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Phosphate Fertilization and Mycorrhizal Inoculation Increase Corn Leaf and Grain Nutrient Contents

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Abstract: The agricultural use of arbuscular mycorrhizal fungi, such as *Rhizoglomus intraradices*, can increase the efficiency of phosphate fertilization for the benefit of the corn plant and grain nutrition. In this study, a field experiment was conducted in an area of Selvíria/MS, Brazil, in the years 2019 and 2020, to verify the effects of reduced doses of phosphorus combined with the inoculation of corn seed with *R. intraradices* on corn plant growth and grain nutrient contents. The experiment was laid in a randomized block design in subdivided plots with four repetitions and twenty treatments resulting from combining five doses of P_2O_5 (0%, 25%, 50%, 75%, and 100% of the recommended dose) with four doses (0, 60, 120, and 180 g ha^{-1}) of an inoculant containing *R. intraradices*. Leaf and kernel macro- and micronutrient contents were evaluated. The foliar P content in 2020 was a function of the interaction between phosphate fertilization and AMF inoculation, with the highest leaf P content observed at the 100% of P_2O_5 combined with AMF inoculation between 120 and 140 g ha^{-1}. In the grains Mg content, an interaction was observed between the two factors in 2020 and the response surface, showing that the highest Mg content was obtained when maximum doses of P_2O_5 and maximum doses of inoculant were combined. A response surface showed that, in 2020, the highest leaf Zn content occurred when 35–55% P_2O_5 is applied with no inoculation and when P_2O_5 is limited to 20–30%, and there is inoculation with doses between 90 and 150 g ha^{-1}. Phosphate fertilization increased foliar K (2019) and Mg (2020) contents, with maximum points at doses of 76.57% and 88.80%, respectively.

Keywords: phosphorus; arbuscular mycorrhizal fungi; *Zea mays*; *Rhizoglomus intraradices*; plant nutrition

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1. Introduction

Phosphorus (P) is an important plant macronutrient because it is a structural component of sugar molecules and a range of other metabolic intermediates in the respiratory and photosynthetic metabolic pathways. Furthermore, P is a structural component of phospholipids in cell membranes, nucleotides, and ATP for energy metabolism (Taiz et al., 2017). However, P fertilization efficiency is commonly low due to high P adsorption to soil particles, mainly Fe and Al oxides in the clay fraction [1–3]; such binding renders P unavailable for plant uptake. Additionally, P reserves for manufacturing fertilizers are becoming increasingly scarce as natural deposits are rapidly depleted around the world [4]. Therefore, P fertilizer supply faces increasingly difficult challenges to meet crop demands.

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that depend on plants to complete their life cycle and multiply [5]. While plants provide AMF with nourishment by supplying the necessary carbohydrates for their development [6], fungi are beneficial to their host plants by developing hyphae that extend into the soil and increase the volume of soil explored by plant roots and, consequently, enhancing plant water and nutrient uptake, especially P [7–9]. Thus, symbiosis increases photoassimilate production and biomass accumulation in plants [10,11].

Phosphate fertilization is related to plant nutrition; indeed, an adequate P supply promotes root development, which in turn leads to enhanced nutrient uptake [12]. Moreover, P is essential for the active uptake and assimilation of certain nutrients; additionally, it exhibits antagonistic (P–Zn, [13,14]) and synergistic (P–Mg, [15]) effects with other nutrients. Similarly, mycorrhizal symbiosis benefits plants nutritionally; for example, the N, S, Zn, Fe, and P uptakes by plants colonized by AMF are higher than those by plants which do not host AMF [9,16–18].

Proper plant nutrition is essential to ensure optimum plant development and maximum agricultural crop productivity, particularly in the crops most extensively grown around the world, such as soybean, corn, wheat, and rice [19]. Plan nutritional deficiencies can lead to reduced plant growth, development, and, consequently, productivity, whereas well-nourished plants grow and develop adequately, thereby resulting in high agricultural productivity [20].

This study aimed to see how phosphate fertilization, seed inoculation with an AMF, and the interaction between the two can influence the plant and grain nutritional status in corn when the inoculation of *R. intraradices* is conducted in an agricultural soil with an established native AMF community. The hypothesis of the work is that the combination of an intermediate dose of phosphorus with the inoculation of *R. intraradices* provides the same nutritional results in maize as the maximum dose of phosphorus in the absence of inoculation.

2. Materials and Methods

2.1. Experimental Site

The experiment was conducted at a second crop in 2019 and 2020 in two different areas of the Teaching, Research, and Extension Farm of FEIS/UNESP in Selvíria/MS, Brazil. The region is located at an average altitude of 358 m and is part of the Cerrado biome. Alvares et al. (2013) described the region as having a type Aw climate according to the classification by Köppen, with an average annual precipitation of 1322 mm and an average annual temperature of 23 °C [21]. Data on the average temperature, rainfall, relative humidity, and insolation during the experiment are shown in Figure 1. The two areas are approximately 800 m apart; they have similar soil fertility, management, and cultivation histories, including a no-tillage system at the implementation phase and the cultivation of *Pennisetum glaucum* as a green cover before corn cropping.

The chemical attributes of the soil in the arable layer (0.00 to 0.20 m) were determined according to the methodology proposed by Raij et al. (2001) [22]. The chemical attributes were as follows. In 2019, the following soil fertility levels were determined at the 0.00–0.20 m topsoil layer: 16 mg P per dm^{-3} of soil (determined in resin); 6 mg S-SO_4 per dm^{-3} of soil; 21 g organic matter per dm^{-3} of soil; pH 5.2 (determined in $CaCl_2$ solution); 1.8, 28.0, 18.0, and 31.0 mmolc of K, Ca, Mg, and H+Al, respectively, per dm^{-3} of soil; 3.6, 21.0, 23.4, and 0.9 mg of Cu, Fe, Mn, and Zn, respectively, per dm^{-3} of soil (determined in DTPA—diethylenetriaminepentaacetic acid); 0.24 mg B per dm^{-3} of soil (determined in hot water); and 61% of base saturation. In 2020, the following soil fertility data were obtained: 25 mg P per dm^{-3} of soil (determined in resin); 3 mg S-SO_4 per dm^{-3} of soil; 18 g organic matter per dm^{-3} of soil; pH 5.0 (determined in $CaCl_2$ solution); 0.7, 19.0, 16.0, and 31.0 mmolc of K, Ca, Mg, and H + Al, respectively, per dm^{-3} of soil; 1.6, 25.0, 11.1, and 0.6 mg of Cu, Fe, Mn, and Zn, respectively, per dm^{-3} of soil (determined in DTPA—diethylenetriaminepentaacetic acid); 0.27 mg B per dm^{-3} of soil (determined in hot water); and 54% of base saturation. In both areas, soils were classified as Typic Haplorthox (Oxisol) according to the Soil Survey Staff (2014) [23]. With regard to the number of mycorrhizal spores in the soil, a total of 630.8 and 672.3 spores per 50 mL of dry soil were obtained in the 0.00–0.20 m depth layer, determined by the method described in Novais et al. (2017) [24].

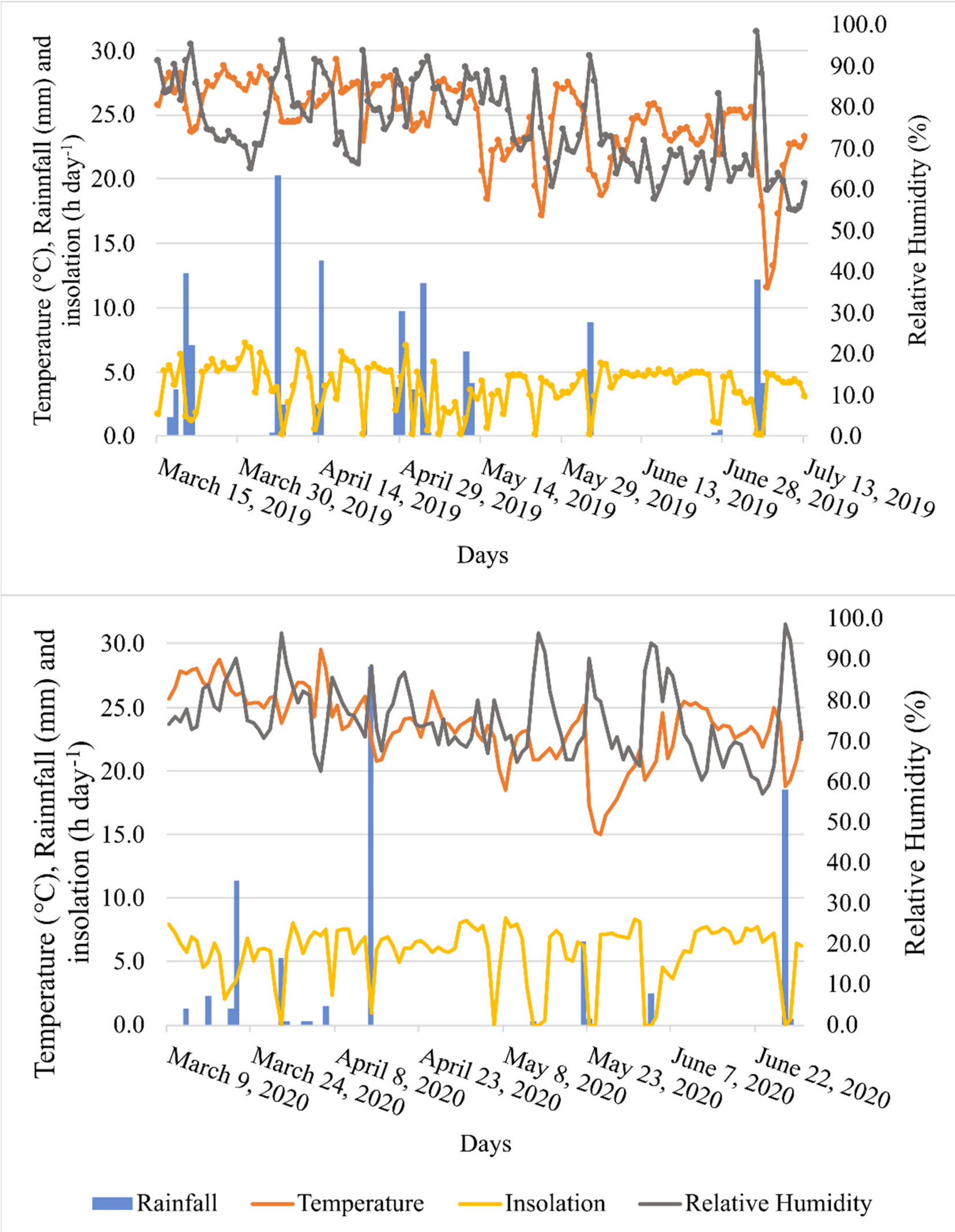

Figure 1. Climatic data: precipitation (mm), average temperature (°C), insolation (h day^{-1}), and average relative humidity (%) over the experimental period. Selvíria/MS, 2019. Based on data available at clima.feis.unesp.br.

2.2. Biological Material

In 2019, the single-cross corn (*Zea mays* L.) hybrid AG 7098® (commercial product) was used. Seeds were treated with metalaxyl + thiabendazole + fludioxonil (2.0 g + 15.0 g + 2.5 g per 100 kg), deltamethrin (0.2 g per 100 kg), and pyrimiphos methyl (0.8 g per 100 kg). In 2020, a coded experimental single-cross corn (*Zea mays* L.) hybrid (obtained from Bayer but not yet released commercially) was used, and the seeds were not chemically treated. In both years, the hybrids used were chosen based on the recommendation for the region of Brazil where the experiment is located and the availability of seeds.

For seed inoculation, a commercial solid powder inoculant (Rootela®) was used. It was obtained from Ground-Work BioAg (Israel) [25] and contains 20,800 infectious propagules per gram of the arbuscular mycorrhizal fungus *Rhizoglomus intraradices* (Schenck & Smith) (Walker & Schüßler) in a mixture of 82% vermiculite, 6% clay, and 12% unidentified particles [26]. In Brazil, this is the first and only commercial product registered for large-scale commercial use.

2.3. Experimental Design and Treatments

The experiment was set up according to a randomized block design in subdivided plots. The plots were treated with P_2O_5, while the subplots were treated with a mycorrhizal inoculant. The P_2O_5 doses were 0%, 25%, 50%, 75%, and 100% of the recommended dosage for corn [27], according to soil fertility and expected yield. Thus, in 2019, the corresponding P_2O_5 doses were 0, 30, 60, 90, and 120 kg ha^{-1}, while, in 2020, they were 0, 15, 30, 45, and 60 kg ha^{-1}. The inoculant *R. intraradices* doses were 0, 60, 120, and 180 g ha^{-1}.

Each plot received a dose of the corresponding phosphate fertilizer and was divided into four subplots with four lines that were 7 m each, with a 0.85 m spacing between lines, in which mycorrhizal inoculant doses were randomized. The two central lines of each subplot comprised the sampling area, disregarding 0.5 m from the subplot ends.

2.4. Experiment Set-Up and Management

The experiments were established on 15 March 2019 and 10 March 2020. Before the seeds were sown, mulch was managed using a knife roller. Considering the soil fertility and expected yield, the fertilization at sowing in 2019 consisted of 45 kg N ha^{-1} and 60 kg K_2O ha^{-1}. In 2020, the fertilization at sowing consisted of 33 kg N ha^{-1} and 60 kg K_2O ha^{-1}, respectively, according to the recommendations of Raij and Cantarella (1997) [27]. The N and K sources were urea and KCl, respectively.

For phosphate fertilization, the P_2O_5 source used in both years was monoammonium phosphate (52% P_2O_5 and 11% N), which regulated the fertilizer flow according to the dose for each plot. All fertilizers were mechanically applied using a no-tillage seed drill supplied with fertilizer but not with seed.

The seeds were inoculated before sowing, and a sugar solution (10%) was used at a dose of 300 mL 50 kg^{-1} of seed to ensure that the inoculant adhered to the seeds. Non-inoculated seeds were coated with the sugar solution.

The seeds were sown with a manual sowing machine at 0.20 m between hills for a total planting density of 55,000 to 60,000 plants ha^{-1}. Each experimental block was sown by a single person to ensure homogeneity within the block. The area was irrigated with 15 mm of water. A fixed-sprinkler irrigation system was used in 2019 to meet the water demand of the corn crop throughout the crop cycle, with an average delivery rate of 15 mm h^{-1}; in turn, a central pivot was used in 2020, with an average application rate of 13 mm h^{-1}. Irrigation management was performed according to Andrade and Albuquerque (2017) [28], who, for a high evaporative demand, define the following crop coefficients: 0.78 for Kc1 and Kc2, 1.29 for Kc3 and Kc4, and 0.35 for Kc5. With this, the management was performed, and a total application of 525 mm and 495 mm of water was obtained in 2019 and 2020, respectively.

Seedlings emerged on 20 March 2019 and on 15 March 2020. Nitrogen fertilization was split in two. When the plants were at the V_4 stage, 60 and 50 kg ha^{-1} of N were applied in

2019 and 2020, respectively. Subsequently, when the plants were at the V_8 stage, N was applied again, with an additional 60 kg N ha^{-1} (urea) supplied in both years.

Plant protection against pests and disease and weed management were performed in both years according to the recommendations for the crop. Predominant pests and weeds in the area were countered using the recommended products. Fungicide application was not necessary at any time during the crop cycle. Phytosanitary and weed management in 2019 was carried out with a spray of chlorantraniliprole (25 g ha^{-1} of the active ingredient [i. a.]) + imidacloprid (50 g ha^{-1} of the a.i.) to control defoliating caterpillars and sucking insects at 16 days after emergence (DAE). Subsequently, at 21 DAE, thiamethoxan + lambda cyhalothrin + methomyl (35.25 + 26.5 + 129 g ha^{-1} of a.i.) was applied for pest control. In the same operation, herbicides were added to control weeds: atrazine + tembotrione (1000 + 84 g ha^{-1} of a.i.). In 2020, a field spraying was carried out with chlorantraniliprole + thiamethoxan + lambda cyhalothrin (25 + 35.25 + 26.5 g ha^{-1} of a.i.) for pest control and with atrazine + tembotrione (1000 + 84 g ha^{-1} dos ai) for weed control at 11 DAE. Then, at 21 DAE, imidacloprid + triflumuron (70 + 48 g ha^{-1} of a.i.) was applied to control pests and glyphosate was applied (792.5 g ha^{-1} of a.i.) to control plants. In all operations, the adjuvant Aureo® (soybean oil methyl ester, 720 g L^{-1}) was added in the proportion of 250 mL 100 L^{-1} of solution and the volume of solution used was 200 L $ha^{-1.}$

Silking occurred at 50 and 45 days after emergence (DAE) in 2019 and 2020, respectively. The crop was harvested manually at 115 and 107 DAE in 2019 and 2020 (after grain maturation), respectively. All ears contained in the two rows of 5 m in the sampling area of each plot were collected.

2.5. Evaluations

(a) Foliar nutrient (N, P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn) contents: During full flowering, the middle third of the blades of 20 leaves located opposite and below the main ear were collected from the sampling area in each plot. The leaf samples were washed with water, oven-dried at 65 °C until a constant mass, and ground in a Wiley mill. Foliar nutrient contents were determined according to the methods of Malavolta, Vitti, and Oliveira (1997) [29].

(b) Grain nutrient (N, P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn) contents: After harvest, the ears were threshed to separate grains and cobs. A sample of approximately 20 g was separated, oven-dried at 65 °C until a constant mass, and ground in a Wiley mill. Subsequently, nutritional composition was analyzed according to the methods of Malavolta, Vitti, and Oliveira (1997) [29].

2.6. Statistical Analysis

Analysis of variance (ANOVA) was initially performed, and the normality of residuals and homoscedasticity were examined using the R software. The data were analyzed with the F-test of the ANOVA for the studied factors and their interactions. When ANOVA revealed significant effects ($p < 0.05$), polynomial regression tests on the doses of phosphorus or the inoculant as independent factors were conducted. When the interaction between factors was significant, a response surface adjustment test was performed. For the ANOVA and polynomial regression tests, the statistical software SISVAR® was used [30]. For the adjustment of response surfaces, the software Minitab® was used. The pooled data from the two experimental years were not statistically analyzed because the experiment was conducted in different areas and using different genotypes.

3. Results

3.1. Macronutrient Contents in Plants

The corn foliar-nitrogen content was not influenced by the treatments tested during the two years of the study (Table 1). However, the foliar P content as a function of the interaction between phosphate fertilization and AMF inoculation significantly ($p < 0.05$) differed between the two years. The response surface could be adjusted (Table 2 and

Figure 2A), from which the highest leaf P content was observed at the maximum doses of phosphate fertilization (the region with approximately 100%) combined with AMF inoculation between 120 and 140 g ha^{-1}. Another region that showed a high foliar P content corresponded with the combination of P doses between 20% and 60% and AMF inoculation between 20 and 80 g ha^{-1}.

Table 1. Macronutrient foliar contents in corn at flowering as a function of P_2O_5 doses and seed inoculation with *Rhizoglomus intraradices*.

Treatments	N		P		K		Ca		Mg		S	
	g kg^{-1}											
Phosphorus (P)	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
0.0	34.39	34.14	3.79	3.03	17.33	16.77	3.90	1.98	3.64	2.25	3.08	2.30
25.0	34.31	35.15	4.18	3.21	18.30	16.55	3.45	2.11	3.23	2.31	3.26	2.42
50.0	34.07	34.14	3.88	3.30	20.34	17.22	3.27	2.22	3.28	2.66	2.85	2.40
75.0	34.32	34.37	3.54	3.16	21.81	16.47	3.43	2.17	3.36	2.64	3.02	2.35
100.0	33.79	34.32	3.72	3.31	19.89	16.50	3.88	2.18	3.67	2.63	3.04	2.48
***R. intraradices* (M)**												
0	34.41	34.34	3.74	3.19	20.39	16.39	3.70	2.10	3.58	2.53	2.97	2.38
60	33.53	34.08	3.77	3.17	19.12	17.04	3.73	2.17	3.50	2.47	3.13	2.41
120	35.01	34.73	4.02	3.15	18.70	16.68	3.49	1.99	3.32	2.39	3.12	2.37
180	33.75	34.54	3.76	3.30	19.93	16.70	3.43	2.25	3.34	2.61	2.98	2.42
F Test (*p*-value)												
P	0.951	0.695	0.198	0.206	0.036 *	0.844	0.013 *	0.567	0.152	0.045 *	0.197	0.167
M	0.095	0.880	0.510	0.449	0.417	0.742	0.301	0.284	0.060	0.230	0.417	0.960
P × M	0.088	0.36	0.587	0.026 *	0.477	0.372	0.756	0.858	0.651	0.904	0.743	0.400
General Average	34.17	34.42	3.822	3.20	19.53	16.70	3.59	2.13	3.43	2.50	3.05	2.39
CV 1	6.25	5.63	15.37	9.12	14.88	11.06	11.05	16.96	13.76	13.73	12.06	6.83
CV 2	5.01	6.57	15.39	8.7	15.43	9.52	14.01	17.52	8.58	11.61	11.16	11.32

CV1 and CV2: Coefficient of variation (%) of plots and subplots. *: Significant at 5% by the ANOVA F-test, respectively.

Table 2. Values of the equations referring to the response surfaces adjusted for the effect of the interaction between P_2O_5 doses and the mycorrhizal inoculant used on leaf phosphorus, leaf zinc, and grain magnesium contents in the 2020 corn experiment.

Variable	Leaf P	Leaf Zn	Grain Mg
Intercept	2.8794×10^{0}	2.733×10^{1}	2.3226×10^{0}
P	2.5529×10^{2}	-1.496×10^{0}	-3.8428×10^{-3}
M	-2.4677×10^{1}	1.60×10^{-1}	-2.3298×10^{-3}
PM	-4.2782×10^{-2}	2.32×10^{-2}	-6.2296×10^{-4}
P^2	-5.0687×10^{0}	9.77×10^{-2}	3.4209×10^{-4}
M^2	1.2785×10^{-1}	-3.42×10^{-3}	-9.3915×10^{-7}
P^3	3.0046×10^{-6}	-1.717×10^{-3}	-2.8267×10^{-6}
M^3	1.0338×10^{-7}	1.40×10^{-5}	7.0657×10^{-8}
P^2M	1.6135×10^{-5}	-1.791×10^{-3}	2.5165×10^{-5}
PM^2	4.6327×10^{-6}	1.94×10^{-4}	1.0328×10^{-5}
P^3M	-1.8309×10^{-7}	3.20×10^{-5}	-1.9235×10^{-7}
P^2M^2	-3.9954×10^{-7}	1.00×10^{-6}	-4.2016×10^{-7}
PM^3	-3.4518×10^{-8}	1.00×10^{-6}	-3.49×10^{-8}
P^3M^2	3.932×10^{-9}	-	3.249×10^{-9}
P^2M^3	1.928×10^{-9}	-	1.462×10^{-9}

Table 2. *Cont.*

Variable	Leaf P	Leaf Zn	Grain Mg
P^3M^3	-1.80×10^{-11}	-	-1.10×10^{-11}
P^4	-	9.00×106	-
R^2	44.19%	49.34%	40.81%

This table demonstrates the equation representing the response surface of the variables Leaf P, Leaf Zn, and Grain Mg as a function of the interaction between P_2O_5 (P) and AMF (M) doses. For example, in Leaf P, the equation is given by: $y = 2.8794 + 0.025529 \cdot P - 24.677 \cdot M - 0,042782 \cdot MP - 5.0687 \cdot P^2 + \cdots + 1.928 \cdot 10^{-9} \cdot P^2 M^3 - 1.8 \cdot 10^{-11} \cdot P^3 M^3$.

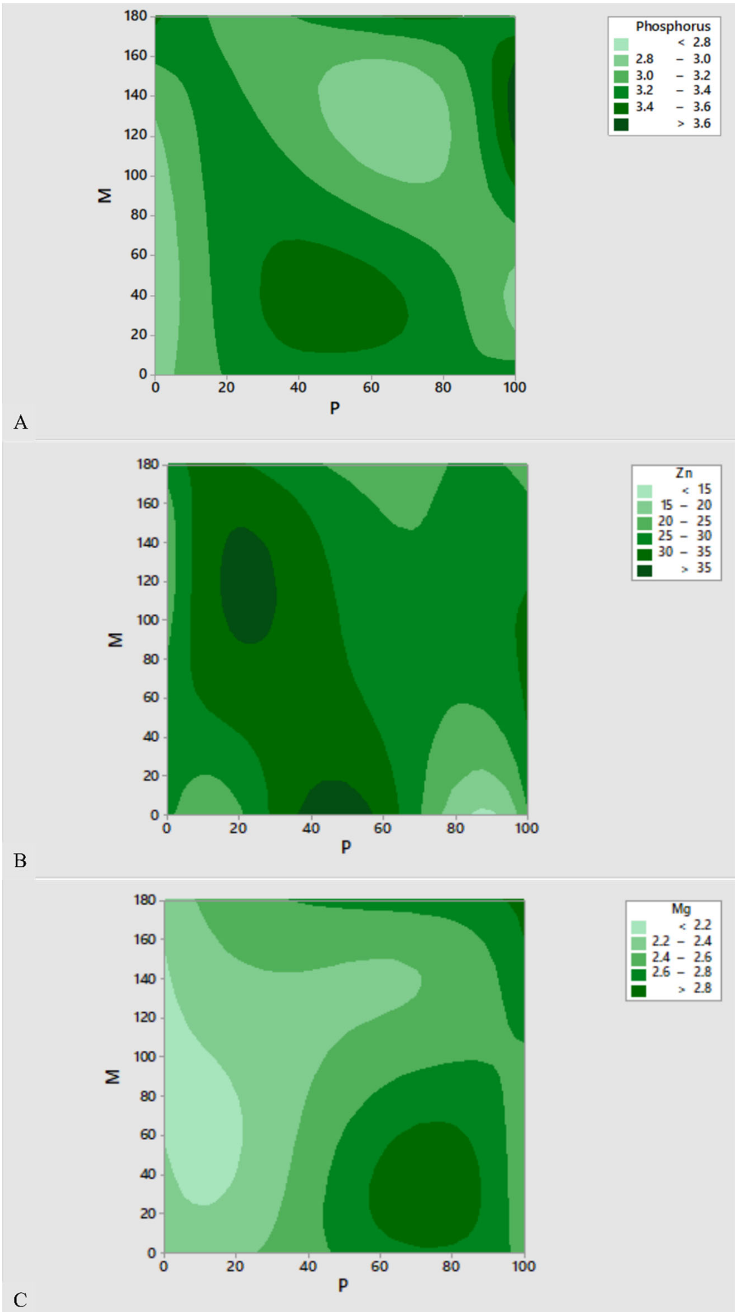

Figure 2. Response surfaces for foliar P content (**A**), foliar Zn content (**B**), and grain Mg content (**C**) as a function of P_2O_5 supplied to the soil and the mycorrhizal inoculant in corn seeds in the 2020 experiment.

Phosphate fertilization affected the foliar K content in 2019 ($p < 0.05$), with a quadratic regression equation fitting ($R^2 = 83.57\%$) and a peak at 76.57% P_2O_5 (Figure 3a). In the same year, P fertilization influenced the Ca leaf content, but this finding did not fit the regression equation with biological significance. No combination treatment affected the K or Ca contents in 2020. Furthermore, phosphate fertilization affected the foliar Mg content in 2020 ($p < 0.05$), with a quadratic regression equation fitting ($R^2 = 85.72\%$) and a peak at 88.80% P_2O_5 (Figure 3b). Treatments with phosphate fertilization, mycorrhizal inoculation, or the interaction between them did not influence foliar S levels in either of the two years of the experiment.

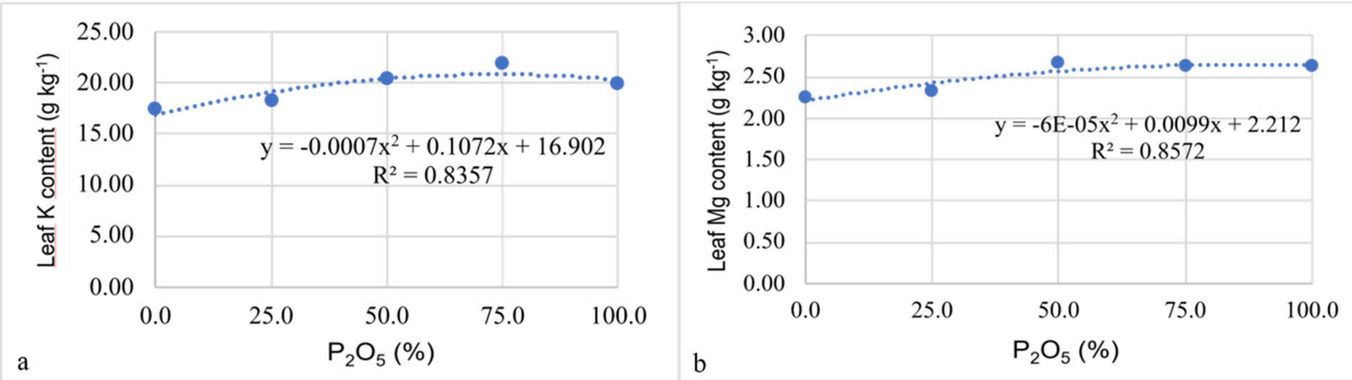

Figure 3. Plots of the fitted regression equations for foliar K in 2019 (**a**) and foliar Mg in 2020 (**b**) in corn plant as a function of phosphate fertilization.

As for the grain nutritional contents (Table 3), the N and K contents as a function of treatment did not differ between the two years. The effect of phosphate fertilization on the grain Ca content was determined in 2020, but the regression equation was not adjusted for the behavior of this variable as a function of treatment. In turn, in the case of Mg, an interaction effect was observed between the two factors in 2020 ($p < 0.05$), and the response surface was adjusted (Table 2 and Figure 2c) to demonstrate the behavior of this variable as a function of the doses of P_2O_5 and the mycorrhizal inoculant used ($R^2 = 40.81\%$). Thus, the Mg concentration was highest when the highest doses of P_2O_5 and the inoculant were combined. In addition to this region, another region had a high Mg content in the grains, defined by high but not full (60–80%) P_2O_5 doses and an AMF inoculation dose lower than 60 g ha^{-1}.

3.2. Micronutrient Contents in Plants

As for foliar micronutrient contents, the Cu and Mn contents did not vary as a function of treatment in either 2019 or 2020 (Table 4). However, in 2019, phosphate fertilization affected the foliar Fe content ($p < 0.05$), but no adjustment of the regression equation for the behavior was performed. Differences were observed for the leaf Zn content as a function of the interaction between phosphate fertilization and mycorrhizal inoculation ($p < 0.05$). Thus, the response surface was adjusted for the behavior of this variable in relation to the interaction between the two factors (Table 2 and Figure 2b). It is observed that the response surface ($R^2 = 49.34\%$) indicates that the highest foliar Zn contents occur when there is no inoculation, but there is phosphate fertilization at intermediate doses (approximately between 35–55% of P_2O_5) and when phosphate fertilization is limited between 20–30% of P_2O_5 and there is mycorrhizal inoculation with doses between 90 and 150 g ha^{-1}.

Table 3. Macronutrient contents in harvested corn grain as a function of P_2O_5 doses and seed inoculation with *Rhizoglomus intraradices*.

Treatments	N		P		K		Ca (g kg^{-1})		Mg		S	
Phosphorus (P)	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
0.0	17.23	16.62	3.53	4.03	4.32	4.68	0.015	0.428	1.51	4.56	1.53	1.10
25.0	16.72	17.77	3.63	4.06	4.37	4.95	0.025	0.425	1.55	4.55	1.56	1.05
50.0	16.37	18.04	3.84	4.21	4.28	4.93	0.023	0.453	1.56	4,66	1.51	1.07
75.0	16.43	17.31	3.63	4.01	4.18	4.79	0.026	0.413	1.49	4.63	1.46	1.17
100.0	16.58	16.99	3.81	3.64	4.32	5.02	0.017	0.492	1.55	4.90	1.45	1.19
***R. intraradices* (M)**												
0	16.87	16.81	3.78	4.25 [1]	4.47	5.09	0.020	0.454	1.62	4.85	1.59 [2]	1.13
60	16.32	16.58	3.45	3.76	4.10	4.80	0.018	0.457	1.42	4.55	1.45	1.06
120	16.77	18.27	3.68	3.87	4.26	4.70	0.023	0.441	1.49	4.59	1.44	1.14
180	16.71	17.73	3.84	4.07	4.35	4.92	0.023	0.418	1.59	4.64	1.52	1.13
F Test (*p*-value)												
P	0.909	0.956	0.968	0.172	0.993	0.385	0.414	0.009 **	0.995	0.006 **	0.620	0.098
M	0.655	0.374	0.651	0.010 *	0.421	0.061	0.753	0.247	0.370	<0.001 **	0.029 *	0.120
P x M	0.298	0.247	0.203	0.068	0.192	0.519	0.340	0.206	0.188	<0.001 **	0.089	0.829
General Average	16.67	17.34	3.69	3.99	4.29	4.88	0.02	0.44	1.53	4.66	1.50	1.12
CV1	14.68	29.14	34.89	12.54	23.62	9.07	76.41	9.01	28.92	3.61	13.01	11.64
CV2	7.58	17.00	24.55	10.08	14.38	7.93	71.87	12.83	21.54	3.63	9.91	9.17

CV1 and CV2: Coefficient of variation (%) of plots and subplots. * and **: Significant at 5% and 1% by the ANOVA F-test, respectively. [1] $y = 0.000049x^2 - 0.009449x + 4.227399$ ($R^2 = 0.9113$)—regression equation for P content in corn grains in 2020 as a function of AMF doses used. [2] $y = 0.000016x^2 — 0.003193x + 1.592400$ ($R^2 = 0.9888$)—regression equation for S content in corn grains in 2019 as a function of AMF doses used.

Table 4. Micronutrient foliar contents in corn at flowering as a function of P_2O_5 doses and seed inoculation with *Rhizoglomus intraradices*.

Treatments	Cu		Fe (mg kg^{-1})		Mn		Zn	
Phosphorus (P)	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
0.0	16.53	3.25	165.47	124.17	112.56	52.00	33.67	26.08
25.0	15.06	3.58	179.14	140.33	118.36	55.42	34.69	31.43
50.0	15.67	3.50	159.67	159.42	98.92	51.17	32.67	30.32
75.0	14.50	3.58	158.75	118.33	108.67	49.00	34.25	24.33
100.0	15.58	3.33	160.67	137.17	115.08	58.42	32.75	26.94
***R. intraradices* (M)**								
0	15.31	3.40	163.13	136.13	111.80	51.27	32.47	27.60
60	15.27	3.67	165.11	123.67	113.07	56.00	34.13	29.54
120	15.73	3.33	165.22	147.20	108.87	54.27	33.73	28.40
180	15.55	3.40	165.49	136.53	109.13	51.27	34.09	25.75
F Test (*p*-value)								
P	0.179	0.900	0.025 *	0.570	0.365	0.726	0.772	0.115
M	0.899	0.577	0.962	0.749	0.762	0.244	0.521	0.102
P x M	0.446	0.740	0.558	0.579	0.258	0.367	0.423	0.012 *
General Average	15.47	3.45	164.74	135.88	110.72	53.20	33.60	27.82
CV 1	11.81	30.23	7.93	46.20	20.98	33.68	13.81	22.84
CV 2	12.38	20.27	8.19	43.01	11.51	14.09	10.25	14.74

CV1 and CV2: Coefficient of variation (%) of plots and subplots. *: Significant at 5% by the ANOVA F-test, respectively.

Lastly, neither phosphate fertilization, mycorrhizal inoculation, or the interaction between them affected the grain micronutrient (Cu, Fe, Mn, Zn) contents in 2019 or 2020 (Table 5).

Table 5. Micronutrient contents in harvested corn grain as a function of P_2O_5 doses and seed inoculation with *Rhizoglomus intraradices*.

Treatments	Cu		Fe		Mn		Zn	
	mg kg^{-1}							
Phosphorus (P)	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**	**2019**	**2020**
0.0	2.94	10.33	20.25	52.92	10.25	8.83	39.00	42.92
25.0	5.92	13.39	19.08	56.00	10.33	9.17	38.17	41.67
50.0	3.33	13.42	19.50	56.75	10.00	9.00	36.08	41.83
75.0	1.58	9.00	21.33	53.33	9.75	8.92	35.67	42.75
100.0	3.33	13.08	20.25	59.36	10.08	9.29	35.25	39.99
***R. intraradices* (M)**								
0	3.53	13.00	21.67	54.93	10.67	9.23	37.73	43.26
60	3.53	10.60	18.80	55.55	9.27	8.73	34.93	41.00
120	3.20	9.60	19.13	54.40	10.20	9.07	34.93	42.07
180	3.42	14.98	20.73	57.80	10.20	9.13	39.73	41.00
F Test (*p*-value)								
P	0.257	0.536	0.994	0.580	0.998	0.933	0.973	0.795
M	0.967	0.339	0.861	0.337	0.669	0.614	0.402	0.145
P x M	0.530	0.250	0.724	0.062	0.141	0.174	0.145	0.250
General Average	3.42	12.04	20.08	55.67	10.08	9.04	36.83	41.83
CV 1	124.34	71.20	64.33	18.88	49.52	15.92	45.68	15.01
CV 2	60.25	72.13	52.19	9.61	31.13	11.83	24.48	7.17

CV1 and CV2: Coefficient of variation (%) of plots and subplots.

4. Discussion

Neither phosphate fertilization nor AMF inoculation influenced foliar N content, as observed for foliar Cu and Mn contents, although the literature shows effects of phosphate fertilization and mycorrhizal inoculation on the leaf contents of N, Cu, and Mn [17,31–33]. In the present work, this may be a consequence of the high abundance of native AMF in the soil at the experimental site, which is benefited by the no-till farming system used in the region, in addition to the cultivation of cover crops and rational soil fertility management, as affirmed by Lehman et al. (2012) and Cameron (2016) [34,35]. This may have favored the occurrence of mycorrhizal symbiosis in corn plants at levels sufficient to allow for N, Cu, and Mn uptakes equivalent to those of inoculated plants. Besides this, the levels of available P in the soil of the two years of cultivation are considered intermediary [36]. One hypothesis for this is that the available nutrients in the soil may have been sufficient to ensure adequate root development and allow for the uptake of these nutrients in plots treated with reduced doses of P_2O_5, at levels similar to those of plants that received 100% of the recommended nutrient content. For this conclusion, further work should be done to evaluate the effects of these treatments in soils with different levels of available P and to verify the development of the plant root system in these cases.

Cu and Mn did not differ depending on the treatments used, and this may be due to the fact that the levels of these nutrients in the soil, available to plants in the 0.00–0.20 m layer, are considered high, i.e., sufficient to meet the demand of corn plants. [36].

The interaction between phosphate fertilization and AMF inoculation observed in relation to the foliar P contents was consistent with the literature, which affirms that phosphate fertilization provides more phosphorus to the roots, resulting in enhanced absorption and an increased P concentration in plant tissues [32,37]. However, Bressan et al. (2001) demonstrated that inoculation with AMF also increases the P content in plant tissues because mycorrhizal symbiosis promotes root growth, and mycorrhizal hyphae extend through the soil beyond the root depletion zone; consequently, more nutrients and water, especially P, are absorbed by plants [38]. Thus, mycorrhizae can increase the efficiency of P uptake by plants, thereby improving the utilization efficiency of phosphate fertilizers [8,31]. The results of this study showed that the combination of the highest P_2O_5 dose with the highest AMF inoculation provided the highest P uptake and increased corn-leaf nutrient contents.

The foliar K and Mg contents showed a similar behavior as a function of phosphate fertilization. Maximum contents were detected at 76.57% and 88.80% of the recommended P_2O_5, respectively, probably because the enhanced availability of P allowed the plants to develop a large root system and explore a large volume of soil. Thus, soil nutrients are efficiently absorbed by plants, resulting in increased leaf nutrient concentrations [33]. In the case of Mg, there is also synergism with phosphorous, that is, the presence of phosphorous induces a greater absorption of Mg [15]. Moderate and low soil K contents were detected in 2019 and 2020, respectively, while high soil Mg contents were observed in both years [36].

The foliar Zn content was also influenced by the interaction between phosphate fertilization and AMF inoculation; in particular, the highest foliar Zn content was detected in the presence of 35–55% P_2O_5 and less than 20 g ha^{-1} AMF and in the combination treatment including 20–30% P_2O_5 and 90–150 g ha^{-1} AMF. Zn was reduced at high P_2O_5 doses because of the antagonism of these two nutrients during absorption by the roots due to the formation of insoluble $Zn_3(PO_4)_2$ [39]. The presence of inoculated AMF results in a high Zn content because of the benefits of mycorrhizal symbiosis for root development and the promotion of increased nutrient uptake, including Zn, as stated by Smith and Read (2010) and González-Guerrero et al. (2005) [8,16].

With respect to the nutritional content of harvested grain, the experimental treatments did not influence N, K, or micronutrient contents. According to Lima (2011) and Silva and Vahl (2002), it happened because the soil P_2O_5 content was sufficient to meet plant nutrient requirements, thereby allowing for adequate root development and favoring the consequent uptake of these nutrients to satisfy the demand of grain formation [32,37]. The high amount of native AMF in the soil contributed to the natural occurrence of symbiosis such that no difference was observed for the nutrients in question between the treatments without inoculation and the treatments including inoculation with AMF [40].

The high Mg content in the presence of the combination of the maximum P_2O_5 dose with AMF inoculation is due to the combined function of promoting enhanced root development, soil exploration, and nutrient uptake, in addition to the synergistic effect between P and Mg on root uptake [8,15,32].

In general, inoculation with AMF improved the nutritional state of the plants, because greater accumulations of some nutrients were observed in the leaves and grains. This result is corroborated by works such as that of Buzo et al. (2022), who, when working with beans and P doses in a soil with a clayey texture, demonstrated increases in P, Mg, S, and Mn [41]. The work of Ma et al. (2022) also obtained similar results, with increases in the nutrients N, P, and K in maize inoculated with three different species of AMF (*Rhizophagus aggreatus*, *Claroideoglomus etunicatum*, and *Funneliformis mosseae*) [42]. However, the literature explains that these inoculation results can vary and depend on factors inherent to the genotypes of the plant and the studied AMF and how they interact with each other, in addition to external factors such as the agricultural management adopted, the population of native AMF (their quantity and diversity), and the availability of nutrients in the soil [8,43]. Thus, new work should be done in order to broaden the understanding of the influence of each of these factors on the results that MFA inoculation can have on an agricultural crop.

Likewise, it is known that phosphate fertilization is essential to ensure good plant nutrition, since P influences root development and, consequently, the volume of soil explored by the plant, while participating in the processes of active nutrient uptake and interacting with certain ions in the soil, and may benefit or hinder their uptake by plants [15,33,39]. However, the results of this practice can be more or less expressive, depending on the previous availability of P in the soil, the presence of decomposing organic material in no-till areas and with the use of mulches, and the microorganisms that interact with plants, including P-solubilizing bacteria and, especially, arbuscular mycorrhizal fungi [31,34,35]. The greater the presence of these items, the more P the plants will obtain from the soil itself and the fewer evident nutritional differences there will be between the plants that will receive some P and those that will not receive any P via fertilizer. Thus, although the dynamics of phosphorus with plant and soil have been studied extensively, further research is needed to better understand the interactions between phosphate fertilization and AMF under different conditions of soil P availability, crop management, and inoculated AMF species.

5. Conclusions

High phosphate fertilization levels enhanced soil nutrient uptake—specifically, that of P, K, and Mg. Inoculation with *R. intraradices* can affect nutrient uptake, especially P and S; however, this practice did not always result in evident benefits because of the rich, native soil-AMF community. Inoculation with AMF also increased the efficiency of phosphate fertilization such that the combination treatment allowed for higher P and Mg levels in plant tissues than those obtained by fertilization or inoculation separately.

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