of 100 mL per 100 kg of seed, and fipronil (250 g L^{-1}) with 200 mL per 100 kg of seed (EMBRAPA, 2011).

In both seasons, ten days before sowing ghyfosate herbicide (3 l ha^{-1} Roundup Ready) and ghyfosate herbicide in V5 stage (1.5 l ha^{-1} Roundup Ready) were applied. It was first deployed soybean cultivar with Vmax RR (SYN 7059RR) on October 2011, in summer 2011/2012 season for assessment in first year, with 14 plants per meter. During the 2012 winter crop sowing corn P3340HX, which is recommended for the region as agroclimatic zoning of Paraná, was performed. Subsequently, treatments were tested in the cultivation of summer crops 2012/2013, with the sowing of transgenic soybean cultivar Potencia RR on October 2012 (Embrapa, 2011), with 14 plants per meter. The use of different cultivars was to alter the genetic material and reduce pest problems related with soil conditions that occur with the use of successive soybean in summer.

During period of the experiment we performed, soybean sowing in 0.45 m spacing, density of 15 seeds per meter and final stand of 266,666 plants ha^{-1} in both summer crops. The plots were total area of 12.00 m^2 and usable area of 5.40 m^2. Monitoring of pests, diseases and weeds and need for control was performed according to the recommendations for soybean (Embrapa, 2011) and for maize cultures (Rodrigues and Silva, 2011). The sowing of soybean was performed with precision seed drill Marchesan Ultra Flex with 11 soybean lines coupled to tractor.

The fertilization of soybean crop in 2011/2012 250 kg ha^{-1} of superphosphate fertilizer was used with 19% P_2O_5. In the second crop corn harvest 250 kg ha^{-1} was used as fertilizer formulated 16-16-16. The next crop of soybeans in the 2012/2013 crop, 250 kg ha^{-1} of this fertilizer 4-15-10 was used with 25% OM (organic matter), Minorgan Organofós (Minorgan, 2013).

In the 2011/2012 harvesting, grains collected had weight of 100 (M 100), and the levels of nitrogen (N) of grains were also determined.

In the second cropping of soybeans in the 2012/2013, leaf samples were collected in full bloom as third trifoliate, as recommended procedures to time and sample leaf described by Malavolta et al. (1997).

Soybean leaves collected were dried in a forced-air oven for three days at 65 ± 2°C, ground to smaller size and analyzed. First, 0.02 g of dry mass was digested by 4 mL HNO_3+$HClO_4$ (3:1) for determining K, Ca, Mg, Cu, Fe, Mn and Zn. Concentrations of Ca, Mg, Cu, Fe, Mn and Zn were determined by flame atomic absorption spectrophotometry, K determined by flame photometer, P and S were determined by colorimetry, as previously described by Embrapa (2009). Concentrations of N in grains in 2011/2012 and leaves in 2012/2013 were determined by 2 mL H_2SO_4 digestion of 0.02 g dry mass and vapor distillation of Kjeldahl (Tedesco et al., 1995).

At the point of harvesting of crop, on February 5, 2013 in the 2012/2013, plants were collected at the weight of 100 grains (M 100) and yield. In both seasons, plants were threshed on “Vencedora B-150” to obtain the grains, for determining the weight of grains and standardizing the moisture content of the samples to 14%.

Table 1. Nitrogen content in grain (grain N), weight of 100 grains (M 100) and soybean yield in the 2011/2012 season based on the presence and absence of Fertiactyl leg® (*Bradyrhizobium japonicum* inoculant with Extender, to keep the bacteria viable for more time and with cobalt (Co) and molybdenum (Mo), with natural sources of amino acids, humic and fulvic acids) grown on Oxisol in the city of Terra Roxa/PR, 2012.

Treatment	Soybean (2011/2012)		
	N grain	**M 100**	**Yield**
	g kg^{-1}	**g**	**kg ha^{-1}**
Witness	17.72b	10.42	1,170.70
Inoculant	24.63a	10.30	1,191.50
Source of variation		F Values	
Treatments	5.03 *	0.23^{ns}	0.36^{ns}
CV (%)	32.52	5.33	6.54

*Significant at 5% by F test; ^{ns}not significant at the 5% level by F test.

Statistical analysis of the results was performed with the GENES program (Cruz, 2006), so the data were subjected to analysis of variance. In case of significant effects, we used the actual F-test to verify the probability of a significant difference between the means, because with only one degree of freedom for the source of variation, the F test is conclusive.

RESULTS AND DISCUSSION

The inoculation with *B. japonicum* in addition to fertilization with cobalt and molybdenum showed greater accumulation of N in grain of soybeans in 2011/2012 harvest, differing ($P<0.05$) from the control. However, this was not reflected in greater mass of 100 grain and crop yield (Table 1). Meschede et al. (2004) reported that seed treatment with cobalt (Co) and molybdenum (Mo) improved seed quality in relation to protein content, that is related with N content in grain. Albareda et al. (2009), Zilli et al. (2010a) and Favero and Lana (2014) also observed that positive effects of inoculation increased levels of N in soybeans, when seeds were inoculated with *B. japonicum* without seed treatment while Lantmann et al. (1989) observed increases of 3-6% of protein in grains, when Mo was used in soybean. Diesel et al. (2010) did not find significant results with application of foliar molybdenum and cobalt for the weight of 100 grains.

The absence of effects on the weight of 100 grains and yield with inoculation was also observed by Silva et al. (2011a) regardless of inoculum dose and way of application of Co and Mo in the first crop. Pessoa et al. (1999) in an Oxisol also observed no interference of Mo foliar application in *B. japonicum* inoculation. On the other hand, Hungria et al. (2013) related problems of inocula-tion with *B. japonicum* on seed of soybean with fungicide

Table 2. Weight of 100 grains (M 100) and soybean yield in the 2012/13 season based on the presence and absence of Fertiactyl leg® (*Bradyrhizobium japonicum* inoculant with Extender, to keep the bacteria viable for more time and with cobalt (Co) and molybdenum (Mo), with natural sources of amino acids, humic and fulvic acids) grown on Oxisol in the city of Terra Roxa/PR, 2012.

Treatment	Soybean (2012/2013)	
	M 100 (g)	Yield (kg ha^{-1})
Witness	15.41	4,290.56
Inoculant	15.45	4,292.44
Source of variation	F Values	
Treatments	0.01^{ns}	0.01^{ns}
CV (%)	4.92	4.62

*Significant at 5% by F test; ^{ns}not significant at the 5% level by F test.

and insecticide, in minimizing favorable effects of biological nitrogen fixation, and research possibility by in-forrow inoculation.

The inoculation of *B. japonicum* using fertilization with Co and Mo for soybean crop in 2012/2013 did not influence variable mass of 100 grains and yield (Table 2) in clayey Oxisol at pH 4.80 for $CaCl_2$. Silva et al. (2011a, b), who grow soybeans in dystrophic typic with a pH $CaCl_2$ of 4.6 with V% of 40, found different results. In the second year of cultivation, the application of Co and Mo in soybean increased the mass 100 grains, irrespective of the method of application, but, there was no increase in grain yield. Similarly, Silva et al. (2011b) reported that the weight of 100 grains is a characteristic value of each cultivar, however, does not prevent difference with changes of environmental and/or management to which the culture is subjected. In this research, in both seasons glifosate herbicide was applied before sowing and V5 stage of soybean. It is an important information, because recent research shows that BNF and yield parameters were more affected by location, cropping season and cultivar than by the applied glifosate herbicide in the culture or weed-management strategy (Nakatani et al., 2014; Hungria et al., 2014). Glifosate did not interfere in the results, therefore, Malty et al. (2006) did not see any effect on nodulation in the study with doses of glifosate applied before sowing soybean.

Meschede et al. (2004) said the expected response to Mo and Co in soybean nodulation is lower in soils with high organic matter content, high fertility and acidity adjusted. So much so that Lantmann (2002) found greater responses to the application of Mo in conditions of pH $CaCl_2$ less than 4.3, for Latosolic Alic, and less than 4.8 for the Oxisol Dark Alic. However, Marcondes and Caires (2005) found that application of Mo and Co in the seed grown in soil with pH $CaCl_2$ 5.2 did not affect nodulation of soybean plants. Thus, the pH may be less relevant when it is growing under no-tillage for several years (Pessoa et al., 1999).

On the other hand, an increase in yield was observed by Zilli et al. (2010a) and with a low content of organic matter in soil and the soil pH $CaCl_2$ of 5.2. In addition, Barbaro et al. (2009) observed response of soybean cultivars to inoculation and application of cobalt and molybdenum in pasture areas of reform. The application of molybdenum and cobalt via seeds and/or the V4 leaf stage provided a significant increase in soybean yield, with increases of up to 240 kg ha^{-1} in grain production in the culture area of reform pasture in dystrophic Red-Dark texture sandy $CaCl_2$ at pH 5.2. The agronomic parameters evaluated were positively affected by the application of Co and Mo even in Red Hapludox clayey with pH $CaCl_2$ 4.4, particularly when applied as a foliar both by seed (TS + V4), including grain yield. The way of application was not significantly different, that is both the application via seed and foliar were efficient in providing these nutrients for soybean (Dourado Neto et al., 2012). Recently, Favero et al. (2013) found that the use of *B. japonicum* inoculation + Co and Mo together in seed treatment with fungicide and insecticide increased the yield of soybean, as observed reduction in plants with "crazy soybean II" in soil with pH 4.7. It affected plants record, high abortion rate of flowers and pods, which prevents natural maturation of the plant, and remains green even after desiccating herbicides. According to the authors, these results may have been evidenced due to the low pH of the soil, which reduces the availability of Mo on the ground, damaging the fixation of N_2 in soil.

Other researchers, like Campos (1999) also found similar results in their survey in Typic dark, with five years in no-tillage, where there was no significant effect on grain yield of soybean with application of up to 1 kg of peat inoculant for each 50 kg seeds. Pessoa et al. (1999), in an experiment conducted on an Oxisol in pH H_2O at 5.3, said no response was observed for seed treatment with *B. japonicum* and Mo or when it was used in foliar fertilization. Chueiri et al. (2005), in areas with high acidity and pH less than 5.5 in water, recommend applying double dose of inoculant *B. japonicum*, because the acidity of the soil interferes with the survival of bacteria and also increases competition among native strains and selection. Therefore, it is expected that this increases the amount of inoculant to compensate for the loss of viable cells.

The difference in soybean yield obtained in 2011/2012 (Table 1) and 2012/2013 seasons (Table 2) is probably related to rainfall condition in every culture since in the first and second crops, prolonged drought was satisfactory rainfall conditions for the development of culture (Figure 1). Water is necessary for increasing microbiology activity and for photosynthesis (Taiz and Zeiger, 2013); also, growth is sustained by nitrogen fixation rates and photosynthesis activity. Luca and

Table 3. Levels of nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg) and sulfur (S) in soybean leaves in the 2012/13 season based on the presence and absence of Fertiactyl leg® (*Bradyrhizobium japonicum* inoculant with Extender, to keep the bacteria viable for more time and with cobalt (Co) and molybdenum (Mo), with natural sources of amino acids, humic and fulvic acids) grown on Oxisol in the city of Terra Roxa/PR, 2013

Treatment	N	K	P	Ca	Mg	S
	$g\ kg^{-1}$					
Witness	51.20	13.40 a	3.68 a	10.46	2.12	12.04 a
Inoculant	51.60	11.53 b	3.16 b	9.66	2.35	10.91 b
Source of variation	F Values					
Treatments	0.22^{ns}	8.09*	6.78*	3.31^{ns}	0.21^{ns}	4.99*
CV (%)	3.59	11.76	13.09	11.98	49.96	9.90

*Significant at 5% by F test; ^{ns}: not significant at the 5% level by F test.

Hungria (2014) related that there are lower plants of soybean in areas of quadruple photosynthesis and nitrogen fixation rates. In this research, 14 plants per meter can be reduced; therefore, the efficiency of water use present in the soil was elevated mainly in 2011/2012.

In general, lack of response to the addition of chemical elements Co and Mo may be associated with adequate levels of availability in the soil or seed with sufficient concentrations of the need for culture (Ishizuka, 1982). In fact, Pessoa et al. (1999) found that the source of basalt soil, rich in micronutrients, with high fertility, acidity corrected by liming and pH $CaCl_2$ 5.3 in no tillage system probably has Mo enough to meet the requirement of soybean.

As reported by Gris et al. (2005) lack of response between treated and untreated seeds with inoculant is likely to occur in soil that already has established populations of bacteria that provide adequate nodulation. As the place used in this work has grown soybean for the last 30 years, there is likely going to be the establishment of populations of nitrogen fixing bactéria. Thus, the increase in yield resulting from inoculation in areas already previously cultivated with soybeans, are less expressive than in areas of first year (Campos, 1999; Campos and Gnatta, 2006). However, average gain of 4.5% was observed in grain yield with inoculation already cultivated areas (Embrapa, 2003).

Critical periods of leguminous vegetable other than the nitrogen biological fixation of nitrogen have been reported.

For example, at the early stage of plant growth when symbiosis is being developed, the N-fixed cannot supply the amount of N to meet the demand of the plant (Gan et al, 2003). In addition, when the culture is achieved, the filling phase of pods can result in nodule senescence. In these situations, and when the supply of soil N is not high enough, one option would be to supplement it with mineral N fertilizer of leaves.

Regarding the contents of macronutrients in leaves of soybean evaluated, only K, P and S (Table 3) decreased with inoculation with Co and Mo, specifically in the 2012/2013 harvest. Vieira Neto et al. (2008) actually found a low effect on levels of macronutrients N, P and S in leaves of soybean. Tiritan et al. (2007) observed 6% increase in the content of S, Mo and Co in the seed.

The reduction in the contents of K, P and S in the leaf tissue of soybean inoculated may be related to the supply of N to the roots of soybean inoculated (Gris et al., 2005). Intensifying the metabolic activity in roots can increase the demand for P and K by this organ. Different results were found by Marcondes and Caires (2005) that the levels of macronutrients in leaves of soybean were slightly influenced by the application of molybdenum in the seeds.

Vieira Neto et al. (2008) working with soybean plants obtained from seeds subjected to different treatments of inoculation, noted that there was no difference between treatments for N, P and S; however, the K observed that treatment (fertilization with N-fertilizer (200 kg ha^{-1}) was lower than the others.

Lack of effect of leaf N (Table 3) was also observed by Toledo et al. (2010) who found no interference with the values obtained by inoculation with *B. japonicum*. The results corroborate those obtained by Marcondes and Caires (2005), showing that the presence of molybdenum did not change the nitrogen concentration in soybean leaves. Albino and Campo (2001) also found no change in the levels of nitrogen in soybean plants by applying sodium molybdate immediately after inoculation on seeds. Favero and Lana (2014) did not observe effect of leaf N when they inoculated soybean seed with *B. japonicum* + Co + Mo + insecticides + fungicides at the moment of sowing.

Regarding the levels of micronutrient on soybean leaves, only Cu (Table 4) was similar (P<0.05) and increased with inoculation together with cobalt and molybdenum used in the 2012/2013 harvest. Vieira Neto et al. (2008) found that micronutrient absorption by soybean plants was not affected by the treatments involving inoculation with *B. japonicum*, showing no

Table 4. Levels of copper (Cu), zinc (Zn), manganese (Mn) and iron (Fe) in soybean leaves in the 2012/13 season based on the presence and absence of Fertiactyl leg® (*Bradrhyzobium japonicum* inoculant with Extender, to keep the bacteria viable for more time and with cobalt (Co) and molybdenum (Mo), with natural sources of amino acids, humic and fulvic acids) grown on Oxisol in the city of Terra Roxa/PR, 2013.

Treatment	Cu	Zn	Mn	Fe
	mg kg^{-1}			
Witness	20.89 b	22.82	31.95	103.19
Inoculant	44.54 a	19.40	31.74	106.32
Source of variation	F Values			
Treatments	7.89 *	0.71 ^{ns}	0.01 ^{ns}	0.16 ^{ns}
CV (%)	57.55	42.76	48.80	16.86

*Significant at 5% by F test; ^{ns}:not significant at the 5% level by F test.

changes in foliar levels of soybean. Marcondes and Caires (2005) also found that the concentrations of Cu, Zn and Mn on soybean leaves were not influenced by the application of molybdenum in the seeds. This is similar to results obtained in this work, except for Cu, which showed a higher content when Fertiactyl leg® was used.

According to Souza et al. (2010), plants fixing N has the ability to reduce the pH of the soil, especially next to the root system of the plants. This reduction is responsible for the increased absorption of some nutrients in the soil. Thus, in the soil the increased absorption of Cu may be associated with organic matter, which retains this nutrient in the form of the inner sphere complex (Novais et al., 2007); it may be available to the soil solution to increase acidity in the rhizosphere provided by the nutrient uptake in soybean crop in the treatment with seed inoculation.

An important factor that deserves to be highlighted is that the inoculation was performed 12 h before sowing with the Fertiactyl ® leg with cellular additives (Timac Agro Brazil, 2013), which serve as food for bacteria to survive up to seven days. Thus, the absence of significant results regarding grain yield may be related to poor survival of bacteria after inoculation (Embrapa, 2011), as sowing was not performed immediately after treatment. Also and mainly because seed treatment with fungicide, evidenced by Zilli et al. (2010a) and Embrapa (2011), who identified inhibition of nodulation of bacteria in this situation, has been performed and also Pereira et al. (2010) observed a reduction in nodulation and growth of soybean plants by seed treatment with carbosulfan insecticide, clothianidin, fipronil, imidacloprid and thiamethoxam depending on the strain used for inoculation. Negative effects have also been observed in the BNF in the associated use of *B. japonicum* with fungicides. Bueno et al. (2003) and Zilli et al. (2010b) tested the effect of fungicides on survival and nodulation of *B. japonicum* and obtained a reduction of microorganisms in combinations of fungicides carboxim+thiram. Moreover, Marks et al. (2013) found that the use of cell additive in combination with inoculant and specifically Maxim fungicidal seed treatment of soybean improved survival and longevity of *B. japonicum* in the seed, and maximized BNF technology.

Another possibility is that these results demonstrated that, probably, the nutritional needs of N by soybean were supplied by the process of symbiotic N_2 fixation, regardless of the use of seed treatment with *B. japonicum* with Co and Mo, as has been cultivated soybean for several years, and the type of soil, minimizing the effects of inoculation. In this situation, it is pertinent to assess new work in the field, with the prior execution of seed treatment fungicides and insecticides, and inoculation with *B. japonicum* with Co and Mo at the time of sowing date. Seeing that the beneficial effects of using insecticides and fungicides for seed treatment is imminent, it is necessary to continue studies to find the ideal setting for inoculation which really identifies the time, since reduction of inoculation may occur to effectuate it in the same instant that the fungicide and insecticide are used. Therefore, recent research shows physiological and yield performance with in-furrow inoculation has been useful alternative for soybean, because it does not use seed treated with fungicides, decreases nodulation (Zilli et al., 2010b) with fungicides/insecticides, does not interfere with on leaf N and yield. Although there is observed green stem and leaf retention in soybean, similarly it uses seed without anything in the sow (Favero and Lana, 2014).

Conclusion

Seeds treated with fungicide and insecticide together with *B. japonicum* inoculation, cellular additives, cobalt and molybdenum (Fertiactyl® leg) used in soybean seeds 12 h before sowing provide greater N accumulation in the grain, increase Cu and reduce K, P e S in leaves, but do not influence the weight of 100 grains and yield in soybean.

It is recommended that farmers should not sow soybean with seeds treated with fungicide and insecticide for 12 hours together with *Bradyrhizobium japonicum* inoculation, cellular additives, Co and Mo; this is because they cause nutritional changes without interfering in the yield under no-tillage in Oxisoil.

Conflict of interests

The authors did not declare any conflict of interest.

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