



Agronomic characteristics and management of diseases in maize with chelate-based products containing calcium, copper, manganese, and zinc

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ABSTRACT. The aim of this work was to verify the potential of chelate-based products containing calcium, copper, manganese, and zinc for the management of *Pantoea ananatis*, *Puccinia polysora*, *Cercospora zea-maydis*, *Exserohilum turcicum*, *Diplodia macrospora*, and *Pseudomonas avenae* in crop maize growth in the summer season, as well as their influence on agronomic characteristics. The treatments included commercial chelate-based products of amino acids with the elements calcium (15%), copper (5%), manganese (15%), and zinc (10%) at doses of 0.5 kg ha⁻¹, 0.3 L ha⁻¹, 0.4 kg ha⁻¹, and 1 L ha⁻¹, respectively; fungicides (20% azoxystrobin and 8% cyproconazole at a dose of 0.3 L ha⁻¹ + 25% propiconazole at a dose of 0.4 L ha⁻¹); and water. The tests were carried out under field conditions for two consecutive years with two simple hybrids. The plant height, stem diameter, number of rows per ear, number of grains per ear row, productivity and mass of one thousand grains, as well as the severity of leaf diseases, were all evaluated, and chemical analysis of the leaves was performed. In the 2016/2017 growth season, for the number of rows per ear and number of grains per row, the fungicide treatment showed the highest values, whereas for the mass of one thousand grains and productivity, the chelate treatments did not differ from the fungicide treatment and were different from the water treatment. In the 2017/2018 growth season, for the mass of one thousand grains and yield, only the fungicide treatment was different from the water treatment. For all the chelates studied for both hybrids, there was no difference in nutrient content before and after foliar application. It can be concluded that calcium, copper, manganese, and zinc products may influence agronomic traits but not the severity of the diseases evaluated in these two hybrids of maize under the edaphoclimatic conditions in which the study was carried out.

Keywords: alternative control; nutrition and disease; nutrients; *Zea mays* L.

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Introduction

Maize (*Zea mays* L.) is a highly important crop (Galvão, Miranda, Trogello, & Fritsche-Neto, 2014) that can be exposed to factors that limit the maximum expression of its productive potential, such as the presence of disease-causing pathogens (Brito, Pinho, Pereira, & Balestre, 2013).

The main phytopathogens and their diseases found in maize leaves are *Cercospora zea-maydis* (T. Daniels) (gray leaf spot), a complex of *Pantoea ananatis* and *Phaeosphaeria maydis* (P. Henn.) (white spot), *Exserohilum turcicum* (Pass.) (northern leaf blight), *Puccinia polysora* (southern rust), *Puccinia sorghi* Schw. (common rust), and *Stenocarpella macrospora* (Earle) Sutton (*Diplodia* leaf streak) (Carvalho, Pereira, & Camargo, 2016).

To reduce the damage caused by these diseases, in addition to the use of fungicides and resistant genotypes, other methods have been researched; one alternative method is nutrient management since the nutritional status of the vegetables is considered one of the main factors responsible for plant defense against phytopathogens (Motter et al., 2012). Thus, the nutritional balance may alter the chemical, morphological, histological (Marschner, 2012), and microbial activity of the rhizosphere of plants (Bedendo, Amorim, & Mattos Júnior, 2018), reducing their predisposition to pathogenic infection.

Calcium, copper, manganese, and zinc are important nutrients that are highly indicated in applications to promote plant health and increase the productivity of corn crops; therefore, there is substantial interest in the need for these nutrients and results related to their use.

respectively. A treatment with fungicide based on 20% azoxystrobin and 8% cyproconazole at a dose of 0.3 L ha⁻¹ + 25% propiconazole at a dose of 0.4 L ha⁻¹ and a treatment with water were used as controls.

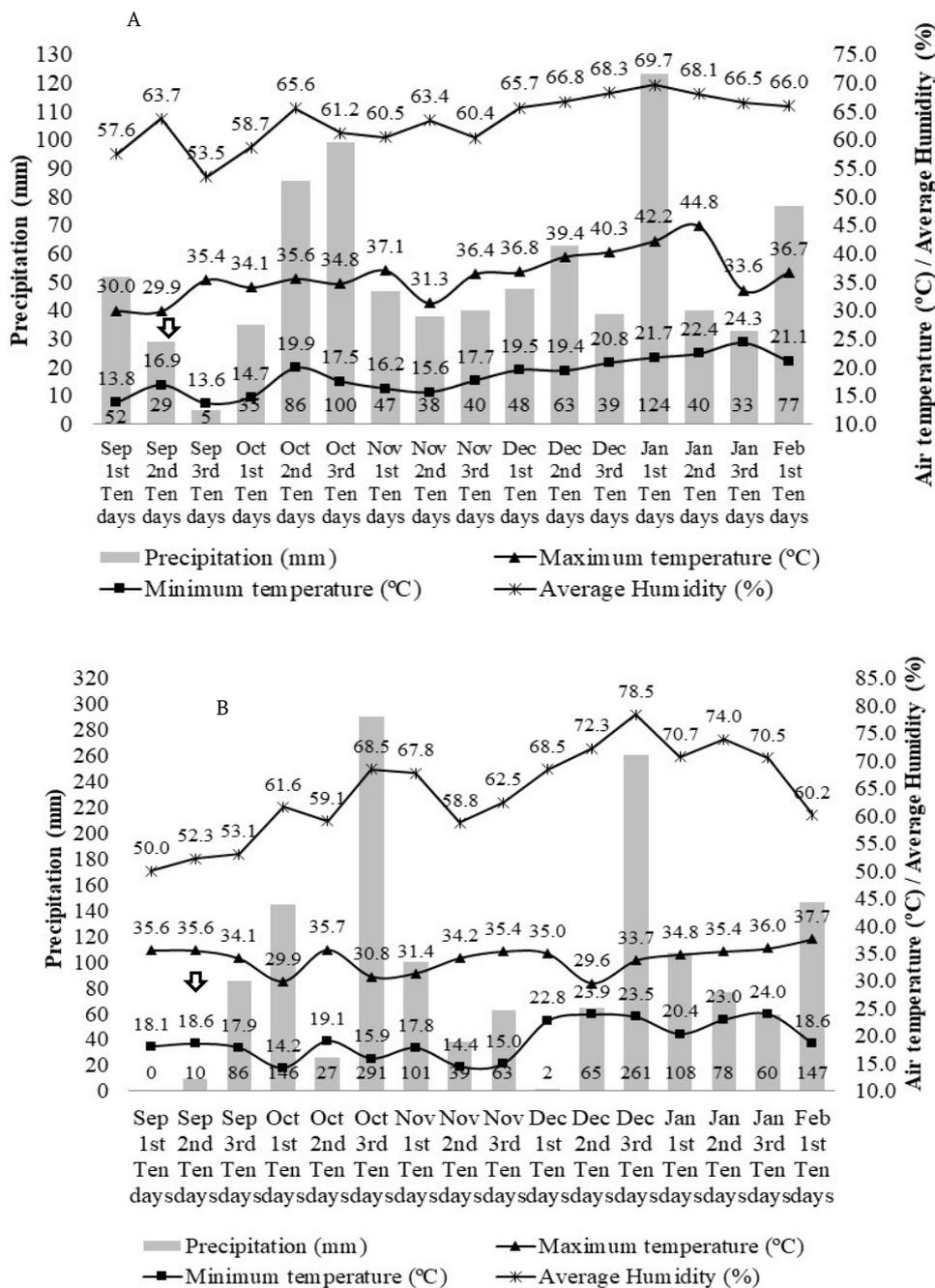


Figure 1. Maximum and minimum temperature and total rainfall, by 10-day period, recorded during the period of the first harvest of 2016/2017 (A) and the first harvest of 2017/2018 (B). The arrows (↓) indicate the 10-day period of sowing, September 13th, 2016 (A), and September 7th, 2017 (B). Novo Sarandi, Toledo, Paraná State, Brazil.

The products were applied 38 and 58 days after emergence, when more than 50% of the plants were in the V8 and VT phenological stages, respectively. In both stages, the application was performed by a costal sprayer with an empty conical jet spray nozzle using a 100 L ha⁻¹ spray volume.

The agronomic characteristics evaluated were plant height, stem diameter, number of rows per ear, number of grains per row, productivity, and mass of one thousand grains.

Plant height was obtained by measuring from the soil to the curvature of the flag leaf one week after flowering. Stem diameter was obtained in full female flowering (stigma/style visible) using a digital caliper to measure in the middle of the first expanded internode, and then the mean basal diameter of the stems for each plot was obtained. For both parameters, the same 10 random plants within the useful area of the plots were evaluated.

After manual harvest, the number of rows was counted to obtain the average number of rows per ear, and the number of grains per row was counted to obtain the average number of rows of grain per ear; both parameters were evaluated in 20 ears randomly sampled within the useful area of each plot for each treatment and replicate.

To evaluate productivity, the harvested ears were threshed, and the mass and moisture of the grains were measured. Based on these data and the useful area of the plot, the productivity per unit area was calculated and recorded in the form of kg ha^{-1} with moisture corrected to 14%, according to the Brazilian Rules of Seed Analysis (Brazil, 2009).

The mass of one thousand grains was determined through the analysis of eight samples of 100 grains for each replicate of each treatment. The samples, after being identified and weighed on a precision scale, were dried in an oven with forced air circulation at 105°C until constant mass was obtained. Subsequently, the samples were again weighed, and the moisture content of the grains was converted to 14% (wet basis) and then extrapolated to obtain the mass of 1,000 grains.

The severity of the diseases was evaluated according to the spontaneous appearance of lesions, and no inoculation was performed. The percentage of leaf area with symptoms of the diseases was measured through a diagrammatic scale specific to each disease, when available in the literature, every seven days from the moment of symptom appearance. The leaves below and opposite the ear of 10 plants within the useful area of each plot were evaluated. By using the severity data, the area under the disease progress curve was calculated using the Shaner and Finney (1977) equation.

In the 2016/2017 season, white spot caused by *P. ananatis*, southern rust caused by *P. polysora* and gray spot caused by *C. zeaе maydis* occurred. Thus, we used the diagrammatic scales proposed by Sachs, Neves, Canteri, and Sachs (2011) for white spot and rust. For gray spot, since no diagrammatic scale was found, the values were estimated.

In the 2017/2018 season, white spot and gray spot occurred, and the severities were evaluated in the same way as in the previous season; northern leaf blight caused by *E. turcicum* was evaluated by the scale proposed by Lazaroto, Santos, Konflanz, Malagi, and Camochena (2012); for Diplodia leaf streak (*D. macrospora*) and bacterial leaf blight (*Pseudomonas avenae*), there are no scales available in the literature, and the severity values were estimated.

In the 2017/2018 season, foliar chemical analysis was performed to determine the elements calcium, copper, manganese, and zinc. Before and 24 hours after the treatments, one-third of 10 leaves from each plot was collected for destructive analysis. The leaves were immediately cleaned in abundant tap water and ultrapure water, and then, using a piece of cotton soaked in 3% (v/v) HCl solution, possible traces of dirt were removed. The leaves were rinsed with ultrapure water and air dried in the shade. The central rib was removed, and the material was packed in paper bags and kept in an air circulation oven for drying at 65°C .

After drying, the samples were ground in a Willey mill with stainless steel blades, and 0.2 g of each sample was placed in digestion tubes with 4 mL of nitroperchloric acid, composed of 3 mL of nitric acid and 1 mL of perchloric acid. The material was left in the tubes for 12 hours for predigestion, and then the digestion was performed using one digester block for 40 digestion tubes; the initial 1 h of digestion was performed at 150°C , and then the temperature was gradually increased to 400°C until the extract became completely clear (colorless) and white HClO_4 fumes were obtained.

The remaining extract after cooling was adjusted to 50 mL by adding ultrapure water, and then the nutrient contents were measured in an atomic absorption spectrophotometer. The macronutrient content was expressed as g kg^{-1} , whereas the micronutrient content was expressed in mg kg^{-1} .

The data were submitted to analysis of variance, and the means were compared by the Tukey test. The 2 x 6 factorial design consisted of two hybrids (30F53 Leptra RR and SX 7331 VIPTERA) and six treatments (calcium, copper, manganese, zinc, fungicide, and water). In the 2017/2018 season for northern leaf blight and bacterial leaf spot only, a simple randomized block design was used, since the occurrence of these diseases was detected in only one of the hybrids under study. For the determination of elements, Student's t test was used to compare the concentrations of each nutrient for each hybrid before and after foliar application of the treatments. The software Genes was used (Cruz, 2016).

The above analyses were carried out rather than a joint analysis because two different agricultural years with very discrepant climatic conditions were studied, and therefore, the diseases that occurred were not exactly the same.

Results and discussion

For the 2016/2017 growth season, the analysis of variance indicated differences among the treatments for the number of rows per ear, grains per row, mass of a thousand grains and productivity, while for the hybrids, all analyzed variables were significant; however, these data are not shown or discussed because the focus of this study was not to verify the varietal differences between the genotypes used. There was no significant effect of the interaction between treatments and hybrids on any of the analyzed variables, which shows that there was no dependence; in other words, independent of the hybrid, the behavior of the treatments was the same.

In the 2017/2018 growth season, there was a difference among the treatments only for the mass of a thousand grains and productivity, while for the hybrids, all variables analyzed were significant. The interaction between treatments and hybrids had no significant effect on any of the analyzed variables, which shows that there is no dependence between these factors.

For the 2016/2017 growth season (Table 2), for the number of rows per ear and the number of grains per row, the fungicide treatment presented a higher value than the water treatment, while the chelate-based products (calcium, copper, manganese, and zinc) had the same effect as the fungicides and water. For the mass of one thousand grains and productivity, the water treatment differed from all other treatments, presenting the lowest values, while the chelate and fungicide treatments had equivalent effects on these variables.

This treatment effect on the mass of a thousand grains and productivity may have occurred because the chelate-based products helped the plants remain healthy, activating plant defense mechanisms through the induction of resistance or strengthening the defense of the plant, such as by supplying calcium and manganese; the products may also have direct fungitoxic action against certain pathogens, as copper does.

Thus, nutrients may be related to the reduction of the severity of some diseases (Lima et al., 2010) acting either in the whole plant or in areas of contact with the pathogen (Bedendo et al., 2018).

Table 2. Number of ear rows (NER), number of grains per row (NGR), mass of one thousand grains (MTG) (g) and productivity (kg ha⁻¹) for maize treated with chelate-based products containing calcium, copper, manganese, and zinc. Novo Sarandi, Toledo, Paraná State, Brazil, 2016/2017.

Treatments	NER	NGR	MTG	Productivity
Calcium	15.46 ab	38.47 ab	400.37 a	14,552.28 a
Copper	15.46 ab	38.14 ab	405.75 a	14,294.99 a
Manganese	15.56 ab	38.01 ab	398.37 a	14,097.79 a
Zinc	15.35 ab	37.44 ab	401.62 a	13,943.43 a
Fungicide	15.92 a	38.96 a	403.50 a	14,597.55 a
Water	14.63 b	36.44 b	378.50 b	12,722.57 b
MSD	1.28	2.29	16.38	1,065.88

*Means followed by the same lowercase letter in the column do not differ statistically by the Tukey test ($p < 0.05$). MSD: minimum significant difference.

Regarding the 2017/2018 growth season, for the mass of a thousand grains and productivity, the fungicide treatment had higher values than the water treatment. All other treatments did not differ from the fungicide or water treatments (Table 3).

Table 3. Mass of a thousand grains (MTG) (g) and productivity (kg ha⁻¹) of maize treated with chelate-based products containing calcium, copper, manganese, and zinc. Novo Sarandi, Toledo, Paraná State, Brazil, 2017/2018.

Treatments	MTG	Productivity
Calcium	392.35 ab	14,650.71 ab
Copper	396.02 ab	14,707.20 ab
Manganese	391.11 ab	15,113.48 ab
Zinc	399.58 ab	15,050.52 ab
Fungicide	408.66 a	15,429.38 a
Water	386.17 b	14,419.49 b
MSD	22.17	960.44

*Means followed by the same lowercase letter in the column do not differ statistically by the Tukey test ($p < 0.05$). MSD: minimum significant difference.

Agronomic characteristics, such as productivity, are dependent on the correct management of diseases and essential nutrients (Zambolim & Ventura, 2012). According to Gonçalves et al. (2012), the use of the fungicides pyraclostrobin + epoxiconazole provides an increase in the mass of a thousand grains, number of

grains per ear and yield of maize. Henriques et al. (2014) and Rosa, Duarte Júnior, Queiroz, Perego, and Mattei (2017) also observed an effect of fungicide treatment on agronomic variables such as number of rows per ear, mass of a thousand grains and productivity, which occurs because the fungicide allows the plant to reach its maximum photosynthetic capacity by the conservation of leaf area, causing more flow of photoassimilates in the plant at the most critical moments, such as flowering and grain filling.

Rosa et al. (2017) found maize yield increases of up to 31% compared to a water treatment when fungicides (azoxystrobin + cyproconazole) were used in maize crops. Bampi et al. (2012), also studying a maize crop, found that preventive applications of systemic fungicides, including those used in our work, promoted a 65% decrease in disease incidence, increasing the performance of important agronomic variables, such as the mass of a thousand grains and productivity.

According to Brito, Silveira, Brandão, Gomes, and Lopes (2011), the period from preflowering until the moment when grain filling begins can be defined as a critical period for maize crops since any stress, such as a lack of water or reduction in photosynthetic area, leads to major impacts on production. This critical period may be one of the reasons why the fungicide treatment showed significant effects on several variables; that is, even though the productive potential of the maize had already been defined, the use of fungicides made it possible to preserve this productive potential, avoiding productivity losses due to diseases.

Another possible justification for the results is that simple hybrids with uniformity and a high propensity for disease incidence were used and these characteristics contribute to more evident positive results regarding the application of fungicides (Rosa et al., 2017).

Another factor that may also have influenced the results was the soil amendment with calcitic limestone performed before crop planting and the use of base fertilizer containing the tested micronutrients. Thus, foliar application of nutrients may have been unnecessary because their levels were in accordance with the needs of the plant, which had already absorbed the necessary nutrients even before the foliar application of chelate-based products.

According to Biscaro, Prado, Motomiya, and Robaina (2013), little is known about leaf fertilizers and how they complement soil-applied fertilizers in the pursuit of higher nutrient use efficiency, higher productivity and consequently higher profitability.

The incidence of pathogens in the 2016/2017 and 2017/2018 growth seasons was distinct. In the first growth season, white spot, gray leaf spot and common rust were recorded. In this harvest, from the third 10-day period in November, the maximum temperatures were higher than those in 2017/2018; combined with other environmental factors, such temperatures may contribute to the onset of these diseases. In the second growth season, white spot, *Diplodia* leaf streak, gray leaf spot, bacterial leaf blight and northern leaf blight were found, probably due to the higher rainfall volume and higher relative air humidity recorded from the third 10-day period in November.

The fact that distinct diseases occurred in the years of study may be justified by the disease triangle and the temperature and rainfall recorded during these seasons. Disease occurrence requires not just a host but also the presence of a pathogen and a favorable environment, and if one of these three factors is absent, the disease will not appear. Thus, certain diseases may have been favored or not because despite having the same place of cultivation and host, the pathogen was not necessarily present, and the environment (temperature and precipitation) was different during these two years.

Concerning the disease occurrence in the 2016/2017 growth (Table 4), there were differences among treatments for white spot and gray leaf spot. The hybrids had significant effects on white spot and common rust; however, these data are not discussed, nor the averages presented, because these results were not the objective of this study. There was no significant interaction for any of the analyzed variables. In the 2016/2017 season, for white spot disease, only the fungicide treatment differed from the water treatment, presenting lower values, but the other treatments were statistically equivalent to both the water and fungicide treatments. For gray leaf spot, only the treatment with fungicide differed from that with water.

Regarding the 2017/2018 growth season (Table 5), there was a difference among the treatments for white spot and gray leaf spot, while for *Diplodia* leaf streak, only the hybrids were significant; therefore, these data are not shown or discussed. Only the treatment with fungicide reduced the severity of both white spot and gray leaf spot.

Table 4. Area under the white spot and gray leaf spot progress curves for maize treated with chelate-based products containing calcium, copper, manganese, and zinc. Novo Sarandi, Toledo, Paraná State, Brazil, 2016/2017.

Treatments	White spot		Gray leaf spot	
Calcium	100.95	ab	57.37	a
Copper	86.85	ab	56.50	a
Manganese	102.90	ab	58.25	a
Zinc	100.85	ab	56.87	a
Fungicide	58.85	b	24.87	b
Water	125.35	a	61.00	a
MSD	45.98		17.33	

*Means followed by the same lowercase letter in the column do not differ statistically by the Tukey test ($p < 0.05$). MSD: minimum significant difference.

Table 5. Area under the white spot, gray leaf spot and northern leaf blight progress curves for maize treated with chelate-based products containing calcium, copper, manganese, and zinc. Novo Sarandi, Toledo, Paraná State, Brazil, 2017/2018.

Treatments	White spot		Gray leaf spot		Northern leaf blight	
Calcium	34.37	a	76.00	a	72.50	a
Copper	34.00	a	75.00	a	75.00	a
Manganese	38.00	a	72.25	a	77.50	a
Zinc	38.00	a	71.00	a	71.00	a
Fungicide	20.50	b	21.50	b	42.00	b
Water	42.00	a	78.12	a	79.25	a
SMD	12.28		24.05		24.90	

*Means followed by the same lowercase letter in the column do not differ statistically by Tukey's test ($p < 0.05$). SMD: significant minimum difference.

Only the Supreme hybrid presented bacterial leaf blight and northern leaf blight, so it was not possible to perform a statistical analysis using a factorial design. Therefore, a simple randomized block design was applied, using only the values for this genotype. For leaf bacterial blight, there was no difference among treatments, whereas for northern leaf blight, there was a significant effect only for the fungicide (Table 5). The lack of difference in leaf bacterial blight must have occurred because this fungicide exerts no control on this pathogen, which was expected since the pathogen is a bacterium and the product is intended to control certain fungi.

The fact that no bacterial leaf blight was observed in the 30F53 Leptra RR hybrid may have occurred due to the genetic characteristics of the hybrid, but no reports of material resistance to this pathogen were found in the literature. On the other hand, regarding northern leaf blight, the 30F53 Leptra RR hybrid is considered resistant, while the Supreme hybrid is considered susceptible to this pathogen.

For white spot, gray leaf spot and northern leaf blight, the severity decreased only under fungicide application. Corroborating these results, Wesp-Guterres, Bruinsma, and Seidel (2015), studying two maize hybrids, found a reduction in the severity of *P. polysora*, *E. turcicum* and *P. maydis* infection by applying epoxiconazole + pyraclostrobin and fluxapyroxad + pyraclostrobin at phenological stage V8. Additionally, Brito, Pinho, Pereira, and Balestre (2013), using azoxystrobin + cyproconazole in V₁₀ and pre-V_T also found a reduction in white spot gray leaf spot severity. According to Vilela et al. (2012), application of the fungicides pyraclostrobin + epoxiconazole and azoxystrobin + cyproconazole to maize during the pre-tearing stage decreases the incidence and severity of leaf diseases, as observed in this study.

Nutrients may also affect plant development, which may have an influence on disease because plant growth can modify the local microclimate by favoring or disfavoring certain diseases (Zambolim & Ventura, 2012). However, plants that receive an adequate amount of nutrients do not have advantages when receiving higher doses of calcium, copper, manganese or zinc; on the contrary, with respect to diseases, it might be more advantageous if nutrients stayed longer on the leaf surface to have some preinfection action. No action of a specific chelate-based product was verified in this work. Specific studies of pathogenesis-related enzyme analysis could be performed to confirm the hypotheses of this study since according to the same authors, nutrients can influence the resistance mechanisms of plant defense. One of these mechanisms could be calcium deposition or the regulation of a pathway for the synthesis of certain antimicrobial compounds, such as phytoalexins.

Due to the fertilization corrections made on the basis of the chemical soil analysis data, and taking into consideration the fertilization approach used, the effect of the chelate-based products was not evident because the plants did not require more the studied nutrients and there was probably no need

for supplementation. According to Pauletti and Motta (2017), the levels of calcium, manganese, and zinc were high, and the copper level was very high, which supports the hypothesis that the maize plants had no need for these nutrients, a fact that may justify the observed agronomic characteristics and severity of the diseases studied.

In the determination of calcium, copper, manganese, and zinc, it was found that for both hybrids and all chelate-based products, there was no difference between the nutrient amounts before and after application (Table 6).

Table 6. Calcium (g kg^{-1}), copper (mg kg^{-1}), manganese (mg kg^{-1}), and zinc (mg kg^{-1}) content in leaf tissue of two maize hybrids (Supremo and 30F53 Leptra RR) before and after treatment with chelate-based products. Novo Sarandi, Toledo, Paraná State, Brazil, 2017/2018.

	30F53 Leptra RR		SX 7331 VIPTERA	
	Before	After	Before	After
Calcium	4.19	3.87 ^{ns}	3.06	3.62 ^{ns}
Copper	0.05	0.07 ^{ns}	0.01	0.04 ^{ns}
Manganese	35.00	33.12 ^{ns}	36.87	30.75 ^{ns}
Zinc	42.50	32.50 ^{ns}	46.37	31.87 ^{ns}

^{ns}: not significant by Student's t-test.

Chelates may not have shown a very satisfactory effect on the severity of the diseases evaluated in this work. According to Gott et al. (2014), factors such as higher nutrient absorption are provided by the nutritional balance of the crop and not by an isolated action or the application of a single nutrient, so it is necessary to study the whole system to better understand this interaction of factors. Leaf analysis is an efficient and low-cost tool that can be used to improve nutritional management (Camacho, Silveira, Camargo, & Natale, 2012) since this type of analysis helps in the planning and monitoring of fertilization-related actions (Dias, Wadt, Tucci, Santos, & Silva, 2013).

Souza, Novais, Alvarez, and Villani (2010) also state that many factors, such as pH, may interfere with nutrient absorption and availability, so the concentration of a particular nutrient in leaves may also vary depending on the soil and environment in which the plant is located.

The other nutrients present in the system may also have contributed to the results obtained; for example, the nutritional demand for phosphorus (P) varies substantially among hybrids with high productive potential according to the cultivation region (Setiyono, Walters, Cassman, Witt, & Dobermann 2010). Iron (Fe) and manganese (Mn) are elements with high concentrations, which negatively influence interactions (Borges, Pinho, & Pereira, 2009), and copper (Cu) has affinity with nitrogen (N); therefore, Cu is displaced in the xylem and phloem by soluble nitrogen compounds (Souza et al., 2010).

Borin, Lana, and Pereira (2010) found that the absorption of nutrients in maize occurs throughout the crop cycle; however, this absorption occurs at different speeds depending on the current stage of the cycle, and the translocation of these nutrients also changes.

Conclusion

For the edaphoclimatic conditions in which the experiment was carried out, supplementation of chelate-based products containing calcium, copper, manganese, and zinc via foliar application had little effect on agronomic traits and disease severity in maize genotypes in the summer season.

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