Effects of foliar nickel (Ni) application on mineral nutrition status, urease activity and physiological quality of soybean seeds

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Abstract

Nickel (Ni) at low concentrations is an essential micronutrient for higher plants. Nickel is a cofactor of the enzyme urease and plays a critical role in the germination of seeds. This study aims to evaluate the effect of foliar Ni application on mineral nutrition status, urease activity and the physiological quality of soybean seeds. The study was conducted using different levels of Ni (0, 10, 20, 40, 60, 80 and 100 g ha^{-1}) were applied in the presence or absence (0 or 75 g i.a. ha^{-1}) of the fungicide pyraclostrobin. Leaf nitrogen concentrations showed a linear increase in response to Ni application irrespective of pyraclostrobin application. Foliar sulfur concentrations inversely proportional to Ni application in the absence of pyroclostrobin, but were proportional to Ni application when pyroclostrobin was also applied. Nickel application showed no effects on foliar concentrations of other macronutrients (P, K$^+$, Ca^{2+}, Mg^{2+}). Nickel concentrations in the leaves and seeds were proportional to nickel applications levels. There was a highly significant correlation between foliar Ni concentrations in the leaves and seed Ni levels (r = 0.99), indicating extensive translocation of Ni from leaf to seed. Urease activity increased proportionally up to 20 g ha^{-1}, with peak activity between 20 and 40 g ha^{-1} of Ni. Urease activity correlated highly with seed yield (r = 0.88) and pod number (r = 0.84). Seed germination and emergence rate increased proportionally with Ni application. However, when Ni was combined with pyraclostrobin, the germination percentage displayed a parabolic curve with an increase of germination up to 40 g ha^{-1} of Ni. The electrical conductivity for a maximum was between 40 and 60 g ha^{-1} of Ni. Nickel treatments had no significant effect on seedling length or seedling dry weight. Foliar application of Ni up to 20 g ha^{-1} in the presence or absence of pyraclostrobin was beneficial to soybean plants based on seed yield, mineral nutrition status and physiological quality of soybean seeds.

Keywords: Pyraclostrobin, micronutrient, urease, physiological quality, soybean.

Introduction

One of the most important steps in soybean production is to obtain high quality seeds. However, several factors affect the quality of seeds, among them fertilizer highlighting (Sá, 1994; Kappes et al., 2008). The occurrence of micronutrient deficiencies can limit productivity, decrease seed quality, and interfere with phytosanitary matters (Kappes et al., 2008).
Nickel was originally classified as non-essential and toxic to plants (Malavolta and Moraes, 2007). However, its essentiality was confirmed by direct demonstration of its role as a cofactor for the enzyme urease (EC 3.5.1.5., urea amidohydrolase) (Dixon et al., 1975), and experimental correlation of Ni with seed germination in soybeans (Eskew et al., 1983) and cowpeas (*Vigna unguiculata*) (Eskew et al., 1984). Urea is produced in plant tissues as a product of arginine catabolism, which is particularly important in the nitrogen remobilization process during seed germination and senescence, and is present in all higher plants (Walker et al., 1985; Witte, 2011; Kutman et al., 2013a). Soybean plants have two additional pathways of urea biosynthesis: one is

purine catabolism, leading to formation of ureides (allantoin and allantoic acid), which comprise the main form of reserve for translocation of nitrogen from the roots to shoots (a characteristic trait of legumes); from the other involves the amino acid, canavanine, found only in cowpea and soybean plants which is involved in glutamine synthesis biosynthesis (Walker et al., 1985; Zonia et al., 1995). In higher plants, urea is recycled only by the action of urease (Polacco et al., 2013), which catalyzes the hydrolysis of urea into ammonia and carbon dioxide (Dixon et al., 1975). Urease has two Ni ions in the active site (Ciurli, 2001). Foliar applications of Ni have been demonstrated to increase foliar Ni concentration and foliar urease activity (Ojeda-Barrios et al., 2016). Plants growing in Ni deprivation conditions produce seeds with low germination rates and reduced growth of seedlings. This situation is reversed by the addition of low concentrations of Ni to liquid fertilizers (Eskew et al., 1983; Brown et al., 1987). Other experiments, however, have shown that the Ni concentration in seeds has a greater influence on germination

and seedling vigor. In addition, foliar application of Ni can prevent toxic symptoms caused by urea accumulation in leaf tissue, and glyphosate drift symptoms (Eskew et al., 1984; Kutman et al., 2013b; Kutman et al., 2014). Urease inhibitors prevent the germination of seeds, and this effect is reversed with the addition of NH_4NO_3 and amino acids (Zonia et al., 1995). In this sense, Ni availability to soybean plants could Ni levels in the seeds by promoting urease activity and contribute to production of seeds with better physiological quality (Lavres et al., 2016).

Thus, optimal concentrations of micronutrients in seeds can enhance the growth and productivity of crops, particularly in nutrient-poor soils (Kutman et al., 2014). In addition, the foliar applications of Ni at low concentrations can play various roles in increasing resistance to fungal disease (Reis et al., 2014; Langenbach et al., 2016). Another important aspect of seed production refers to the plant's vitality, and the physiological quality of seeds. In order to improve both it is common to apply foliar fungicides for fungal soybean disease control (Godoy et al., 2016).

It is known that some types of fungicides have physiological effects on plants. The application of pyraclostrobin to soybean plants can promote an increase in the photosynthetic rate and the foliar nitrate reductase activity, and a decrease in the respiratory rate (Fagan, 2010; Carrijo, 2014). In this study, we hypothesize that the application of foliar Ni combined with pyraclostrobin during the anthesis stage, could enhance the urease activity and contribute to improved physiological quality of soybean seeds and yields. This is the first report in the literature showing the synergistic effect of Ni combined with pyraclostrobin on physiological quality of soybean seeds. This study aims to evaluate the interactions between mineral nutrition status, urease activity and physiological quality of soybean seeds in response to foliar Ni application in the presence or absence of pyraclostrobin.

Results and Discussion

Mineral nutrition status of soybean leaves and grains

Leaf nitrogen concentrations in soybean plants showed a proportional linear increase in response to foliar Ni application regardless of pyraclostrobin status (Fig. 1a). Nickel plus pyraclostrobin exhibited an additive effect on leaf nitrogen concentrations (Fig. 1a). Similar results were obtained for foliar Ni application in tomato (Tan et al., 2000) and soybean plants (Kutman et al., 2014). Increases in foliar nitrogen concentrations in banana plants treated with pyraclostrobin were also observed by Lima et al. (2012). The mechanism of action of pyraclostrobin is to increase the nitrate reductase activity, which improves the nitrogen assimilation in plants (Rodrigues, 2009; Fagan, 2010; Carrijo, 2014).

Foliar sulfur concentrations linearly decreased with Ni foliar spray application. However, sulfur concentrations linearly increased when the foliar Ni application was combined with pyraclostrobin (Fig. 1f). In general, the literature points to sulfur interactions with other nutrients such as iron, molybdenum, selenium and zinc (Kabata-Pendias and Pendias, 2011). There is a lack of information in the literature regarding the interaction between Ni and sulfur. Lavres et al. (2016) observed an increase in foliar sulfur concentrations in soybean plants by applying Ni through seed priming technique. On the other hand, Rodak (2014) observed no significant effect of Ni application via soil on soybean foliar sulfur concentrations. No previous studies regarding the effect of pyraclostrobin on foliar sulfur

concentration in soybean plants and its interaction with Ni have been done. Further studies are needed in order to elucidate the interactions between Ni, pyraclostrobin and sulfur metabolism in higher plants.

According to Malavolta and Moraes (2007), nitrogen and sulfur may interact synergistically. The three-dimensional structure of nitrate reductase contains a sulphite oxidase (Campbell, 1999), and the molecule had sulfur contanining amino acids. Some studies have found decreased nitrate reductase activity under sulfur-limiting conditions (Thomas et al., 2000; Prosser et al., 2001).

Studies have shown that Ni has no effect on nitrate reductase activity in sugar beets (Kevresan et al., 1998) and soybeans (Rodak, 2014), whereas pyraclostrobin increases nitrate reductase activity, leading to an increase in foliar nitrogen concentration in plants (Rodrigues, 2009; Fagan, 2010). The increase in foliar sulfur concentrations may occur through the indirect influence of Ni on foliar nitrogens concentration and of pyraclostrobin on nitrate reductase enzyme. However, the effects of Ni concentration may vary between plants of the same genus, or even between different cultivars of the same species (Barsukova and Gamzikova, 1999; Seregin Kozhevnikova, 2006).

Foliar Ni application showed no effect on foliar concentrations of other macronutrients such as phosphorus, potassium, calcium and magnesium (Figs. 1b, 1c, 1d, 1e). Foliar boron concentrations increased linearly with increased Ni application. Whereas when Ni is combined with pyroclostrobin, there was a significant decrease in boron levels at high Ni concentrations (Fig. 2a). Franco (2015) also observed increases in foliar boron concentrations in soybean plants in response to Ni levels. Rodak (2014) reported boron levels to be inversely proportional to Ni levels in both leaves and seeds. There is little in the literature regarding the interaction between Ni and boron and what is available often reports contradictory observations.

In the absence of pyroclostrobin, foliar copper concentrations showed a parabolic response to Ni application with peak concentrations at about 40 g ha^{-1} whereas when combined with pyroclostrobin, the initial copper concentration started higher then decreased with increasing Ni concentrations. (Fig. 2b). The antagonistic relationship between Cu and Ni has been show in soybeans (Cataldo et al., 1978) and barley (Korner et al., 1987). In this study, the highest copper concentrations in soybean leaves was observed between 40 and 60 g ha^{-1} of foliar Ni application. Some studies suggest that Ni exerts synergistic effects on copper absorption (Küpper and Andresen, 2016). This synergistic effect was also observed in *Alyssum inflatum*, a hyperaccumulator species of Ni (Ghasemi et al., 2009; Ghasemi et al., 2015).

In the absence of pyroclostrobin, foliar iron concentrations demonstrated a modest parabolic response with peak iron concentrations obtained at 40 g ha^{-1}. However, when combined with pyroclostrobin, iron concentrations demonstrated an inverted parabolic curve with the highest concentrations occurring at 10 g ha^{-1} (Fig. 2c). Similar results related to increases in iron concentration in soybean leaves were observed by Rodak (2014) and (Franco, 2015). This happens because tropical soils have iron availability for plants (Kämpf et al., 2012). In plants grown in nutrient solution Rahman et al. (2005) found lower absorption of iron, which causes the two cations to compete with each other for absorption sites (Nishida, 2012). These authors found that the accumulation of Ni induces the expression of IRT1, an iron 1 transport regulator gene and member of the ZIP family, causing more Ni to be absorbed by that carrier.

Table 1. Relationship among N – nitrogen (g kg^{-1}); Mn - manganese (mg kg^{-1}); Zn – zinc (mg kg^{-1}); Ni – nickel (mg kg^{-1}); RL – root lenght (cm); SDW - shoot dry weigh (g); RDW – root dry weight (g); SPAD – chlorophyll index; urease (µmol N-NH$_4^+$ g^{-1} FW h^{-1}); yield (kg ha^{-1}), and pod.

|	N	Mn	Zn	Ni-leaf	RL	SDW	RDW	SPAD	urease	yield	pod	grains	Ni-grain
N	1												
Mn	ns	1											
Zn	ns	ns	1										
Ni-leaf	0.96*	ns	-0.86*	1									
RL	ns	ns	ns	ns	1								
SDW	ns	ns	ns	ns	ns	1							
RDW	ns	ns	ns	ns	ns	0.93*	1						
SPAD	0.83*	ns	-0.90*	0.92*	ns	ns	ns	1					
urease	ns	ns	ns	0.81*	ns	ns	ns	ns	1				
yield	ns	ns	ns	ns	ns	ns	ns	ns	0.64*	1			
pod	ns	ns	ns	ns	0.88*	ns	ns	ns	0.88*	0.84*	1		
grains	ns	ns	ns	ns	-0.89*	ns	ns	ns	ns	ns	-0.83*	1	
Ni-grain	0.98*	ns	-0.79*	0.99*	ns	ns	ns	ns	ns	ns	ns	ns	1

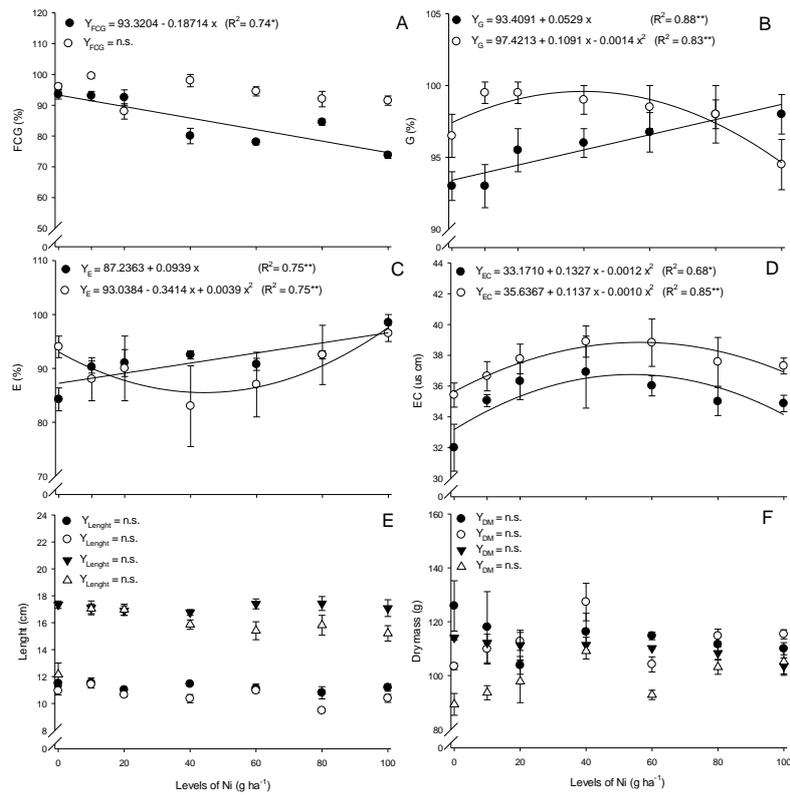*ns* - Not significant; * Significant at 1%, (p ≤ 0.0001)
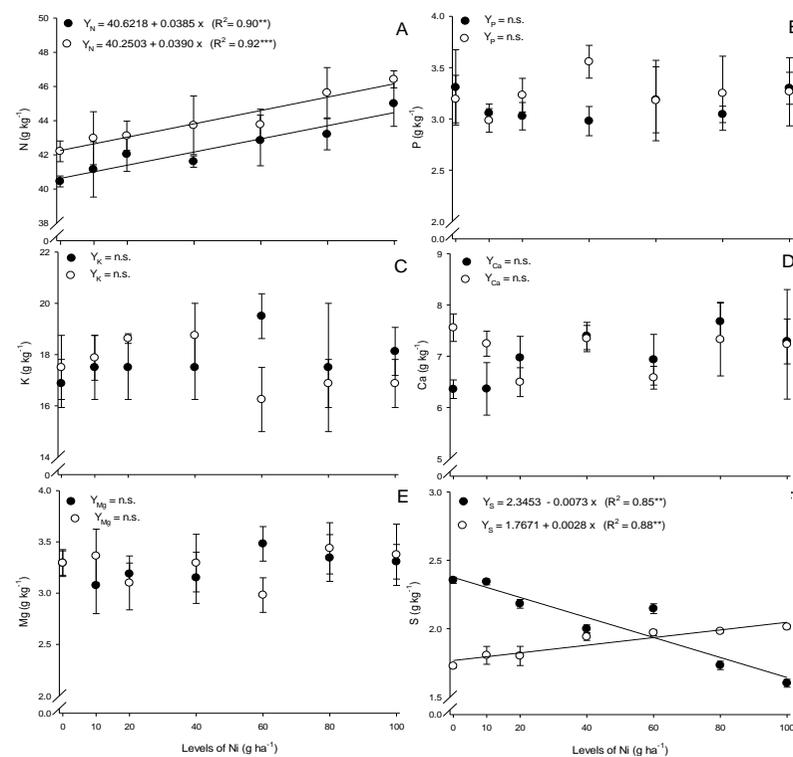
Fig 1. Nitrogen (N) [A], phosphorus (P) [B], potassium (K) [C], calcium (Ca) [D], magnesium (Mg) [E], and sulfur concentration (S) [F] in soybean leaves in response to Ni application with or without pyraclostrobin. Open circles: Ni + pyraclostrobin; dark circles: Ni only. The error bars indicate the standard deviation of four replicates (*n* = 4).
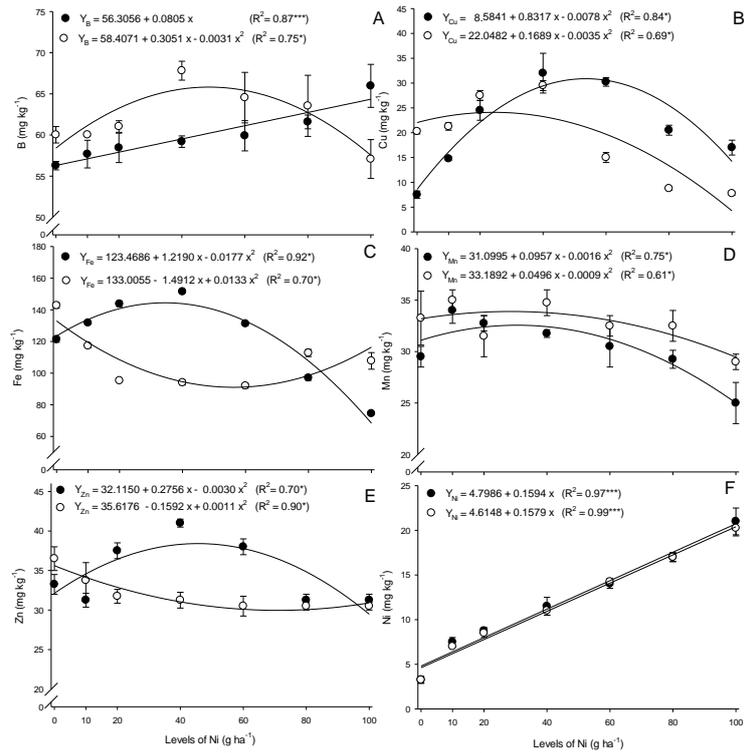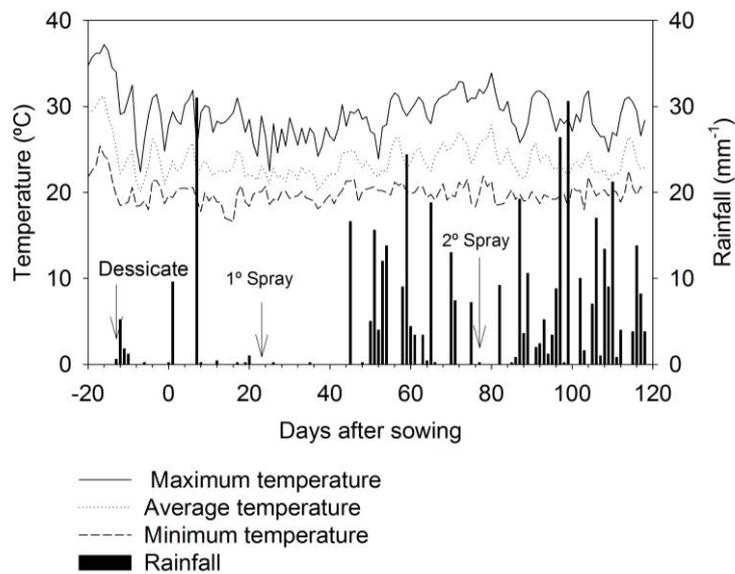
Fig 2. Boron (B) [A], copper (Cu) [B], iron (Fe) [C], manganese (Mn) [D], zinc (Zn) [E] and nickel concentration (Ni) [F] in soybean leaves in response to Ni application with or without pyroclostrobin. Open circles: Ni + pyraclostrobin; dark circles: Ni only. The error bars indicate the standard deviation of four replicates ($n = 4$).
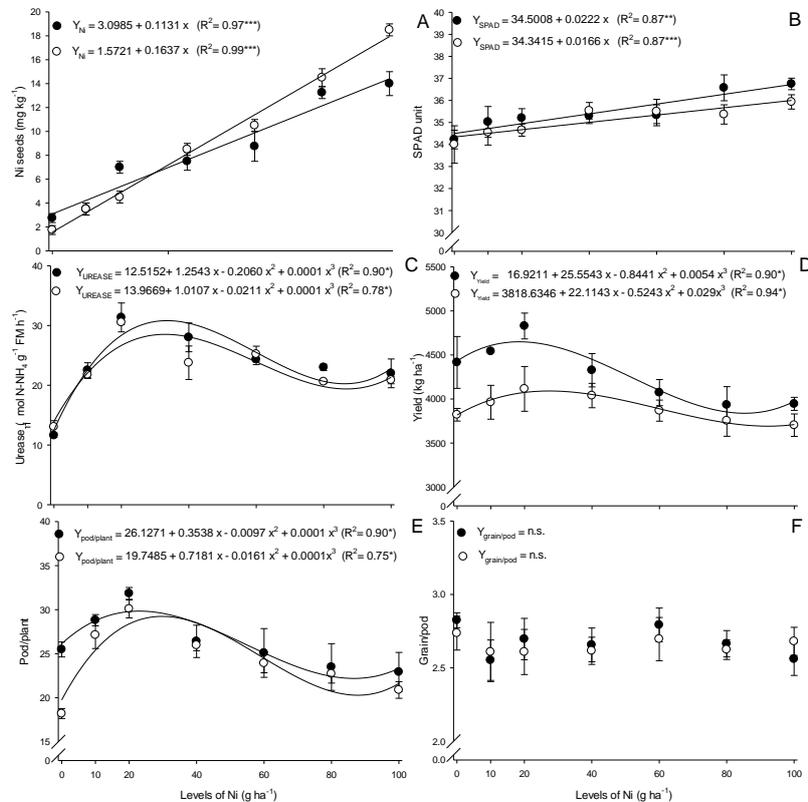
Fig 3. Nickel concentration in seeds [A], SPAD unit [B], urease activity [C], yield [D], number of pods per plant [E], and number of seed per pod [F] in response to Ni application with or without pyroclostrobin. Open circles: Ni + pyraclostrobin; dark circles: Ni only. The error bars indicate the standard deviation of four replicates ($n = 4$).

Fig 4. First count of germination (5 days) (PCG) [A], germination (8 days) (G) [B], emergence (E) [C], electrical conductivity (E) [D], seedling length [E], and seedling dry weight [F] in response to Ni application with or without pyroclostrobin. Open circles: Ni + pyraclostrobin; dark circles: Ni only. The error bars indicate the standard deviation of four replicates ($n = 4$).

Fig 5. Rainfall and temperature average during experiment conduction at Chapadão do Sul, State of Mato Grosso do Sul, Brazil.

The foliar zinc concentration (Fig. 2e), showed a similar response to those obtained for foliar iron concentration (Fig. 2c) showing a parabolic response in response to Ni application and an inverted parabolic response when Ni was combined with pyraclostrobin. The highest zinc concentration was obtained at 40 g ha^{-1} of Ni in the absence of pyroclostrobin. In addition, in the absence of Ni, pyraclostrobin application produced higher foliar zinc concentrations. Nickel also uses zinc transporters, members of the ZIP family, and there is greater Ni accumulation in zinc deficient plants (Nishida et al., 2015).

Foliar manganese concentration in soybean leaves showed a parabolic response to the to Ni application (Fig. 2d). The foliar manganese concentration increased up to 40 g ha^{-1} of Ni, decreasing with higher levels. Compared to Cu and Fe, Ni combined with pyroclostrobin promoted higher foliar manganese concentrations. Nickel can compete with manganese for uptake, and White (2012) suggests that ZIP family transporters also carry manganese through the cells.
The effects of Ni application on the mineral nutritional status of ryegrass plants have been described. Iron, zinc and manganese concentrations in shoots were inversely proportional to Ni concentrations (Khalid and Tinsley, 1980). The leaf zinc concentrations were negatively correlated with Ni concentrations in leaves (Table 1, r = - 0.86), and seeds (Table 1, r = - 0.79), showing the antagonistic relationship between these two cations. Nickel does not use specific cation transporters such as YSL, ZIP/Nramp and nicotianamine synthase, thus it can compete with other divalent cations, especially copper, iron, manganese and zinc (Douchkov et al., 2005; Mizuno et al., 2005; Gendre et al., 2006).

In this study, the Ni concentration in leaves increased in response to Ni supply (Fig. 2f), corroborating with other studies the effect of Ni application in soybean plants (Queiroz, 2012; Rodak, 2014; Franco, 2015). According to Cataldo et al. (1978) during senescence soybean plants translocate about 70% of Ni to the seeds, confirming results obtained in this report where foliar Ni concentrations positively correlated with seed Ni concentrations (Table 1, r = 0. 99). Similar results were also observed by Kutman et al. (2014) and Rodak (2014). The Ni concentration in seeds was higher when Ni application was combined with pyroclostrobin, demonstrating a possible synergistic effect of pyraclostrobin in the accumulation of Ni in soybean seeds (Fig. 3a).

Chorophyll and urease activity

The SPAD index was linearly proportional to foliar Ni application (Fig. 3b). This index estimates the amount of chlorophyll present in the leaves and showed a positive correlation with foliar nitrogen concentrations (Table 1, r = 0.83). The foliar nitrogen concentration was positively correlated with the Ni concentration in the leaves (Table 1, r = 0.96) and seeds (Table 1, r = 0.98). Foliar Ni concentration also showed a positive correlation with the SPAD index (Table 1, r = 0.92) and urease activity (Table 1, r = 0.81).
Nickel is a cofactor of urease, playing an important role in nitrogen recycling in higher plants (Polacco et al., 2013; Fabiano et al., 2015). Our results show a correlation between Ni and the chlorophyll content and consequently on the SPAD index.

Urease activity increased up to 40 g ha^{-1} of Ni, showing the highest activity between levels 20 and 40 g ha^{-1} of Ni application (Fig. 3c) followed by a decrease under the highest levels of Ni exposure. Similar results were observed by

Rodak (2014), showing that urease activity in soybean leaves was inhibited in presence of high concentrations of Ni. In this study, the urease enzyme activity correlated with the seed yield (Table 1, r = 0.88) and number of pods (Table 1, r = 0.84).

Soybean yield and physiological quality of seeds

The soybean yield (Fig. 3d) and the number of pods (Fig. 3e) increased in response to Ni application up to 20 g ha^{-1} but were inhibited in the presence of pyroclostrobin. According to Moraes et al. (2010), under Brazilian environmental conditions, soybean yield increased approximately 300 kg ha^{-1} when plants were sprayed with 50 g ha^{-1} of Ni. The increase in soybean yield by the foliar application of Ni can be attributed to the effect of Ni on reducing symptoms of fungal infection (Reis et al., 2014; Godoy et al., 2016; Langenbach et al., 2016).

Regarding the physiological quality of seeds, the first count of germination (PGC) decreased in response to Ni application though PGC was higher in the presence of pyroclostrobin (Fig. 4a). Conversely the percentage of germination (Fig. 4b) and emergence (Fig. 4c) increased linearly with foliar Ni supply in the absence of pyroclostrobin. The germination percentage showed a slight increase up to 40 g ha^{-1} when Ni was combined with pyraclostrobin and emergence showed an inverted parabolic response when Ni and pyroclostrobin were combined. There is a lack of information regarding the effect of Ni fertilization on physiological quality of seeds. Brown et al. (1987) observed that the germination rates of barley seeds increased in plants previously enriched with Ni.
The electrical conductivity of seeds increased in response to levels of Ni up to 40 and 60 g ha^{-1} of Ni regardless of pyraclostrobin status. However the treatments with fungicide showed higher values of electrical conductivity (Fig. 4c). These results suggest that the foliar Ni application combined with pyraclostrobin show an additive effect on seed deterioration, generating seeds with high membrane degradation.

There was no significant effect of Ni on seedling length and seedling dry weight. According to Pearson's correlation, shoot dry weight was positively correlates with root dry weight (Table 1, r = 0.93). Piccini and Malavolta (1992) studied the toxic effect of Ni in *Phaseolus vulgaris* plants and observed a reduction in root growth as well as considerable inhibitory effect on the morphogenesis and plant growth, and seed formation.

There are few studies evaluating the effect of foliar Ni application on the physiological quality of soybean seeds (Kutman et al, 2013a; Rodak, 2014). It is known that seed storage compounds have great influence on physiological seed quality. Therefore, further studies are needed in order to better understand the physiological roles of Ni on soybean seed germination.

Materials and Methods

Plant materials

The experiment was conducted using a no-tillage system on cultural remains of forage turnips. The desiccation of the area was performed 15 days before sowing, using 1440 g of i.a. ha^{-1} glyphosate + 30 g i.a. ha^{-1} carfentrazone-ethyl.

The soybean cultivar that was used was the Anta 82 RR, semi-determined growth habit, which shows an approximate cycle of 115 days. The seeds were pretreated with fipronil, thiophanate methyl, and pyraclostrobin at 5 g i.a., 45 g i.a.

and 50 g i.a. per 100 kg of seed respectively. The seeding fertilization was based on 200 kg ha^{-1} MAP 10-50-00. The inoculation was performed by direct spraying into the planting furrow, using 600 mL ha^{-1} of liquid inoculant containing *Bradyrhizobium japonicum*.

The sowing was carried out aiming for a stand of 340.000 plants ha^{-1}. Each experimental plot consisted of six lines of 6 m each with 0.45 m between each line. The plants harvested for measurement came from the two central lines, excluding 0.5 m on both ends. The data of rainfall and average temperatures during the crop cycle are shown in Fig. 5.

During the plant development cycle, phytosanitary management was carried out twice. The first was done 25 days after sowing using: glyphosate (1.44 g i.a. ha^{-1}), clorantraniliprole (10 g i.a. ha^{-1}) and teflubenzuron (15 g i.a. ha^{-1}). The second application was made 75 days after sowing using clorantraniliprole (10 g i.a. ha^{-1}), teflubenzuron (15 g i.a. ha^{-1}) and acephate (800 g i.a. ha^{-1}). For specific management of soybean rust along with pyraclostrobin, the plots were treated with trifloxystrobin (60 g i.a. ha^{-1}) and prothioconazole (70 g i.a. ha^{-1}) twice. The first treatement was during R1 and the second 20 days later.

Characterization of the experimental area

This study was conducted between November 1st, 2014 to February 27th, 2015 at the Campo Bom Experimental Farm, located in Chapadao do Sul, State of Mato Grosso do Sul, Brazil (Latitude 18°46'24.38" South and Longitude 52°37'23.75" West), with an average altitude of 820 m. The climate is classified as tropical humid with dry winters and rainy summers, with average temperatures ranging between 13 and 28 °C and an average annual rainfall of 1.850 mm (Cunha et al., 2013).

The soil of the area was classified as red latosol (LVd) very clayey, and corresponds to the Oxisol order (Soil Survey Staff, 1999). Soil analysis was performed according to the methodology described by Raij et al. (2001). The soil properties showed the following chemical characteristics: pH(CaCl$_2$) 4.7; calcium 2.6 cmolc dm^{-3}; magnesium 0.5 cmolc dm^{-3}; aluminum 0.18 cmolc dm^{-3}; H$^+$ + Al 6.7 cmolc dm^{-3}; potassium 58 mmol dm^{-3}; phosphorus (Melich) 6 mg dm^{-3}; sulfur 4.8 mg dm^{-3}; boron 0.36 mg dm^{-3}; copper 0.9 mg dm^{-3}; iron 49 mg dm^{-3}; manganese 9.5 mg dm^{-3}; zinc 2.7 mg dm^{-3}; organic matter 25 g dm^{-3}; nickel 0.1 mg dm^{-3}; cation exchange capacity 9.9 cmolc dm^{-3}. The physical properties of soil are: clay 610 g dm^{-3}; silt 50 g dm^{-3}; and sand 340 g dm^{-3}.

Experimental design and treatments

The study was conducted using a randomized plot experimental design; with seven different levels of Ni (0, 10, 20, 40, 60, 80 and 100 g ha^{-1}) using as nickel sulfate as the source (Sigma Aldrich) in presence or absence of fungicide (0 or 75 g i.a. ha^{-1} of pyraclostrobin) (Trademark Comet). The foliar application of treatments was performed during phenological stage R1 with with each treatment scheme performed in quadriplate, resulting in 56 individual plots.

Mineral nutrition analysis

For mineral nutrition analysis the third trefoil was collected from 20 plants per plot at 12 days post Ni application. The soybean plants were in the R2 phenological stage of development. The collected samples were dried and used to quantify leaf concentrations of macro and micronutrients.

The soybean seeds harvested during the R8 phenological stage were also analyzed.

The nitrogen concentration was determined by the semi-micro Kjeldahl method after sulfuric digestion (Malavolta et al., 1997). For the quantification of other nutrients, a nitropercloric digestion was carried out (Johnson and Ulrich, 1959) and incineration in the case of boron (Alcarde, 1969). Nutrient determination was performed using atomic absorption spectrophotometry, except for phosphorus and sulfur, which were determined by colorimetric spectrophotometry.

Chlorophyll Index

During the R2 phenological stage of soybean development, indirect measurements of chlorophyll were made using a portable chlorophyll meter (SPAD 502 digital model) (MINOLTA). An average of 10 readings per trefoil was obtained under field conditions. The SPAD readings were obtained from 15 trefoils per plot.

Urease activity (EC 3.5.1.5)

The third trefoil was collected from the apex in order to measure the urease activity (EC 3.5.1.5.). The samples were collected during R2 phenological stage at 10 days post- Ni application. The samples were transported immediately to the laboratory for extraction and analysis of urease activity.

The urease activity was determined by the production of N-NH$_4$ according to McCullough (1967) and the extract was obtained according to the methodology described by Hogan et al. (1983). After collection, 0.2 g of leaf (fresh weight) was placed in 8 mL of sodium phosphate buffer containing urea at pH 7.4 and was incubated at 30 °C with stirring for 3 hours. To a 0.5 mL aliquot of the extract 2.5 mL of reagent I: [phenol (0.1 mol L^{-1}) and sodium nitroprusside (SNP) (170 μmol L^{-1})] was added, followed by stirring, and then 2.5 ml of reagent II: [sodium hydroxide (0.125 mol L^{-1}), disodium phosphate (0.15 mol L^{-1}) and sodium hypochlorite (3% Cl$_2$)]. The samples were then incubated at 37 °C for 35 minutes. The determination was performed in a spectrophotometer at 625 nm and the results expressed as μmol N-NH$_4$ g^{-1} FW (fresh weight) h^{-1}.

Growth and yield assessment

During the R8 phenological stage, the number of pods and seeds per pod were evaluated. The evaluation was conducted from five plants in the central area of each plot. During this same stage the average seed weight was determined from 100 seeds and an evaluation of soybean yield was also performed with standardized humidity at 13%.

Physiological quality of seeds

In order to carry out the germination tests, seed samples from each plot were collect to perform the vigor tests soon after.

Germination test: 50 seeds were used per replicate and distributed between three sheets of "germitest" paper that were moistened with distilled water at 2.5 times the dry seed weight, and kept in a germinator at a constant temperature of 25 °C. The first count of germination was performed five days and the final count was performed eight days after initiation, expressed as mean percentage of normal seedlings (Brasil, 2009).

Seedling length test: there were four replicates of 20 seeds per treatment, packed in rolls previously moistened with distilled water 2.5 times dry seed weight, grouped, closed in plastic bags and placed in a germinator at a constant temperature of 25 °C. The length of normal seedlings was measured five days after the initiation. The values were summed and divided by the number of measured normal seedlings.

Seedlings used for the evaluation of dry mass were obtained from the length test. For this, the samples were dried in a forced circulation stove at 80 °C for 24 hours, weighed and divided by the number of normal seedlings to give the mean mass per seedling.

Emergence test: 200 seeds per treatment in four replications of 50 were put in expanded polystyrene trays, kept in the greenhouse and irrigated twice a day, with daily assessments until the tenth day after sowing.

Electrical conductivity: 50 seeds per treatment were placed in plastic cups with 75 mL of deionized water and incubated at 25 ºC; electral conductivity was determined after 24 hours of incubation (Vieira and Krzyzanowsky, 1999).

Statistical analysis

The data was submitted to analysis of variance using the SAS statistical program. Using the F test significance level for the treatments (Ni levels and pyraclostrobin), the data were submitted to linear and quadratic regression analysis using the GLM (general linear model). The correlations among dependent variables (leaf nutrients, urease activity, SPAD index, yield, and physiological quality parameters of seeds) were obtained by means of CORR procedure (Pearson correlation coefficient), using the SAS - System for Windows 6.11 (SAS Inst. 1996).

Conclusion

The foliar Ni application showed the most benefits on mineral nutrition status, urease activity and seed physiological quality under low Ni concentrations combined with pyraclostrobin. Nickel foliar application at higher concentrations combined with pyraclostrobin apparently enhances the Ni antagonistic effects on cationic micronutrients in soybean plants. There was no significant effect of the treatments on seedling length and seedling dry weight. Foliar application of Ni up to 20 g ha^{-1} regardless of pyraclostrobin co-treatment was beneficial to soybean plants based on seed yield, mineral nutrition status and physiological quality of soybean seeds.

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