

ISSN 1678-3921

Journal homepage: www.embrapa.br/pab

For manuscript submission and journal contents, access: www.scielo.br/pab

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Received March 2, 2018

Accepted October 15, 2018

How to cite

CAMARGO, A.B. de; SAGGIN JÚNIOR, O.J.; SILVA, E.M.R. da; PINTO, F.A.; SANTOS, J.V. dos; CARNEIRO, M.A.C. Steel slag and phosphate nutrition of corn inoculated with arbuscular mycorrhizal fungi. **Pesquisa Agropecuária Brasileira**, v.54, e00096, 2019. DOI: https://doi.org/10.1590/ S1678-3921.pab2019.v54.00096.

Microbiology/ Original Article

Steel slag and phosphate nutrition of corn inoculated with arbuscular mycorrhizal fungi

Abstract – The objective of this work was to evaluate the effect of the use of steel slag as a soil acidity corrective and of mycorrhizal fungi associated with phosphate fertilization on corn plants. The study was performed in a greenhouse, using 3-kg pots with a Typic Haplorthox, in a 4x5x2 factorial arrangement, with four acidity correction treatments (C1, correction with dolomitic limestone at a dose of 4 Mg ha⁻¹; C₂, correction with steel slag at a dose of 4 Mg ha⁻¹; C3, correction with a 1:1 mixture of 2 Mg ha⁻¹ dolomitic limestone and 2 Mg ha⁻¹ steel slag; and C4, control, without pH correction), five phosphorus doses $(0, 42, 95, 213,$ and 480 mg dm^{-3} , and the presence or absence of two arbuscular mycorrhizal fungi (*Rhizophagus clarus* and *Gigaspora margarita*), with five replicates. Steel slag was efficient in correcting soil pH and providing Ca and Mg for the plants; therefore, it could replace limestone. The inoculation with *R*. *clarus* and *G*. *margarita*, associated with P doses of 42, 95, and 213 mg $dm³$, improved the development of corn plants after 45 days, resulting in greater plant height, stem diameter, leaf area, and shoot and root dry matter.

Index terms: *Zea mays*, acidity, limestone, root colonization, symbiosis.

Escória de siderurgia e nutrição fosfatada de milho inoculado com fungos micorrízicos arbusculares

Resumo – O objetivo deste trabalho foi avaliar o efeito do uso de escória de siderurgia como corretivo da acidez do solo e de fungos micorrízicos associados à adubação fosfatada em plantas de milho. O trabalho foi conduzido em casa de vegetação, tendo-se utilizado vasos com 3 kg de Latossolo Vermelho distrófico, em arranjo fatorial 4x5x2, com quatro tratamentos corretivos da acidez (C1, correção com calcário dolomítico na dosagem de 4 Mg ha-1; C2, correção com escória de siderurgia de aciaria na dosagem de 4 Mg ha-1; C3, correção com a mistura 1:1 de 2 Mg ha-1 de calcário dolomítico e 2 Mg ha-1 de escória; e C4, controle, sem correção de pH), cinco doses de fósforo $(0, 42, 95, 213, e 480 \text{ mg dm}^3)$, e presença ou ausência de dois fungos micorrízicos arbusculares (*Rhizophagus clarus* e *Gigaspora margarita*), com cinco repetições. A escória foi eficiente em corrigir o pH do solo e fornecer Ca e Mg para as plantas; portanto, pode substituir o calcário. A inoculação com *R*. *clarus* e *G*. *margarita*, associada às doses de 42, 95 e 213 mg dm-3 de P, promoveu melhoras no desenvolvimento de plantas de milho após 45 dias, tendo resultado em maiores altura, diâmetro do colo, área foliar e matéria seca das raízes e da parte aérea das plantas.

Termos para indexação: *Zea mays*, acidez, calcário, colonização radicular, micorrização.

Introduction

Tropical soils, in general, have low pH values and a high concentration of iron and aluminum oxides and hydroxides (Novais & Smyth, 1999) that form complexes with phosphorous, favoring the fixation of this nutrient (McBride, 1994). This is important since over 80% of the P applied to crops is estimated to become immobile and unavailable to plants due to adsorption, rainfall, or conversion to the organic form (Schachtman et al., 1998).

However, most tropical soils require pH adjustment for cultivation, considering the ideal value ranges from 6.0 to 6.5 (Ronquim, 2010). Traditionally, pH is increased by using acidity correctives such as limestone. However, steel slag, a by-product of steel production, has a similar corrective capacity (Prado et al., 2003; Ramos et al., 2006; Corrêa et al., 2009) and may be an alternative for agricultural areas close to steel production. Besides its acidity corrective capacity, slag may also increase Ca and Mg contents and base saturation, reducing the concentration of H+Al (Prado et al., 2003; Prezotti & Martins, 2012).

Associated with the interventions on soil physicochemical characteristics through traditional correctives and fertilizers, or even alternative products as steel slag, some edaphic microorganisms, such as arbuscular mycorrhizal fungi (AMF), can play an essential role in plant development by increasing the absorption capacity of water and nutrients, especially P.

Mycorrhizal plants show an increase in their P absorption capacity, which can reach up to 80% (Liu et al., 2016), as well as a usually higher nutrient absorption than non-mycorrhizal plants, reducing costs with fertilizers, mainly those with phosphate. Therefore, AMF should be considered in studies on phosphate nutrition, P availability in the soil, and plant selection.

The objective of this work was to evaluate the effect of the use of steel slag as a soil acidity corrective and of mycorrhizal fungi associated with phosphate fertilization on corn plants.

Materials and Methods

The experiment was conducted in a greenhouse, using 3-kg pots with the B horizon of a Typic Haplorthox collected in the municipality of Lavras, in the state of Minas Gerais, Brazil (21º13'40.1"S,

44º58'46.4"W). The soil was autoclaved at 120°C for 1 hour, and the procedure was repeated after 48 hours for the elimination of the microbial community. Then, the soil was kept at dry and aerated conditions for a week to enable manganese oxidation. The chemical and granulometric characterization of the soil, determined according to the methods described by Donagema et al. (2011), are following: 1.57 g kg⁻¹ organic carbon; pH $(H₂O)$ 2:1, 5.3; 0.011 mg dm⁻³ P_{Mehlich-1}; 0.5, 9.0, 8.3, and 1.0 mmol_c dm⁻³ K, Ca, Mg, and Al, respectively; and 130, 30, and 840 g kg^{-1} sand, silt, and clay, respectively.

The doses of steel slag and limestone used as acidity correctives were defined in pre-trials to increase soil pH (H₂O) to 6.0. The pre-defined calibration curve showed the need to apply $4 \text{ Mg} \text{ ha}^{-1}$ limestone or slag to achieve this value. Table 1 shows the characteristics of the applied limestone and slag. The used slag was obtained from the ArcelorMittal Brasil steel mill, located in the municipality of Serra, in the state of Espírito Santo, Brazil.

The experiment was carried out in a randomized complete block design, in a 4x5x2 factorial arrangement, with four acidity correction treatments (C1, correction with dolomitic limestone at a dose of 4 Mg ha-1; C2, correction with steel slag at a dose of 4 Mg ha⁻¹; C3, correction with a 1:1 mixture of 2 Mg ha⁻¹ dolomitic limestone and 2 Mg ha-1 steel slag; and C4, control, without pH correction), five phosphorus doses $(0, 42, 95, 213,$ and 480 mg dm⁻³, corresponding to 0, 0.22, 0.49, 1.11, and 2.50 g dm⁻³ triple superphosphate), and the presence or absence of two AMF (*Rhizophagus clarus* and *Gigaspora margarita*), with five replicates.

P doses were determined according to a previous evaluation of the adsorption capacity of this element, based on the calculation of the solution equilibrium P (P-rem) (Alvarez V. et al., 2000). P adsorption was higher in the treatment without the application of acidity corrective, which showed a P-rem value of 19.8 mg L^{-1} . From this result, as suggested by Alvarez V. et al. (2000), P doses between 0 and 480 mg $kg⁻¹$ were used.

Initially, the soil was mixed with each acidity corrective. The mixture was moistened and incubated for 40 days, maintaining soil moisture between 75 and 80% field capacity using deionized water. Afterward, P was applied in the form of triple superphosphate and then incorporated into the soil. After 15 days of incubation, three corn seeds of the Eldorado variety were sown in each pot.

Mycorrhizal inoculation was performed at sowing time, using 10 mL of an inoculum soil containing 80 spores of the AMF species *R*. *clarus* and *G*. *margarita*, together with hyphae and colonized roots, which also act as propagules. The species *R*. *clarus* [A5 (CNPAB 005)] and *G*. *margarita* [A1 (CNPAB 001)] came from the AMF collection of Embrapa Agrobiologia. Thinning was performed seven days after emergence, leaving one plant per pot.

The plants were then cultivated in a greenhouse for 77 days. Pots were irrigated daily with deionized water, and modified Hoagland nutrient solution (Hoagland & Arnon, 1950) was added once a week without Ca, Mg, and P sources. At the end of the experiment, each pot received a total of 250 mL nutrient solution.

P content, measured in 8-mm diameter leaf discs, was quantified every 15 days according to the methodology of Aziz & Habte (1987). At the end of the experiment, the following variables were evaluated: plant height; stem diameter; leaf area, using the LI-3100C Area Meter (LI-COR, Lincoln, NE, USA); shoot and root dry matter, after drying in forced-air circulation oven at 65ºC; percentage of mycorrhizal colonization (McGonigle et al., 1990), after clarification and staining, based on Grace & Stribley (1991) and Koske $&$ Gemma (1989); and number of AMF spores, separated by wet sieving (Gerdemann & Nicolson, 1963).

Data were subjected to the analysis of variance (Anova), and means were compared by the Scott-Knott test, at 5% probability, or by the regression

Table 1. Characterization of the applied limestone and steel slag.

Caracteristic	Limestone	Slag
CaO $(\%)$	25.0	40.0
MgO(%)	17.0	9.0
SiO(%)	-	11.0
Reactivity $(\frac{9}{0})^{(1)}$	98.3	70.4
Arsenic (mg kg^{-1})		3.2
Lead (mg kg^{-1})		4.5
Total chromium (mg kg^{-1})		446.7
Selenium (mg kg^{-1})		6.7
Hexavalent chromium (mg kg^{-1})		0.7

(1)Determined according to the procedures defined by Ministério da Agricultura, Pecuária e Abastecimento (Brasil, 2006).

analysis for P doses; the Sisvar software was used for all analyses (Ferreira, 2011). The numbers of AMF spores and mycorrhizal colonization were transformed to $(x+0.5)^{0.5}$ and arcsin $(x/100)^{0.5}$, respectively.

Results and Discussion

A summary of the Anova of the studied variables is shown in Table 2. No triple interaction was detected between the evaluated factors. However, double interactions were observed between acidity correction treatments and P doses for root dry matter (RDM), spore density, and mycorrhizal colonization; between acidity correction treatments and mycorrhizae, for leaf area and RDM; and between P and mycorrhizae, for stem diameter, leaf area, shoot dry matter (SDM), RDM, P in leaf discs, and shoot P content.

At the beginning of cultivation, the correctives were efficient in increasing soil pH, which reached between 5.8 and 6.8 (Table 3). At 77 days after cultivation, pH values decreased, especially when limestone was applied. The control treatment (without corrective) had the lowest pH values over the experimental period. Differences between the reactivity of limestone and slag have already been reported in other studies, which also confirmed that the latter was efficient in correcting soil pH (Prado et al., 2003; Corrêa et al., 2009; Sobral et al., 2011; Deus & Büll, 2013).

The P contents available in the soil of the treatments with the P doses of 213 and 480 mg $dm³$ were classified as very good, according to Alvarez V. et al. (1999). After cultivation, P contents decreased, which may be related to P adsorption by the soil minerals. The highest reductions were verified in the treatments that received the highest P doses (Table 3); however, the P available in the soil did not differ among acidity correction treatments.

Ca contents were higher in the treatments that received the highest P doses. This was due to the composition of triple superphosphate, which has about 16% Ca. Ca contents also decreased at the end of the experiment, although at a lower proportion than that observed for P. Mg contents were influenced by the composition of the correctives, showing higher values in the treatments with dolomitic limestone and lower ones in those with steel slag (Table 3).

P and Ca contents in the soil reduced at the end of the experiment due to the extraction of these nutrients

by corn plants. Since P was the limiting factor in the soil, increasing the application of this nutrient strongly affected plant development, resulting in increments in all evaluated variables (Figure 1). Bastos et al. (2010) studied six soils with P doses ranging from 135 to 463 mg kg⁻¹ and did not find any effects on plant development for the same soil; however, the increase in P doses resulted in a higher content of the nutrient in the shoot.

The presence of AMF spores or mycorrhizal colonization was not observed in uninoculated treatments, confirming the asepsis of the experiment. In the AMF-inoculated treatments, the highest sporulation values of 73, 72, and 42 spores per 50 mL soil occurred at the P doses of 42, 95, and 213 mg dm⁻³, respectively. These values are well above those of 28 and 19 spores per 50 mL soil (data not shown), respectively, found in the treatments without P application or with the P dose of 480 mg dm^3 . Mycorrhization is related to the nutritional status of plants, and P availability is considered one of its determining factors (Smith & Smith, 2011; Balota et al., 2012; Deguchi et al., 2012; Gosling et al., 2013; Liu et al., 2016; Ribeiro et al., 2016). It should be pointed out that the absence of P application caused nutritional stress to the plant and stimulated colonization; however, fungi were unable to supply nutritional demands, consequently producing few spores. Conversely, at the highest P dose, the plant was well nourished and less dependent on the symbiosis with AMF, resulting in lower colonization rates (Table 4).

Mycorrhizal colonization, therefore, varied depending on the correctives and P doses used (Table 4). In the presence of limestone, higher colonization rates occurred at the P doses of 42 and 95 mg dm⁻³. In the treatment with slag and the control (without corrective), colonization was higher without P application or with the application of the P dose of 213 mg dm-3. However, when the mixture of limestone and slag was used, the highest colonization rates were obtained at the P doses of 0, 42, 95, and 213 mg dm-3. The colonization rates verified in the present study were close to those observed by Santos et al. (2000), who worked with ten cultures inoculated with *R*. *clarus* and *G*. *margarita* and subjected to the application of low-solubility P.

In this context, considering the practical use of AMF in agriculture, P contents in the soil must be maintained within a range that does not inhibit the efficiency of mycorrhizal symbiosis. In the present study, colonization inhibition occurred at the highest P dose of 480 mg dm^3 , which is in alignment with other works, depending on the genetic characteristics and nutritional status of the plant, as well as on the degree of dependence of the mycorrhizal association (Reis et al., 2008; Hausmann & Hawkes, 2009; Smith & Smith, 2011; Liu et al., 2016).

Corn plants were responsive to P doses and mycorrhizal inoculation (Table 2). The increase in P dose promoted a linear increase in the variables leaf area, SDM, plant height, stem diameter, and RDM for non-mycorrhizal plants (Figure 1). The treatments inoculated with AMF at the P doses of 0, 42, 95, and

Table 2. Summary of the analysis of variance of the studied variables for corn (*Zea mays*) plants cultivated in pots under different treatments of soil acidity correction (C), phosphorus (P) doses, and inoculation with arbuscular mycorrhizal fungi (M).

	Source of variation							Coefficient of	
Variable	Blocks	C	P	M	CxP	CxM	P x M	CxPxM	variation $(\%)$
Height	**	ns	$***$	$***$	ns	ns	ns	ns	38.68
Stem diameter	ns	ns	**	$***$	ns	ns	$***$	ns	23.30
Leaf area	$***$	ns	$***$	$***$	ns	$***$	$***$	ns	24.73
Shoot dry matter	$***$	$***$	$***$	$***$	ns	ns	$***$	ns	59.69
Root dry matter	$***$	$***$	$***$	$***$	$***$	\ast	$***$	ns	58.34
Phosphorus in the leaf disc	ns	ns	$***$	$***$	ns	ns	$***$	ns	30.62
Shoot P content	$***$	\ast	$***$	$***$	ns	ns	$***$	ns	50.75
Spore density ⁽²⁾	ns	ns	$***$	$***$	$***$	$\overline{}$		۰.	44.70
Mycorrhizal colonization ^{(2)}	ns	$***$	$***$	$***$	$***$	-	۰.	۰	26.28

(1)Analyses performed for factors C, P, and their interaction. ** and *Significant by the F-test, at 1 and 5% probability, respectively. ^{ns}Nonsignificant.

213 mg dm-3 showed greater leaf area, SDM, plant height, plant diameter, and RDM than uninoculated treatments. However, plants cultivated at the highest P dose of 480 mg dm-3 were not affected by mycorrhizal inoculation (Figure 1).

In general, regardless of the type of corrective applied, mycorrhization stimulated plant development, reflecting in higher leaf area, SDM, plant height, stem diameter, and RDM values than uninoculated plants. When AMF associates with roots, they function as extensions of the root system, increasing the capacity of the plants to absorb water and nutrients from the soil, especially those of low mobility, such as P (Hausmann & Hawkes, 2009; Smith & Smith, 2011; Liu et al., 2016; Salgado et al., 2016). This effect is evidenced in the present study since the contribution of the AMF to plant development occurred up to the P dose of 213 mg dm⁻³.

The P contents in leaf discs, for all evaluation periods, increased with the P doses applied to the soil (Figure 2). P contents were higher at 15 days after planting (DAP), reducing at 30 and 45 DAP, with an increase or stabilization on the last two evaluation dates, i.e., 60 and 75 DAP. The reductions in the P contents at 30 and 45 DAP possibly indicate an accelerated plant growth stage or a higher demand for P by other parts of the plant. Considering that leaf area and SDM increased with plant development, P accumulation in the shoot also increased with time, resulting in greater P extraction from the soil over the experiment. This explains the reductions in the P available in the soil after the experimental period (Table 3).

Mycorrhizal inoculation contributed to increasing P contents in leaf discs, compared with uninoculated treatments, even with the one without P application. At the doses of 42, 95, and 213 mg dm^{-3} , the most significant differences were found between the P contents of inoculated and uninoculated plants (Figure 2). It should be highlighted that at the highest P dose of 480 mg dm⁻³, AMF did not significantly affect P contents in leaf discs. However, mycorrhizal plants had higher P contents at 45 days after colonization. Studies have reported periods close to 45 days for the establishment of functional symbiosis, also in corn plants (Castillo et al., 2012; Liu et al., 2016).

Table 3. Soil chemical characteristics under different treatments of soil acidity correction and phosphorus (P) doses before and after corn (*Zea mays*) cultivation.

P doses	Correction ⁽¹⁾	pH (H ₂ O) 2:1		P (mg dm ⁻³)		$Ca (cmolc dm-3)$		Mg (cmol _c dm ⁻³)	
(mg dm ³)		Before	After	Before	After	Before	After	Before	After
$\mathbf{0}$	C1	6.4	6.2	0.9	0.3	2.6	2.6	0.7	1.4
$\boldsymbol{0}$	C ₂	6.2	6.2	2.3	1.3	2.9	2.4	0.2	1.1
$\boldsymbol{0}$	C ₃	6.3	6.4	1.6	1.6	3.1	2.5	0.5	1.1
$\boldsymbol{0}$	C ₄	5.3	5.7	0.9	0.8	1.2	0.9	0.1	0.6
42	C1	6.7	6.5	1.3	0.8	2.9	2.8	0.7	1.7
42	C ₂	6.2	5.9	4.2	2.9	3.0	2.6	0.2	1.0
42	C ₃	6.4	6.3	2.2	1.7	2.9	2.6	0.5	$0.8\,$
42	C4	5.3	5.4	1.9	0.8	1.1	0.8	0.1	0.3
95	C1	6.8	6.4	6.2	2.0	3.2	3.2	0.7	1.3
95	C ₂	6.0	5.9	7.3	3.2	3.0	2.8	0.2	0.7
95	C ₃	6.3	6.3	7.0	3.0	3.3	2.9	0.5	1.1
95	C4	5.3	5.3	6.6	1.2	1.3	1.0	0.1	0.4
213	C1	6.5	6.5	18.4	5.9	3.7	3.5	0.8	1.2
213	C ₂	6.0	5.9	12.9	13.1	3.3	3.1	0.2	1.4
213	C ₃	6.2	6.1	30.5	6.8	4.1	3.3	0.5	1.0
213	C ₄	5.2	5.3	26.1	4.8	2.2	1.6	0.1	0.4
480	C1	6.3	6.2	36.4	20.3	4.9	4.2	0.7	1.1
480	C ₂	5.8	5.7	63.7	22.2	4.4	2.1	0.3	0.5
480	C ₃	6.2	6.0	45.8	16.5	4.4	4.2	0.5	0.9
480	C ₄	5.1	5.1	60.9	14.5	3.1	2.7	0.1	0.6

(0 C1, 4 Mg ha⁻¹ dolomitic limestone; C2, 4 Mg ha⁻¹ steel slag; C3, 2 Mg ha⁻¹ dolomitic limestone + 2 Mg ha⁻¹ steel slag; and C4, control, without acidity correction.

Figure 1. Leaf area (A), shoot dry matter (B), plant height (C), stem diameter (D), and root dry matter (E) of corn (*Zea mays*) plants cultivated under different phosphorus (P) doses and mycorrhizal inoculation. * and **Significant at 5 and 1% probability, respectively.

Table 4. Mycorrhizal colonization in corn (*Zea mays*) roots inoculated with *Rhizophagus clarus* and *Gigaspora margarita*, when cultivated in a Typic Haplorthox under different treatments of soil acidity correction and phosphorus doses (0, 42, 95, 213, and 480 mg dm⁻³)⁽¹⁾.

Corrective	Mycorrhizal colonization $(\%)$						
		42	95	213	480		
Limestone (L)	33.0aB	51.4aA	50.4aA	37.6aB	19.6 _b B		
Slag(S)	58.0aA	29.0 _b B	39.6aB	51.2aA	32.8aB		
$L+S(1:1)$	50.0aA	60.6aA	43.8aA	37.2aA	13.4 _b B		
Control	45.6aA	23.2 _b B	24.0 _b B	40.2aA	10.8 _b B		

(1)Means followed by equal letters, uppercase in the same row and lowercase in the same column, do not differ by Scott-Knott test, at 5% probability. Data transformed by arcsin $(x/100)^{0.5}$.

Figure 2. Phosphorus (P) content in the leaf discs of corn (*Zea mays*) plants developed under different P doses (0, 42, 95, 213, and 480 mg dm-3) and mycorrhizal inoculation. *Significant at 5% probability.

Steel slag application as a soil acidity corrective may be an alternative use for a by-product of the steel industry, since the treatment with it showed no negative effect on the symbiosis between arbuscular mycorrhizal fungi and corn plants; however, the use of this by-product must comply with environmental standards.

Conclusions

1. Steel slag is effective in correcting soil pH and providing Ca and Mg to corn (*Zea mays*) plants and, therefore, can replace or be used together with limestone.

2. Limestone and slag provide similar values of P available in the soil (Mehlich-1), although the latter promotes higher colonization rates at the highest P dose of 480 mg dm⁻³.

3. The inoculation with *Rhizophagus clarus* and *Gigaspora margarita* improves the development of corn plants at 45 days after inoculation, supplying more P to them.

4. Mycorrhizal corn plants show greater development than uninoculated plants at the same P doses, but have no response to or dependence on mycorrhizal symbiosis at the P dose of 480 mg dm⁻³.

Acknowledgments

To ArcelorMittal Brasil, for the donation of the steel slag used in the study; to Embrapa Agrobiologia, for technical support; and to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, process number 310947/2017-0) and to Fundação de Amparo à Pesquisa do Estado de Minas Gerais (Fapemig, process number CAG APQ 01661/2017-0), for financial support.

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