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Yield of maize hybrids: Is there any association among nitrogen rate, *Azospirillum* inoculation and fungicide treatment?

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Optimization of land use can be attained by incorporating technologies to crop production, such as the use of diazotrophic bacteria, fertilizers, and pesticides. Seed inoculation with *Azospirillum* is an alternative that favors the incorporation of green agriculture in regions of conventional farming, such as the Brazilian savannah (Cerrado). However, limited information is available about this bacterium's contribution to agriculture when other technologies are also incorporated. This study evaluated the performance of maize hybrids inoculated, or not, with *Azospirillum brasilense*, with or without fungicide applications, and subjected to different nitrogen rates under Cerrado field conditions. Each factor analyzed contributes to the increased maize grain yield. The use of inoculants containing plant growth promoting bacteria is a good option to ensure high yield of maize. Still, nitrogen should not be replaced, neither totally nor partially, by seed inoculation with *Azospirillum*. Fungicide applications should be done, as required, during maize cycle. Moreover, specific maize breeding programs should consider the affinity between *Azospirillum* strains and maize hybrids, mainly for regions with nitrogen deficient soils, like Cerrado. Thus, by incorporating additional technologies, maize crop farmers can optimize land use and, consequently, reduce the expansion into new agricultural areas.

Key words: Foliar protection, nitrogen use, plant growth promoting bacteria, sustainability, *Zea mays* L.

INTRODUCTION

Optimization of land use has been the focus of international discussions for a long time. Recently, in RIO + 20, once again this aspect was addressed, now emphasizing the idea of "green economy" (Scarano et al., 2012). In fact, many farmers do not take advantage of the area's full potential (Silva et al., 2006; Brannstrom et al.,

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2008; Valipour, 2012, 2013; Sá et al., 2013). Thus, agriculture moves into new areas, turning it in an unsustainable business (Klink and Machado, 2005; Gallardo and Bond, 2011). Furthermore, the global area available for agricultural purposes is becoming increasingly scarce and many experts state that the Brazilian savannah (Cerrado), a biodiversity hotspot, is the last agricultural frontier of the world (CEPF, 2015).

One of the biggest granaries of the world, Cerrado is responsible for most of the Brazilian commodity production, especially soybean and maize (Trivedi et al., 2012; CONAB, 2015a). Current maize production with new hybrids has potential yield between 9 and 15 t ha^{-1}. However, in Brazil, the average production is approximately 5.1 t ha^{-1} (CONAB, 2015b), demonstrating that natural resources are poorly managed. Thus, questions about how to solve this problem and how to give maize a label of green agriculture product become important. The immediate answer is the use of technologies that maximize plant genetic potential. One of these technologies, which has been adopted for some time, is seed inoculation with *Azospirillum* (Bashan et al., 2004; Cavaglieri et al., 2009; Hungria et al., 2010; Hungria, 2011).

Since Cerrado soils are naturally nitrogen deficient (Lopes and Cox, 1977; Araújo and Haridasan, 1988; Haridasan, 1994; Bortolini et al., 2001; Ohland et al., 2005; Bustamante et al., 2006; Souza, 2006; Haridasan, 2008), seed inoculation with *Azospirillum* could result in increased maize production (Cavaglieri et al., 2009; Compant et al., 2010; Hungria et al., 2010). However, the incorporation of a new technology, in general, does not replace other practices used.

Pesticide spraying and fertilization are among the most common practices used in cropping systems. Still, the wide use of pesticides in modern agriculture may cause side-effects on non-target microbiota (Pereyra et al., 2009). In this perspective, seed inoculation with *Azospirillum* is controversial. Some authors state that interactions between pesticides and microbes are compatible, such as for tebuconazole and *A. brasilense* sp245 on wheat (Pereyra et al., 2009); while others assert that these interactions are incompatible, such as for carbofuran, chlormephos, terbufos and benfuracarb with *A. lipoferum* strain CRT1 on maize (Revellin et al., 2001). Similarly, another interesting and controversial issue is the use of fertilizers, especially nitrogen, together with *Azospirillum* inoculation. Although strains of *Azospirillum* can improve plant growth and development (Cassán et al., 2009; Hartmann and Bashan, 2009), some studies suggest that nutrient supplementation with mineral fertilizers is needed for greater grain yields (Díaz-Zorita and Fernández-Canigia, 2009), especially for maize (Mehnaz et al., 2010; Ferreira et al., 2013; Myresiotis et al., 2014), in which practices such as fertilization and pesticide application may impair efficacy of treatments with *Azospirillum*. Studies about the interaction among these practices in maize are restricted, mainly on field conditions. Thus, questions are raised by farmers and scientists involved in the maize chain. Therefore, the association of *Azospirillum* seed inoculation, with nitrogen fertilization, and with plant protection in maize production, their combination on yield, and the consequences of such combination in nitrogen use from the physiological point of view were evaluated.

MATERIALS AND METHODS

Site description

The study was done at 18°59'02'' S and 47°27'39'' W during the crop season of 2009/2010, under Cerrado field conditions. The region's climate is classified as humid subtropical (Cwa, according to Köppen's climate classification), with average temperature of 22.8°C and precipitation around 1539 mm per year. Weather was ideal for maize crop during the experiment conduction (Figure 1). Previously to the experiment, soil samples were taken arbitrarily from spatially distributed points, from the 0-20 cm layer, and chemically and physically analyzed. Chemical parameters evaluated were soil pH (in H_2O), exchangeable P (in $Mehlich^{-1}$), exchangeable K, Ca, Mg and Al. All parameters were analyzed according to the Committee of Soil Fertility of Minas Gerais State (CFSEMG) (1999). The main chemical and physical characteristics of the soil at the establishment of the experiment are shown in Table 1. The soil of the experimental area is classified as an Oxisol.

Experimental model design

A randomized block design was set up, with six replications, in a 4 × 2 × 2 × 6 factorial structure. Four maize hybrids inoculated, or not, with *Azospirillum brasilense*, with or without fungicide applications, and subjected to different nitrogen rates (50, 100, 150, 200, 250 and 300 kg N ha^{-1}) were evaluated.

Each plot consisted of six 5.2 m long rows, 0.6 m apart, covering an area of 18.7 m^2 per plot and an experimental area of 5,391.4 m^2. The four central rows were used for evaluations, discarding 1 m from each row end.

Seed inoculation

Maize seeds were inoculated with strains of the bacterium *A. brasilense* (Ab-V5 and Ab-V6) in a minimum concentration of 2×10^8 viable cells ml^{-1}. Mixture was carefully done, in plastic bags, to ensure a uniform distribution of the liquid inoculant on the seeds, at a dose equivalent to 100 ml ha^{-1}. Therefore, theoretical estimate of bacterium cells per seed was 285,714. Maize hybrids used in the study (coded 1 to 4) are genetically modified materials of high yield potential and belong to four different maize breeding companies. The hybrids were selected because they are recommended for Cerrado conditions.

Experiment conduction

Sowing was done immediately after seed inoculation with *Azospirillum*, in a no-tillage system and an approximate stand of 70,000 plants ha^{-1}. Basic fertilization was applied at sowing consisting of 625 kg ha^{-1} of the NPK formula 08-20-20 + 0.5% Zn. When maize plants were at V_6 stage (Ritchie et al., 1992), 78 kg K_2O ha^{-1} were applied broadcast, as well as different rates of urea

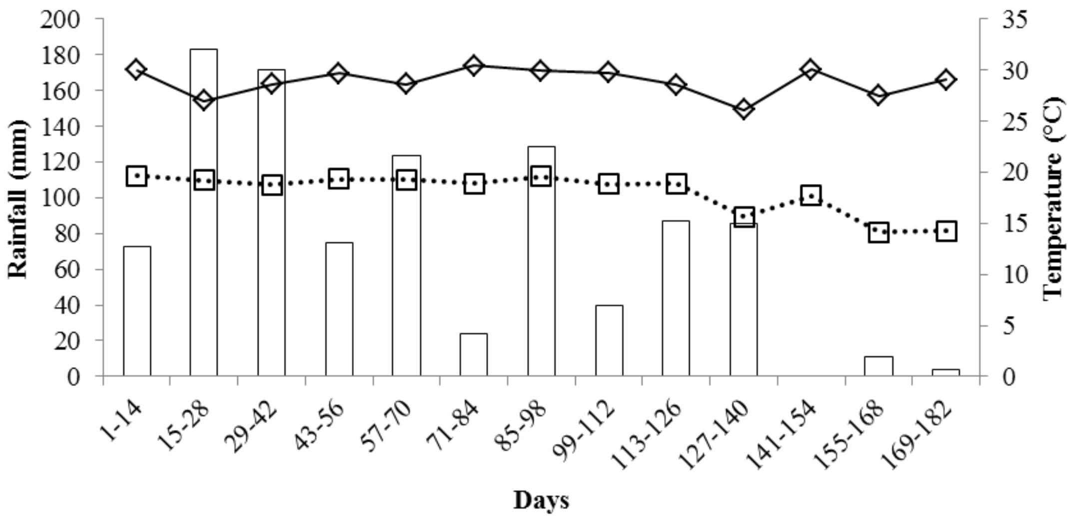

Figure 1. Pluvial precipitation, minimum and maximum temperatures from 17 Nov 2009 to 19 May 2010 at the site where the experiment was conducted (18°59'02'' S and 47°27'39'' W). Source: Laboratory of Climatology of the Federal University of Uberlândia, MG, Brazil.

Table 1. Soil chemical and physical properties (0-20 cm) at the experiment site.

Chemical											Physical
pH	P	K^+	Ca^{2+}	Mg^{2+}	Al^{3+}	Al+H	CEC^z	T_{CEC}^{z}	BS^z	OM^z	Clay
(H_2O)	(mg kg^{-1})	(mmol$_c$ kg^{-1})							(%)	(dag kg^{-1})	(g kg^{-1})
5.5	15.1	4.0	24.0	6.0	0.0	36.0	70.0	34.0	48	3.4	411

^{z}Cation Exchange Capacity (H+Al+Ca+Mg+K); T_{CEC} (Ca+Mg+K); Base Saturation (T_{CEC}/CEC) × 100; Organic Matter.

according to the treatment. Herbicides were used for weed control and insects were controlled with biological and chemical insecticides, according to technical recommendations for the crop, described by the pesticides' manufacturers. Fungicide applications were done at V_8, V_T and R_3 stages with a triazole + strobilurin-based product (in treatments with foliar protection). The dose of the commercial product and mineral oil used were 300 ml + 600 ml ha^{-1}, respectively, at the spray volume 150 L ha^{-1}.

Harvest

Ears from each experimental plot were mechanically harvested and processed when maize grains with 23% of moisture. Weight and humidity were determined on onboard scale and grain-moisture tester, in the harvester. Data were extrapolated to a one-hectare area and corrected to 13% moisture content, rendering productivity values in kg ha^{-1}.

Statistical analysis

All assumptions required for the analysis of variance (ANOVA) were confirmed. The error normality was evaluated by Kolmogorov-Smirnov and the variance of homogeneity by Levene, both at 0.01 significance level. Subsequently, the data set was submitted to the ANOVA (Table 2). When significant differences were detected ($P \leq 0.05$), averages of inoculation effect and of foliar protection were compared by the Tukey test and averages of nitrogen rates by polynomial regression. All analyses were done at 0.05 significance level.

RESULTS

Effects of nitrogen fertilizer, *Azospirillum* inoculation, and foliar protection on maize grain yield

Each factor analyzed contributed to the increase of maize grain yield. Besides isolated effects, productivity was affected by the inoculation with *Azospirillum* combined with the fungicide application, and by the latter factor with the hybrids studied (Table 2). However, the second interaction will not be addressed in this paper since the focus is not the recommendation of maize hybrids, neither the study of their performance in the field, considering that new hybrids are constantly developed and released on the market. Thus, the effects of technologies on maize crop production are emphasized, regardless of the hybrid used by farmers.

Nitrogen fertilization promoted greater maize yield. Crop production peaked up to 9.41 t ha^{-1} at 256 kg N ha^{-1}

Table 2. Analysis of variance.

Source of Variation	DF	Mean Square Grain Yield	*F*
Hybrid (Hyb)	3	48,365,385.94*	260.22
Azospirillum (Azos)	1	36,147,851.49*	194.48
Nitrogen (N)	5	2,495,142.77*	13.42
Fungicide (Fung)	1	273,671,648.58*	1,472.43
Hyb x Azos	3	216,316.95ns	1.16
Hyb x N	15	201,803.18ns	1.09
Hyb x Fung	3	24,523,347.17*	131.94
Azos x N	5	258,140.05ns	1.39
Azos x Fung	1	1,560,221.66*	8.39
N x Fung	5	317,722.49ns	1.71
Hyb x Azos x N	15	91,945.92ns	0.49
Hyb x Azos x Fung	3	191,265.04ns	1.03
Hyb x N x Fung	15	105,917.16ns	0.57
Azos x N x Fung	5	101,598.25ns	0.55
Hyb x Azos x N x Fung	15	41,968.96ns	0.23
Block	5	1,528,514.80	8.22
Error	475	185,864.27	260.22
CV (%)		4.64	

* and ns: significant and not significant by the *F* test (Snedecor statistics) at 0.05 significance level; DF: Degree of freedom; CV: Coefficient of variation.

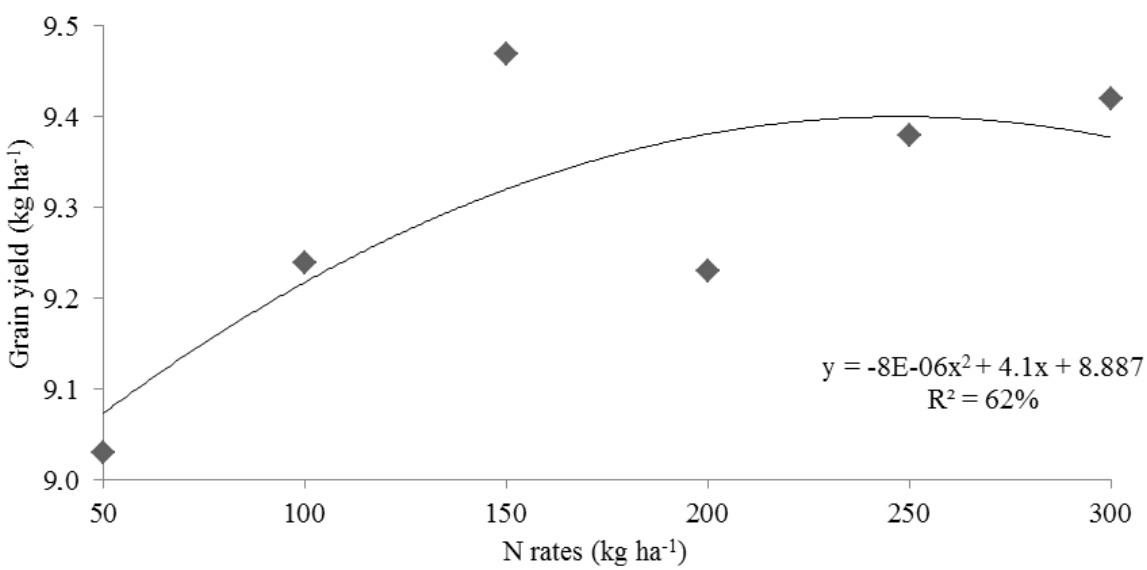

Figure 2. Grain yield of maize hybrids in response to nitrogen fertilization.

(Figure 2). In contrast, inoculation of maize hybrids with *A. brasilense* resulted in yield increases varying from 4 to 6% (approximately 400 to 600 kg ha^{-1}) (Figure 3). Fungicide applications also contributed to increased crop productivity, regardless of the hybrid tested. Foliar protection promoted an increment of 30% in maize production (Figure 4). Besides the isolated effects of inoculation and foliar protection on hybrids' yield, a noteworthy increase in maize productivity was obtained when both practices were associated (Table 3). This increase was 22% above the control (with neither fungicide spraying nor *Azospirillum* inoculation).

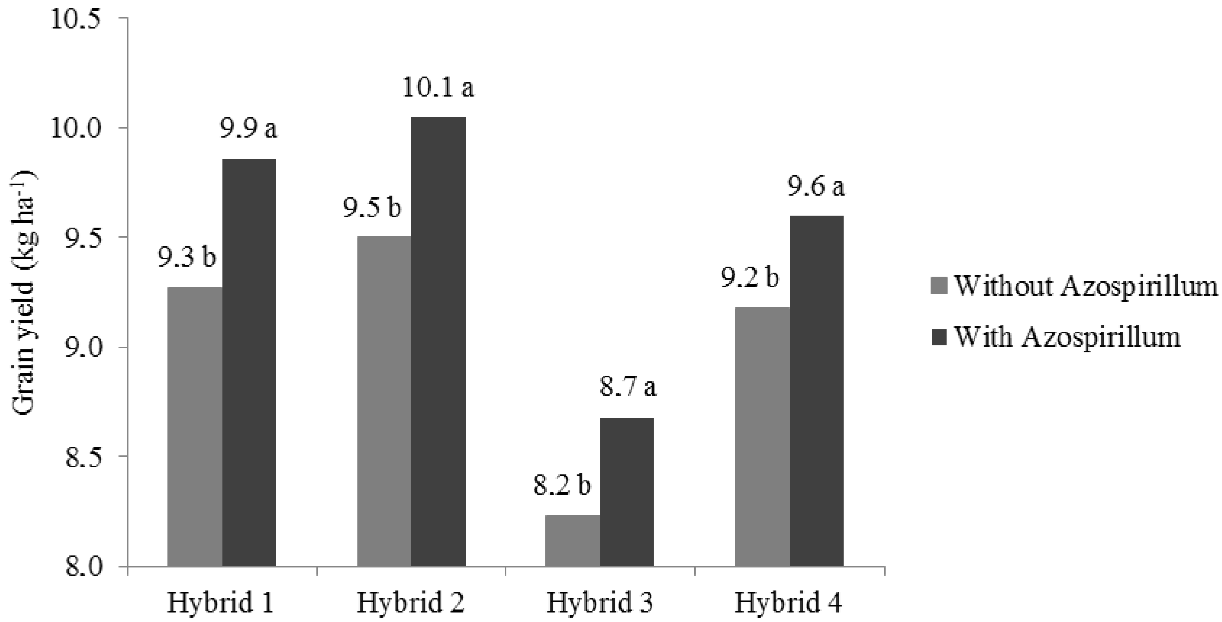

Figure 3. Grain yield of maize hybrids in response to *A. brasilense* inoculation*.*averages followed by different letters, for each hybrid, are statistically different by the Tukey test ($P \leq 0.05$).

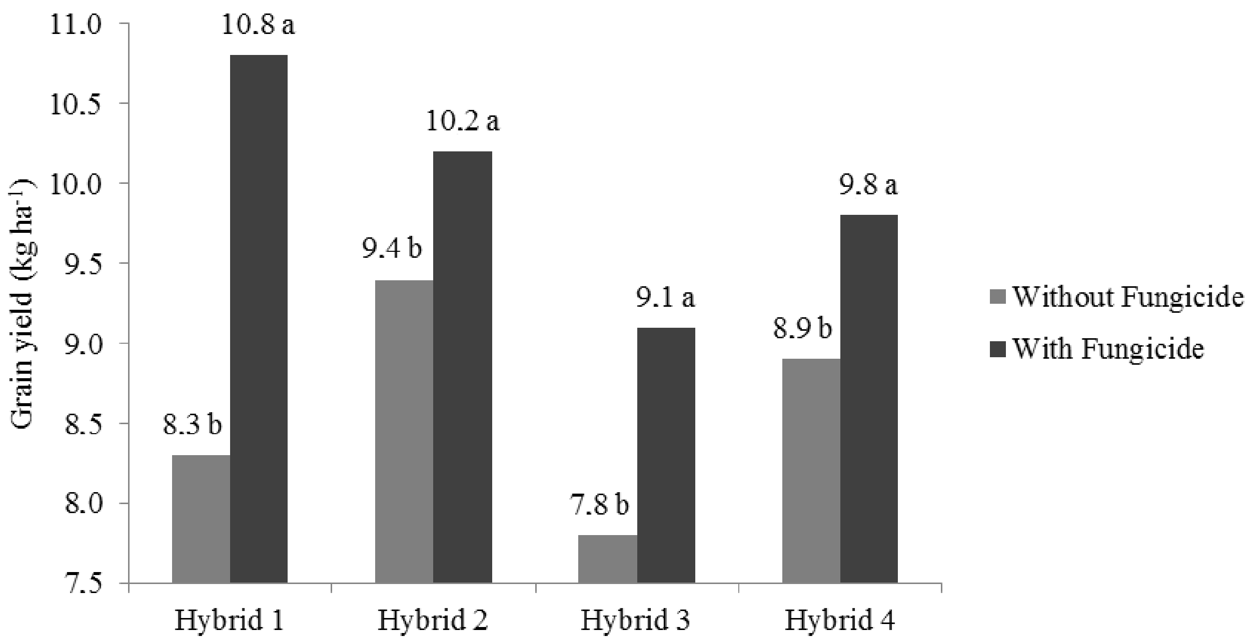

Figure 4. Grain yield of maize hybrids in response to fungicide applications*.*averages followed by different letters, for each hybrid, are statistically different by the Tukey test ($P \leq 0.05$).

Effects of A*zospirillum* inoculation and foliar protection on maize nitrogen use

Regardless of the rate of nitrogen applied, maize grain yield increased due to the inoculation with *A. brasilense* (Figure 5), indicating better nitrogen fertilizer use by the plants. Fungicide spraying also optimized nitrogen use by the hybrids (Figure 6). Analyzing each nitrogen rate (50, 100, 150, 200, 250 and 300 kg ha^{-1}), fungicide use led to increases of 14 to 17% in maize productivity,

Table 3. Grain yield of maize hybrids (kg ha^{-1}) in response to fungicide applications and *A. brasilense* inoculation*.

Azospirillum brasilense	**Foliar protection**	
	With	**Without**
Inoculated	10.3^{aA}	8.8^{aB}
Non inoculated	9.7^{bA}	8.4^{bB}

*averages followed by different letters, in lowercase in the columns and uppercase in the lines, are statistically different by the Tukey test ($P \leq 0.05$).

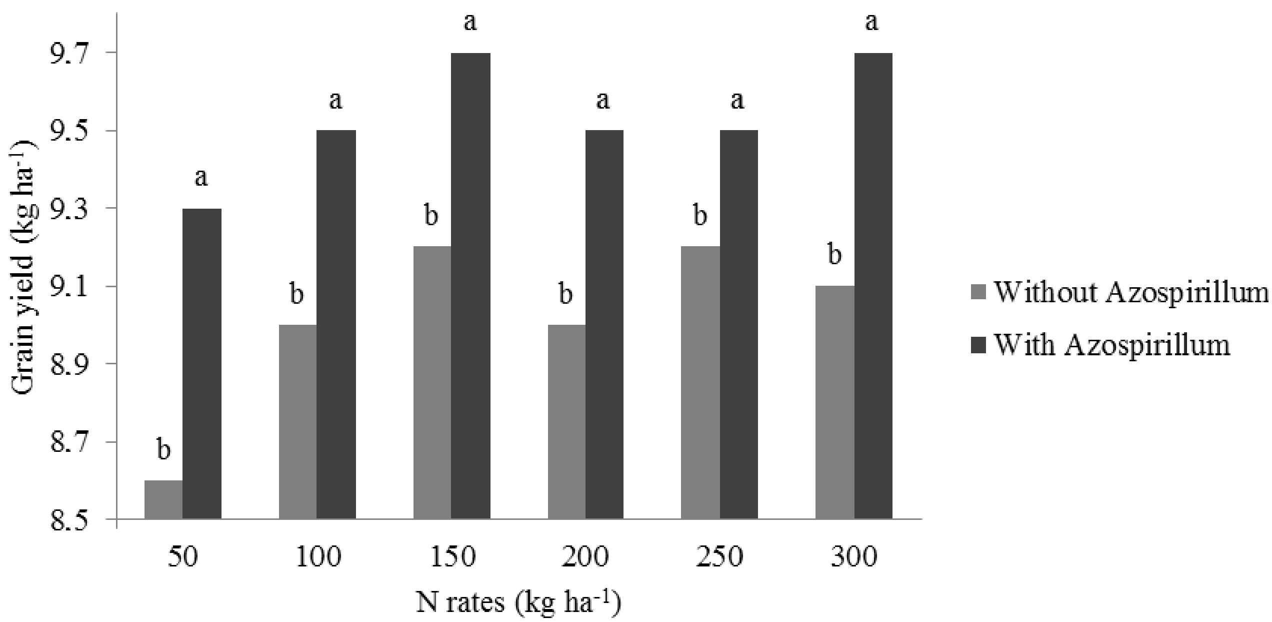

Figure 5. Grain yield of maize hybrids in response to *A. brasilense* inoculation and nitrogen fertilization*.*different letters, for each N dose, are statistically different by the Tukey test ($P \leq 0.05$).

corresponding to 1.2 to 1.5 t ha^{-1}.

DISCUSSION

The quadratic response of maize yield to increasing rates of nitrogen was also reported by Silva et al. (2005). This result could be explained by ammonia volatilization due to urea application to the soil. Urea hydrolysis raises the pH around the fertilizer granules and converts all of its N content into NH_4^+, which reacts with OH^- resulting in H_2O and volatile NH_3^+, which is phytotoxic. Thus, high nitrogen rates applied via urea can impair plant development, and, consequently, decrease production.

The inoculation of maize hybrids with *A. brasilense* led to greater yields. This increase varied among the hybrids tested due to their genetic constitution, which is consistent with several studies demonstrating affinity between *Azospirillum* strains and maize genotypes, altering their responses to inoculation (Salamone and Döbereiner, 1996; Salamone et al., 1996). This also emphasizes that research on selection of bacteria that are able to associate effectively to maize genotypes are essential in order to ensure investment return.

A wide range of responses of cereals to inoculation with *Azospirillum* is reported. Studies show yield increases varying from 5 to 30% (Okon and Labandera-González, 1994) and from 662 to 823 kg ha^{-1} in relation to non-inoculated controls (Hungria et al., 2010). In this study, grain yield increases varied from 4 to 6% (which represents 400 to 600 kg ha^{-1}), meaning that even highly productive maize genotypes, obtained from conventional and biotechnological breeding, can have yield increased by seed inoculation with *Azospirillum*.

Therefore, inoculation enabled yield increases of maize crops growing under Cerrado conditions, which resulted from the affinity between *Azospirillum* and hybrids recommended for the region. Thus, inoculation allowed optimization of land use and even small and medium farmers (in low investment production systems) can obtain greater yields with this technology.

Increased production of maize can be attributed to the

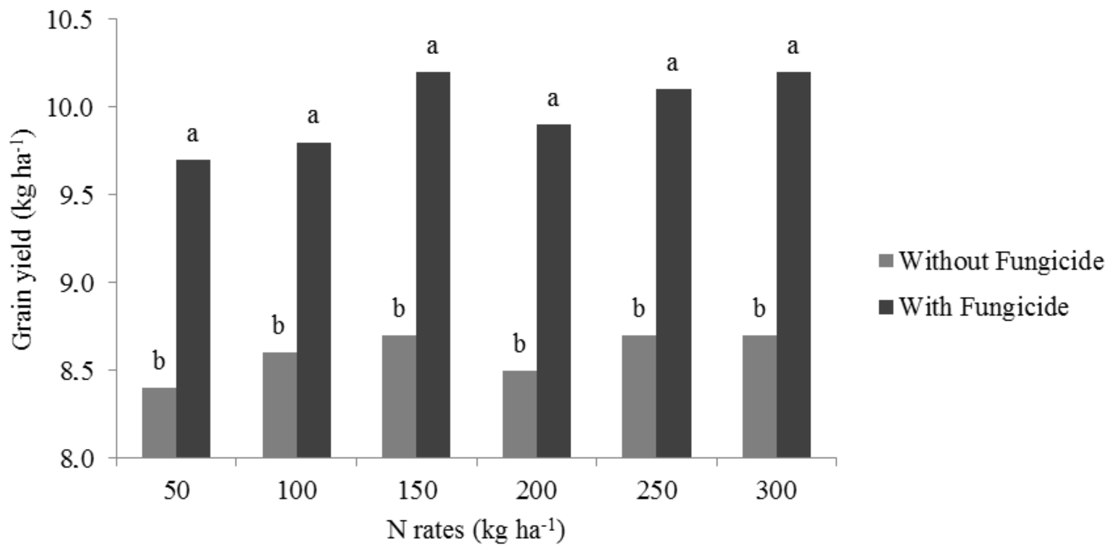

Figure 6. Grain yield of maize hybrids in response to fungicide applications and nitrogen fertilization*. *different letters, for each N dose, are statistically different by the Tukey test ($P \leq 0.05$).

phytostimulatory effects of inoculation with *Azospirillum*, due not only to biological nitrogen fixation in the rhizosphere, but also to plant's greater efficiency in water and nutrient uptake due to greater growth of root system provided by the production of plant growth promoting substances by the bacteria (Döbereiner, 1992; Reis et al., 2000; Cassán et al., 2008). Better nitrogen fertilizer use was observed when maize hybrids were inoculated with *Azospirillum*. From this result it can be inferred that *Azospirillum* inoculation enhances nitrogen use, although it does not replace it. Thus, even if part of the maize nitrogen demand is supplied by association with diazotrophic bacteria, reduction of nitrogen fertilizer rates is not recommended. This result, however, contrasts with the one reported by Hungria (2011), who found substantial reduction of nitrogen fertilization in maize plants inoculated with *Azospirillum*. Applying a nitrogen rate equivalent to half of that recommended for maize in Brazil (100 kg N ha^{-1}), the researcher obtained grain yield of 7.8 t ha^{-1} (Hungria, 2011). However, it must be stated that such production was achieved only with the strain Ab-V5 (+ 54 kg N ha^{-1}) in a single crop season. Besides the previously mentioned aspects, foliar protection affected maize yield as well. The hybrids obtained greater yield potential, reaching up to 10.2 t ha^{-1}, after fungicide applications. This is certainly related to treated plants health. In treatments without fungicide application, hybrid photosynthetic activity may have been compromised, resulting in lower production. This ratifies the idea that investment in plant nutrition is jeopardized if correct phytosanitary management is not adopted. This statement is consistent with studies about effects of fungicide use to increase plant yield (Köhle et al., 2003).

Besides the already known foliar protection, it has been postulated that strobilurin-based fungicides can interfere in the physiology of some crops, such as dry beans (Rava, 2002) and soybean (Fagan et al., 2010), promoting a better fertilizer use by the plants, significantly increasing yields. Therefore, regardless of the N rate applied, this nutrient uptake by maize plants was optimized due to foliar protection (control of diseases) and to physiological effects also provided by the fungicide. These physiological effects comprise an increase in the enzyme nitrate reductase activity (Kaiser and Brendle-Behnisch, 1995), a decrease in ethylene synthesis (Grossmann and Retzlaff, 1997) and a greater plant tolerance to abiotic stresses (Grossmann et al., 1999). This result confirms that of Ruske et al. (2003) while studying the effects of a strobilurin-based fungicide on N uptake, partitioning, remobilization, and grain N accumulation in winter wheat cultivars. Thereby, it is possible to state that foliar protection as well as physiological effects due to the fungicide application positively influenced nitrogen use by maize, increasing yield of the hybrids tested. It is important to emphasize that this better nitrogen use can reduce production costs of maize, avoid degradation of natural resources and increase crop productivity.

Foliar spraying with fungicide was not antagonistic to *Azospirillum* inoculation. Therefore, both technologies can be recommended for greater maize yields. This is important since *A. brasilense* is not restricted to organic crops and, therefore, will be exposed to a wide variety of pesticides commonly used in intensive agriculture.

Generally, agrochemicals have side-effects on non-target micro-organisms (Bashan et al., 2007). However, these authors recognize the lack of studies addressing this important issue and that most of them were performed under *in vitro* conditions. Research on the effects of agricultural pesticides on *Azospirillum* species are available, focusing on herbicides (Jena et al., 1990; Salmeron et al., 1991; Omar et al., 1992; Rivarola et al., 1992; Forlani et al., 1995) and insecticides (Langenbach et al., 1991; Buff et al., 1992; Sánchez et al., 1994).

As to the effect of the fungicide applied in this study, one could wonder whether its absorption and translocation in the plant could affect bacteria development in maize rhizosphere. However, strobilurins and triazoles have low systemic activity, often bound to the outer layers of plant cuticle, showing limited transport on the boundary leaf layer resulting in long-lasting residual effects on plant pathogens (Köhle et al., 1994). Therefore, *Azospirillum* cells do not come into direct contact even with fully systemic fungicides as all of these compounds are translocated acropetally into leaves and the shoot tip (Diedhiou et al., 2004). The low or inexistent basipetal transport may explain why foliar fungicide applications have no direct effect on micro-organisms in the root zone (Sicbaldi et al., 1997; Chamberlain et al., 1998). In addition, indirect effects like greater photosynthesis activity of strobilurin-treated plants (Beck et al., 2002) may also be involved, and should promote *Azospirillum* development due to improved carbohydrate supply to the roots. Those effects could explain the positive interaction observed between *Azospirillum* inoculation and foliar protection on maize yield.

Conclusions

Each factor analyzed contributes to an increase in maize average yield. Greater interest in the use of inoculants containing plant growth promoting bacteria has been observed and will probably increase in the coming years, due to fertilizer cost, awareness about pollution, and emphasis on sustainable agriculture. However, to ensure high yield, nitrogen rates should not be replaced, neither totally nor partially, by seed inoculation with *Azospirillum*. Fungicide applications should be done during maize cycle. In addition, specific maize breeding programs should consider the affinity between *Azospirillum* strains and maize hybrids, mainly for regions with N deficient soils, like Cerrado. Thus, by incorporating additional technologies, maize crop farmers can optimize land use and, consequently, reduce the expansion into new agricultural areas.

Conflict of Interests

The authors have not declared any conflict of interests.

REFERENCES

Araújo GM, Haridasan M (1988). A comparison of the nutritional status of two forest communities on mesotrophic and dystrophic soils in central Brazil. Commun. Soil Sci. Plant Anal. 19:1075-1089.

Bashan LE, Holguin G, Glick BR, Bashan Y (2007). Bacterias promotoras del crecimiento en plantas para propósitos agrícolas y ambientales. In: Microbiología agrícola: hongos, bacterias, micro y macrofauna, control biológico, planta-microorganismo (Eds.: R. Ferrera-Cerrato and A. Alarcon). Editorial Trillas, Mexico City, Mexico pp. 170-224.

Bashan Y, Holguin G, Bashan LE (2004). *Azospirillum*-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). Can. J. Microbiol. 50(8):521-577.

Beck C, Oerke EC, Dehne HW (2002). Impact of strobilurins on physiology and yield formation of wheat. Mededelingen (Rijksuniversiteit te Gent. Fakulteit van de Landbouwkundige en Toegepaste Biologische Wetenschappen) 67(2):181-187.

Bortolini CG, Silva PRF, Argenta G, Forsthofer EL (2001). Rendimento de grãos de milho cultivado após aveia-preta em resposta à adubação nitrogenada e regime hídrico. Pesqui. Agropecu. Bras. 36(9):1101-1106.

Brannstrom C, Jepson W, Filippi AM, Redo D, Xu Z, Ganesh S (2008). Land change in the Brazilian Savannah (Cerrado), 1986-2002: comparative analysis and implications for land-use policy. Land Use Pol. 25(4):579-595.

Buff K, Mano DMS, Langenbach T (1992). Effect of endosulfan on *Azospirillum lipoferum* growth, morphology, nitrogenase activity and protein binding. Appl. Environ. Microbiol. 58:3173-3176.

Bustamante MMC, Medina E, Asner GP, Nardoto GB, Garcia-Montiel DC (2006). Nitrogen cycling in tropical and temperate savannas. Biogeochemistry 79:209-237.

Cassán FD, Perrig D, Sgroy V, Masciarelli O, Penna C, Luna V (2009). *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.). Eur. J. Soil Biol. 45(1):28-35.

Cassán FD, Sgroy V, Perrig D, Masciarelli O, Luna V (2008). Producción de fitohormonas por *Azospirillum* sp. Aspectos fisiológicos y tecnológicos de la promoción del crecimiento vegetal. In: *Azospirillum* sp.: cell physiology, plant interactions and agronomic research in Argentina (Eds.:Cassán FD , Salamone IG). Asociación Argentina de Microbiologia, Buenos Aires, Argentina pp. 59-84.

Cavaglieri L, Orlando J, Etcheverry M (2009). Rhizosphere microbial community structure at different maize plant growth stages and root locations. Microbiol. Res. 164:391-399.

CEPF (Critical Ecosystem Partnership Fund) (2015). Cerrado. http://www.cepf.net/resources/hotspots/South-America/Pages/Cerrado.aspx Downloaded: Mar 01, 2015.

CFSEMG (Comissão de Fertilidade do Solo do Estado de Minas Gerais) (1999). Recomendações para o uso de corretivos e fertilizantes em Minas Gerais, 5ª aproximação. Comissão de Fertilidade do Solo do Estado de Minas Gerais: Viçosa, Brazil. 359 p.

Chamberlain K, Patel S, Bromilow RH (1998). Uptake by roots and translocation to shoots of two morpholine fungicides in barley. Pestic. Sci. 54(1):1-7.

Compant S, Clément C, Sessitsch A (2010). Plant growth-promoting bacteria in the rhizo- and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. Soil Biol. Biochem. 42:669-678.

CONAB (Companhia Nacional de Abastecimento) (2015a). Brazilian Crop Assessment: grains: Fourth Assessment, January/2015. CONAB, Brasília, DF, Brazil. http://www.conab.gov.br/OlalaCMS/uploads/arquivos/15_01_09_09_00_21_boletim_graos_janeiro_2015.pdf Downloaded: Feb 08, 2015.

CONAB (Companhia Nacional de Abastecimento) (2015b). Séries históricas: estimativa de produtividade safra 2014/2015 Milho. CONAB, Brasília, DF, Brazil. http://www.conab.gov.br/conteudos.php?a=1252&t=2&Pagina_objcmsconteudos=2#A_objcmsconteudos Downloaded: Feb 08, 2015.

Díaz-Zorita M, Fernández-Canigia MV (2009). Field performance of a liquid formulation of *Azospirillum brasilense* on dryland wheat productivity. Eur. J. Soil Biol. 45(1):3-11.

Diedhiou PM, Oerke EC, Dehne HW (2004). Effects of the strobilurin fungicides azoxystrobin and kresoxim-methyl on arbuscular

mycorrhiza. J. Plant Dis. Prot. 111(6):545-556.
Döbereiner J (1992). Fixação de nitrogênio em associação com gramíneas. In: Microbiologia do solo (Ed.: Cardoso EJBN). Sociedade Brasileira de Ciências do Solo, Campinas pp. 173-180.
Fagan EB, Dourado Neto D, Vivian R, Franco RB, Yeda MP, Massignam LF, Oliveira RF, Martins KV (2010). Efeito da aplicação de piraclostrobina na taxa fotossintética, respiração, atividade da enzima nitrato redutase e produtividade de grãos de soja. Bragantia 69(4):771-777.
Ferreira AS, Pires RR, Rabelo PG, Oliveira RC, Luz JMQ, Brito CH (2013). Implications of *Azospirillum brasilense* inoculation and nutrient addition on maize in soils of the Brazilian Cerrado under greenhouse and field conditions. Appl. Soil Ecol. 72:103-108.
Forlani G, Mantelli M, Branzoni M, Nielsen E, Favilli F (1995). Differential sensitivity of plant-associated bacteria to sulfonylurea and imidazolinone herbicides. Plant Soil 176(2):243-253.
Gallardo ALCF, Bond A (2011). Capturing the implications of land use change in Brazil through environmental assessment: time for a strategic approach? Environ. Impact Assess. Rev. 31(3):261-270.
Grossmann K, Kwiatkowski J, Caspar G (1999). Regulation of phytohormone levels, leaf senescence and transpiration by the strobilurin kresoxim-methyl in wheat (*Triticum aestivum* L.). J. Plant Physiol. 154:805-808.
Grossmann K, Retzlaff G (1997). Bioregulatory effects of the fungicidal strobilurin kresoxim-methyl in wheat (*Triticum aestivum* L.). Pestic. Sci. 50(1):11-20.
Haridasan M (2008). Nutritional adaptations of native plants of the cerrado biome in acid soils. Braz. J. Plant Physiol. 20(3):183-195.
Haridasan M (1994). Solos do Distrito Federal. In: Cerrado: Caracterização, ocupação e perspectivas - O caso do Distrito Federal (Ed.: M. Novaes-Pinto). Editora Universidade de Brasília/SEMATEC, Brasília pp. 322-334.
Hartmann A, Bashan Y (2009). Ecology and application of *Azospirillum* and other plant growth-promoting bacteria (PGPB) – Special Issue. Eur. J. Soil Biol. 45(1):1-2.
Hungria M, Campo RJ, Souza EM, Pedrosa FO (2010). Inoculation with selected strains of *Azospirillum brasilense* and *A. lipoferum* improves yields of maize and wheat in Brazil. Plant Soil 331:413-425.
Hungria M (2011). Inoculação com *Azospirillum brasilense*: inovação em rendimento a baixo custo. Embrapa-Soja: Londrina, Brazil. 36 p.
Jena PK, Adhya TK, Rao VR (1990). Nitrogen fixing bacterial populations as influenced by butachlor and thiobencarb in rice soils. Zentbl. Mikrobiol. 145(6):457-460.
Kaiser WM, Brendle-Behnisch E (1995). Acid-base-modulation of nitrate reductase in leaf tissues. Planta 196(1):1-6.
Klink CA, Machado RB (2005). Conservation of the Brazilian Cerrado. Conserv. Biol. 19(3):707-713.
Köhle H, Gold RE, Ammermann E (1994). Biokinetic properties of BAS 490 F and some related compounds. Biochem. Soc. Trans. 22(1):65S.
Köhle H, Grossmann K, Jabs T, Gerhard M, Kaiser W, Glaab J, Conrath U, Seehaus K, Herms S (2003). Physiological effects of the strobilurin fungicide F 500 on plants. AgroConcept, pp. 61-74.
Langenbach T, Mano DMS, De-Souza W, Hagler AN (1991). Influence of insecticides on growth, nitrogenase activity and morphology of *Azospirillum lipoferum*. Ciênc. Cult. 43:207-209.
Lopes AJ, Cox FR (1977). A survey of the fertility status of surface soils under cerrado vegetation of Brazil. Soil Sci. Am. J. 41:752-757.
Mehnaz S, Kowalik T, Reynolds B, Lazarovits G (2010). Growth promoting effects of corn (*Zea mays*) bacterial isolates under greenhouse and field conditions. Soil Biol. Biochem. 42(10):1848-1856.
Myresiotis CK, Vryzas Z, Papadopoulou-Mourkidou E (2014). Effect of specific plant-growth-promoting rhizobacteria (PGPR) on growth and uptake of neonicotinoid insecticide thiamethoxam in corn (*Zea mays* L.) seedlings. Pest manag. Sci. 71(9):1258-1266.
Ohland RAA, Souza LCF, Hernani LC, Marchetti ME, Gonçalves MC (2005). Culturas de cobertura do solo e adubação nitrogenada no milho em plantio direto. Ciênc. Agrotec. 29(3):538-544.
Okon Y, Labandera-González CA (1994). Agronomic applications of *Azospirillum*: an evaluation of 20 years worldwide field inoculation. Soil Biol. Biochem. 26:1591-1601.
Omar MNA, Berge O, Hassanein EE, Shalan SN (1992). *In vitro* and *in situ* effects of herbicide thiobencarb on rice-*Azospirillum* association. Symbiosis 13:55-63.
Pereyra MA, Ballesteros FM, Creus CM, Sueldo RJ, Barassi CA (2009). Seedlings growth promotion by *Azospirillum brasilense* under normal and drought conditions remains unaltered in Tebuconazole-treated wheat seeds. Eur. J. Soil Biol. 45(1):20-27.
Rava CA (2002). Eficiência de fungicidas no controle da antracnose e mancha angular do feijoeiro comum. Summa Phytopathol. 28(1):65-69.
Reis VM, Baldani JI, Baldani VLD, Döbereiner J (2000). Biological dinitrogen fixation in gramineae and palm trees. CRC Cric. Rev. Plant Sci. 19:227-247.
Revellin C, Giraud JJ, Silva N, Wadoux P, Catroux G (2001). Effect of some granular insecticides currently used for the treatment of maize crops (*Zea mays*) on the survival of inoculated *Azospirillum lipoferum*. Pest Manage. Sci. 57(11):1075-1080.
Ritchie SW, Hanway JJ, Benson GO (1992). How a Corn Plant Develops. Iowa State University Extension Department: Ames, Iowa, Story County, USA. 26 p.
Rivarola V, Fabra A, Mori G, Balegno H (1992). *In vitro* protein synthesis is affected by the herbicide 2,4-dichlorophenoxyacetic acid in *Azospirillum brasilense*. Toxicology 73:71-79.
Ruske RE, Gooding MJ, Jones SA (2003). The effects of triazole and strobilurin fungicide programmes on nitrogen uptake, partitioning, remobilization and grain N accumulation in winter wheat cultivars. J. Agric. Sci. 140(4):395-407.
Sá SA, Palmer C, Di Falco S (2013). Dynamics of indirect land-use change: empirical evidence from Brazil. J. Environ. Econ. Manag. 65(3):377-393.
Salamone IEG, Döbereiner J (1996). Maize genotype effects on the response to *Azospirillum* inoculation. Biol. Fertil. Soils 21(3):193-196.
Salamone IEG, Döbereiner J, Urquiaga S, Boddey RM (1996). Biological nitrogen fixation in *Azospirillum* strain-maize genotype association as evaluated by ^{15}N isotope dilution technique. Biol. Fertil. Soils 23(3):249-256.
Salmeron V, Martinez-Toledo MV, Gonzalez-Lopez J (1991). Effects of alachlor and metolachlor on the biological activity of *Azospirillum brasilense* grown in chemically defined and dialyzed-soil media. Environ. Toxicol. Chem. 10(4):493-499.
Sánchez CE, Rodelas B, Martinez-Toledo MV, Salmeron V, Gonzalez-Lopez J (1994). Diflubenzuron and the biological activity of *Azospirillum brasilense*. Toxicol. Environ. Chem. 42(3-4):241-247.
Scarano FR, Silva JMC, Guimarães AL, Raik D, Bolt F (2012). Brazil on the spot: Rio+20, sustainability and a role for science. Braz. J. Bot. 35(2):233-239.
Sicbaldi F, Sacchi GA, Trevisan M, Del Re AAM (1997). Root uptake and xylem translocation of pesticides from different chemical classes. Pestic. Sci. 50(2):111-119.
Silva EC, Buzetti S, Guimarães GL, Lazarini E, Sá ME (2005). Doses e épocas de aplicação de nitrogênio na cultura do milho em plantio direto sobre Latossolo Vermelho. Rev. Bras. Ciênc. Solo 29:353-362.
Silva JF, Fariñas MR, Felfili JM, Klink CA (2006). Spatial heterogeneity, land use and conservation in the Cerrado region of Brazil. J. Biogeogr. 33(3):536-548.
Souza JA (2006). Manejo da fertilidade de solo para a cultura do milho. Inf. Agropecuário 27(233):26-37.
Trivedi M, Pinto LFG, Hall A, da Motta RS, Filho LM, Strassburg B, Mitchell A, Ortiz R, Costa D, Oakes N, Ometto J (2012). Think PINC: Securing Brazil's Food, Water and Energy with Proactive Investment in Natural Capital. Global Canopy Programme: Oxford, Oxfordshire, England, UK. 21 pp.
Valipour M (2012). Critical areas of Iran for agriculture water management according to the annual rainfall. Eur. J. Sci. Res. 84(4):600-608.
Valipour M (2013) Need to update of irrigation and water resources information according to the progresses of agricultural knowledge. Agrotechnology S10:e001.