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RESEARCH ARTICLE

Micronized Zn Oxide on Carbonic Anhydrase Activity, Health, and Yield of Ratoon Sugarcane Under Tropical Conditions

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Abstract

Zinc (Zn) is one of the most important micronutrients with a direct effect on sugarcane yield. Our hypothesis is that micronized Zn oxide is an optimal Zn source to improve the zinc status, health, and yield of ratoon sugarcane. The present study assessed the effect of Zn doses, sources, and application times (foliar spraying) on sugarcane yield, carbonic anhydrase activity (CAA), and plant diseases in tropical conditions. A study was developed in three sites (Assis, Ourinhos, and Serrana, São Paulo state), Brazil, during the first ratoon cane in 2019/2020. The study tested four Zn doses (0; 639; 1039; and 1386 g ha^{-1}), two Zn sources (Zn sulfate; and micronized zinc oxide), and three application times [at plant heights of 0.5 (100% dose); 1.0 m (100% dose); and 0.5 (50% dose) + 1.0 m (50% dose)]. The results showed that Zn foliar spraying increased CAA (from 224.8 to 742.1 UE g^{-1}) and leaf Zn content (from 12 to 15 g kg^{-1}). The Zn doses reduced orange rust severity and increased yield. Application at the onset of ratoon development (0.5 m tall stems) was more efficient than the other timing formats. Based on the results obtained, it was concluded that soil fertilization micronized zinc oxide improves the Zn status of sugarcane and CAA, contributing to reducing Zn deficiency and health problems in this crop.

Keyword *Saccharum officinarum* L. · Fertilizers · Micronutrients · Plant nutrition

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Introduction

Brazil is the world's largest sugarcane producer with average production of 71.3 Mg ha^{-1}, considered low when compared to high-performing fields (> 100 Mg ha^{-1}) (CONAB 2022; Otto et al. 2022). In less favorable environments with poor natural soil fertility and climate conditions there are low sugarcane yields (Singh et al. 2019a; Mellis et al. 2022). To increase yield, adequate nutritional management is needed, including liming, fertilizer application (macro and micronutrients), and crop rotation. However, the adequate supply of micronutrients has been neglected, especially zinc (Zn) (Marangoni et al. 2019).

Zn is considered one of the most important micronutrients, with a direct effect on sugarcane yield. Plant Zn deficiency affects tillering and plant growth, two fundamental factors in sugarcane yield (Marangoni et al. 2019). Plant Zn deficiency is directly related to Zn deficiency in humans, with about thirty percent of the world's population exhibiting this deficiency (Hafeez et al. 2014). The average Zn concentration in plant tissue is 0.3 μmol (g dry matter^{-1}), corresponding to 20 μg (g dry matter^{-1}) (Broadley et al.

2012). In plant metabolism, Zn is essential for tryptophan synthesis, a precursor to indole-acetic acid (IAA), which is responsible for producing enzymes that promote cell stretching and growth. This micronutrient is also involved in activating different enzymes that act in photosynthesis and sugar formation (Wei et al. 2022; Hassan et al. 2020).

Among these enzymes, carbonic anhydrase is directly involved in photosynthesis, with Zn deficient plants exhibiting a decline in carbonic anhydrase activity (CAA) and photosynthetic rate (DiMario et al. 2018). Carbonic anhydrases are classified as zinc-metalloenzymes and catalyze the interconversion of CO2 and HCO^{-3} in metabolic pathways, requiring a carboxylation step (Hiruni et al. 2022; Cabot et al. 2019). Kumar et al. (2022) found that Zn-deficient conditions promoted ascorbate peroxidase and catalase activity and expressed additional isoforms of NADPH (nicotinamide adenine dinucleotide phosphate) oxidase and superoxide dismutase in rice plants.

Recent studies with Zn (dose: 10 kg ha^{-1}) in Brazilian sugarcane fields have shown that Zn can increase stalk yields by up to 16% and that Zn application has a residual effect on ratoons (Mellis et al. 2016). Although 10 kg ha^{-1} of Zn is recommended in Brazilian tropical soils, operational difficulties mean that few producers adopt this technology. An alternative approach, particularly in ratoon sugarcane, is foliar Zn application. However, scientific data on the efficacy of this method, particularly regarding dosage, application time, and Zn sources, is scarce and inconclusive for sugarcane crops in tropical conditions (Silva et al. 2022).

Zinc foliar spray is commonly applied to crops as $ZnSO_4$ or synthetic chelates (Zn-EDTA), but these sources may not be as efficient in grasses (reference crop). Doolete et al. (2018) observed limited mobility in wheat plants that received foliar $ZnSO_4$ and Zn-EDTA due to localized zinc toxicity in the leaves, and concluded that foliar fertilizers with slower release doses may be more advantageous, since they mitigate the toxicity that decreases zinc bioavailability. Nicchio et al. (2020) also observed no difference in the yield and quality of sugarcane in tropical soil at a Zn dose of 300 g ha^{-1}. On the other hand, Jamro et al. (2002) demonstrated that 1.5 kg ha^{-1} of (foliar) $ZnSO_4$ increased internode size, height, and tillering in sugarcane in Pakistan. Wang et al. (2005) reported that combining 4.4 kg ha^{-1} (soil) and 1.3 kg ha^{-1} of $ZnSO_4$ (foliar) in acidic and limestone soils improved sugarcane yield and industrial quality parameters. Majeed et al. (2022) demonstrated that Zn had a substantial impact on sugarcane growth parameters and yield when compared to iron, copper, and boron. Research on foliar fertilization with ZnO nanoparticles has shown positive results in several crops (Alvarez et al. 2019, Jalal et al. 2023a, b; Jalal et al. 2022); however, this new technology is costly, limiting its use in sugarcane. In this context, micronized zinc oxide is an economically viable alternative source of foliar Zn fertilization.

Recent research has demonstrated the efficiency of foliar micronized ZnO application in crops such as potatoes, wheat, maize, and apple, among others (Kumar and Sepehya 2020; White et al. 2012; Whiteet al. 2016; Ivanov et al. 2021). In sugarcane (variety RB 85515) grown in sandy soil, Queiroz et al. (2004) found that micronized oxides in suspension (675 g ha^{-1} and 300 L ha^{-1}) did not affect sugarcane yield, while Brajendra et al. (2016) demonstrated that micronized Zn oxide increased grain and straw yields in rice paddies. However, the efficacy of micronized zinc oxide for foliar Zn nutrition in sugarcane remains unknown.

Our hypothesis is that micronized Zn oxide is an optimal Zn source to improve the zinc status, health, and yield of ratoon sugarcane. As such, the present study assessed the effect of Zn rates, sources, and application times (foliar spraying) on sugarcane yield, CAA, and plant diseases under tropical conditions in São Paulo state, Brazil.

Material and Methods

Experimental Characterization

A study was carried out during the first ratoon cane crop at three sites, namely the cities of Assis (altitude 546 m; Site 1), Ourinhos (altitude 483 m; Site 2), and Serrana (altitude 575 m; Site 3), in São Paulo state, Brazil, in the 2019/2020 growing season. Weather data were obtained from weather stations close to the sites and are shown in Fig. 1. Respective annual rainfall and temperature ranges at sites 1, 2, and 3 were 970, 850, and 1135 mm, and 15.0 to 29.3, 17.2 to 31.3, and 17.6 to 31.2 °C.

A randomized block design was used at all sites, with a 4 × 2 × 3 factorial scheme corresponding to (i) four Zn doses (0; 639; 1039; and 1386 g ha^{-1}), (ii) two Zn sources (Zn sulfate, 20% Zn + 9.8% S; and micronized Zn oxide, 40% Zn + 1% N), and (iii) 3 application times, described as Format A (applied at a plant height of 0.5 m; 100% dose), B (plant height of 1.0 m, 100% dose), and C (split application of a 50% dose at a plant of 0.5 m and 50% at 1.0 m). Each treatment had four replications, totaling 96 experimental plots, with each plot composed of five 10 m-long rows. Micronized Zn oxide (Zintrac®) was developed by YaraVita, and Zn sulfate obtained in ICL.

Prior to the experiments, the soil was sampled up to 0.5 m (in 0.25 m intervals) for chemical and physical characterization, in accordance with van Raij et al. (2001), Table 1. The soil was classified as Oxisol (sites 1 and 2) and Entisol (site 3), according to Soil Survey Staff (2014), with sandy (sites 1 and 3) and clay textures (site 2).

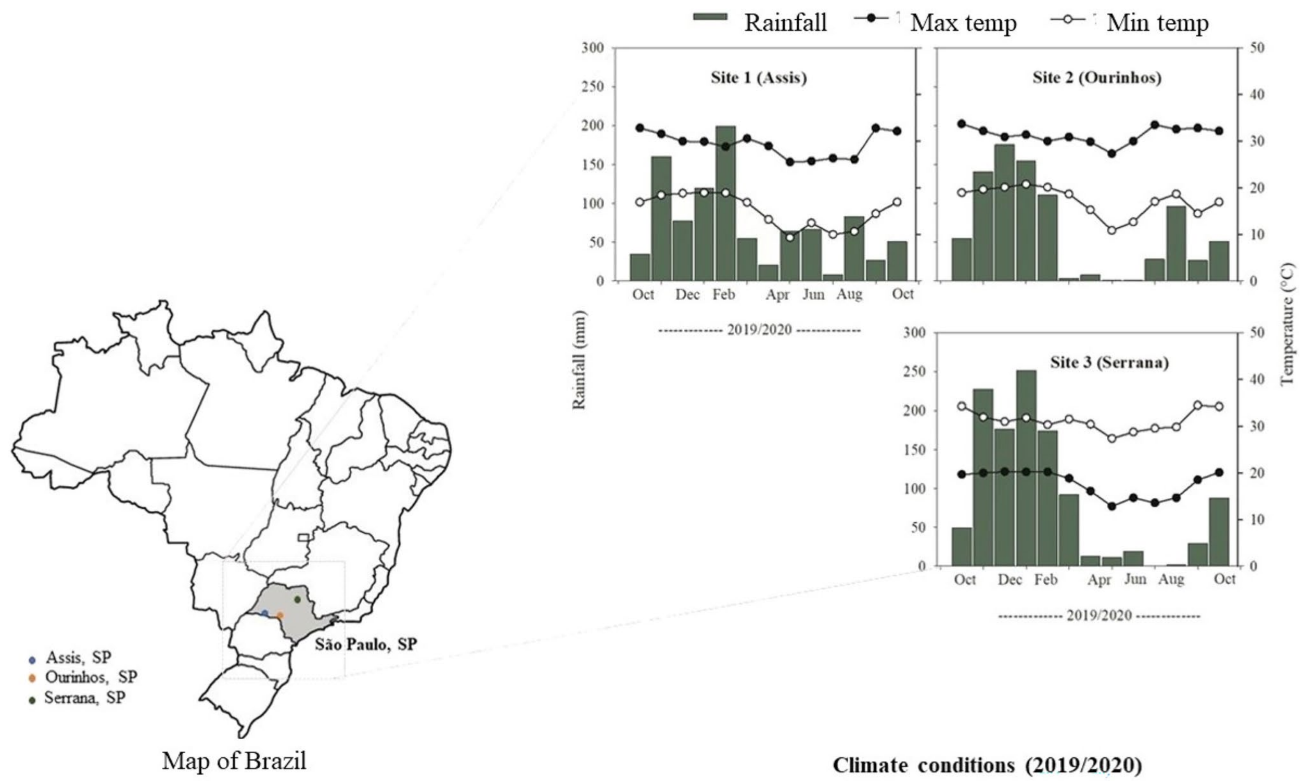


Fig. 1 Rainfall (mm) and temperature (°C) in Assis (altitude 546 m; Site 1), Ourinhos (altitude 483 m; Site 2), and Serrana (altitude 575 m; Site 3), São Paulo, Brazil, in 2019/2020 harvest

Table 1 Soil characterization before the trials were set up in Assis (Site 1), Ourinhos (Site 2), and Serrana (Site 3), Brazil

	Organic matter	pH	P	S	K	Ca	Mg	H + Al	CEC	B	Cu	Fe	Mn	Zn
Soil layers (m)	g kg^{-1}	$CaCl_2$	mg dm^{-3}		mmol$_c$ dm^{-3}					mg dm^{-3}				
Assis, São Paulo (Site 1)														
0.00–0.25	10.0	4.3	9.0	6.0	1.0	15.0	5.0	14.0	35.0	0.4	0.5	20.0	2.9	0.7
0.25–0.50	5.0	4.5	7.0	5.0	0.6	7.0	3.0	23.0	33.6	0.4	0.4	23.0	1.0	0.5
Ourinhos, São Paulo (Site 2)														
0.00–0.25	22.0	4.9	43.0	7.0	2.7	54.0	10.0	47.0	113.7	0.1	0.2	37.0	19.5	1.2
0.25–0.50	21.0	4.9	43.0	8.0	2.8	53.0	10.0	44.0	109.8	0.1	0.2	34.0	23.7	1.1
Serrana, São Paulo (Site 3)														
0.00–0.25	17.0	5.3	135.0	28.0	8.7	40.0	8.0	28.0	84.7	0.3	1.9	154.0	9.0	2.1
0.25–0.50	15.0	5.2	179.0	36.0	6.8	49.0	11.0	26.0	92.8	0.3	1.5	126.0	8.9	1.8

pH in $CaCl_2$ (0.01 mol L^{-1}). Phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg) were determined in Resin; Sulphur (S) was monitored in calcium phosphate solution (0.01 mol L^{-1}), and aluminum (Al) was determined in KCl solution (1 mol L^{-1}). Hydrogen plus aluminum (H + Al). CEC: cation exchange capacity. Boron (B), copper (Cu), iron (Fe), manganese (Mn), and zinc (Zn)

Field Experiment

The experiment was conducted between October (2019) and January (2020) during the first ratoon cane crop, using the RB 867515 (site 1), CTC 9003 (site 2), and RB 855156 varieties (site 3). These varieties are highly susceptible to red rot and a slightly orange rust. All varieties are indicated for regions with low fertility, and sandy soils with water restrictions.

Plant spacing at site 1 was 0.9 × 1.5 (double rows) and 1.5 × 1.5 m at both, sites 1 and 2. Dolomitic lime (1.5 to 2 Mg ha^{-1} per site) was incorporated into the soil 90 days before planting. The soil was fertilized using nitrogen, phosphorus, and potassium (NPK) formulations 06-30-17 (site 1), 05-25-20 + 0.85% B and 10-00-30 + 1% B (site 2), and 30-00-00 (Site 3) at respective doses of 0.32; 0.6 and 0.3; and 0.3 Mg ha^{-1}. The following fertilizer formulations used were: NP 25-00-25 (0.5 Mg ha^{-1}) and NPK 17-08-20 (0.6 Mg ha^{-1}) applied to the soil at site 1; NPK 17-06-21 + 0.3% B and NPK 16-06-20 + 0.20% B at 0.6 Mg ha^{-1} each for site 2; and 0.3 Mg ha^{-1} of N 30-00-00 (corresponding to nitrogen) + 0.5% of Zn at site 3. The formulations were selected to ensure adequate nitrogen, phosphorus, and potassium content. Zn foliar spraying was performed between October 2019 and January 2020 and December/2019 and January/2020 for the first and second applications, respectively.

Sugarcane Measurements

Stalk yield was assessed at all the sites by mechanically harvesting the three center rows of each plot 12 months after the last harvest. The stalks from each plot were weighed in an automated truck (equipped with a cell loading system) to estimate sugarcane yield (Mg ha^{-1}, fresh basis). Ten stalks per plot were randomly collected to determine theoretical recoverable sugar (TRS; kg sugar Mg^{-1} of stalk). Theoretical recoverable sugar was not influenced by Zn management at any of the sites studied, with an average of 126.6–182.7 kg sugar Mg $stalk^{-1}$, and results are presented in Supplementary Table 1S.

Leaves were collected (15 units + first fully expanded leaf) six months after the final Zn application to monitor leaf Zn content. At the same time, two leaves per plot were collected, immediately frozen in liquid nitrogen, and stored at – 80 °C until analysis for CAA quantification. Enzyme activity was analyzed via the colorimetric method, using veronal buffer (02 M; pH 8.0-NaOH), peptone (0.018%), and bromothymol blue (rate: 0.12 mL; 0.2% in ethanol). The CAA results were expressed in units of enzyme per gram of fresh matter.

The incidence of orange rust (*Puccinia kuenhii*) and red rot associated with sugarcane borer (*Colletotrichum falcatum Went*) was monitored, with all sites exhibiting a low incidence of both diseases before the experiments. Five leaves were collected (leaves + 3) from each experimental unit to assess the visual symptoms of orange rust according to Klosowski et al. (2013), with severity classified from 0 to 50%. The percentage of stalk red-rot infestation was determined based on the method described by Pannuti et al. (2013).

Statistical Analysis

Assumptions of normality and homogeneity of variance were evaluated using the Shapiro–Wilk and Oneill–Mathew tests ($P \leq 0.05$), respectively. Outliers identified by the Grubbs test were removed. Data for each year and site were submitted to ANOVA based on the F-test and, when significant ($P \leq 0.1$), the Zn sources and application times were analyzed using the LSD test ($P \leq 0.1$) and Zn doses by regression testing ($P \leq 0.1$). Double and triple interactions between treatments were also evaluated to explain the dataset ($P \leq 0.1$).

The correlation between sugarcane yield, leaf Zn content, CAA, and diseases were analyzed for each site via Pearson's correlation ($P \leq 0.05$). The results were presented in a cycle graph (adapted), divided into two layers: (i) root layer, containing Zn application data; and (ii) layer 1, data on sugarcane yield, leaf Zn content, CAA, and diseases. The k-apices were represented by the average calculated for each site. Statistical analyses were performed in R (version 4.0.0; R Foundation for Statistical Computing) and Python software (version 3.8.3; Python Software Foundation) and the results were presented in graphs using Sigmaplot (version 11.0; Systat Software, Inc.).

Results

Sugarcane Yield

Sugarcane yield ranged from 61 to 136 Mg ha^{-1}, with an overall average of 96 Mg ha^{-1}. The highest yield was recorded at site 2 (125 Mg ha^{-1}), followed by sites 3 (91 Mg ha^{-1}) and 1 (72 Mg ha^{-1}). Zn did not affect sugarcane yield at Site 1, with an overall average from 61 to 86 Mg ha^{-1} (Table 2), while at site 3, Zn doses exhibited a linear response on sugarcane yield (R^2: 79%), which increased by 9% yield from 85.5 (control) to 93.6 Mg ha^{-1} (dose of 1386 g ha^{-1}), Fig. 2.

At site 2, Zn presented a triple interaction for (i) Zn doses, (ii) sources, (iii), and timing on sugarcane yield (Table 2). In all treatments, Zn doses fitted quadratic responses with optimal doses at 540; 670; and 718 g ha^{-1} for micronized Zn oxide applied in format A (R^2: 91%), B (R^2: 35%), and C (R^2: 99%), and at 770; 681; and 710 g ha^{-1} for $ZnSO_4$ via format A (R2: 91%), B (R2: 75%), and C (R2: 94%), respectively (Fig. 2). Among controls, sugarcane yield was lowest at Site 1 (71.6 Mg ha^{-1}), followed by sites 3 (85.3 Mg ha^{-1}) and 2 (123.3 Mg ha^{-1}), Table 2.

Leaf Zn Status

Leaf Zn increased linearly with applications of Zn rates at sites 1 (micronized Zn oxide: R2: 87%; and $ZnSO_4$: R2: 99%), 2 (application at 0.5 + 1.0 m), and 3 (overall average, R2: 67%), from 12 to 15 g kg^{-1} (Fig. 3). However, at site 2, Zn applied at 1.0 m showed a quadratic response (R2:

Table 2 Sugarcane yield (Mg ha^{-1}) with the application of Zn doses (0; 639; 1039; or 1386 g ha^{-1}) and sources (Zn sulfate or micronized Zn oxide), in 3 application time (application on the plant with height of 0.5 (100%; A Format), 1.0 (100%; B Format), or 0.5 (50%) and 1.0 m (50%) (C Format) in Assis (Site 1), Ourinhos (Site 2), and Serrana (Site 3), Brazil

Zn rates (g ha^{-1})	Site 1			Site 2			Site 3		
	Sugarcane yield (Mg ha^{-1})								
	A Format 0.5 m	B Format 1.0 m	C Format 0.5 + 1.0 m	A Format 0.5 m	B Format 1.0 m	C Format 0.5 + 1.0 m	A Format 0.5 m	B Format 1.0 m	C Formt 0.5 + 1.0 m
Micronized Zn oxide									
0	71.6 ± 2.0	71.6 ± 1.1	71.6 ± 2.0	123.3 ± 1.3	123.3 ± 1.3	123.3 ± 1.3	85.3 ± 4.4	85.3 ± 4.4	85.3 ± 4.4
633	72.0 ± 0.5	69.2 ± 1.5	72.0 ± 1.3	119.8 ± 0.3	132.0 ± 1.1	128.0 ± 2.2	89.8 ± 3.4	92.4 ± 10.7	87.9 ± 4.5
1039	75.6 ± 1.1	67.7 ± 3.3	71.6 ± 2.7	127.5 ± 3.1	120.5 ± 1.6	127.0 ± 0.4	95.8 ± 1.9	89.2 ± 1.5	90.9 ± 2.9
1386	68.8 ± 1.6	75.0 ± 2.3	73.8 ± 4.8	131.8 ± 3.1	123.0 ± 1.7	123.0 ± 2.0	98.5 ± 2.9	96.9 ± 1.8	81.3 ± 6.2
Zn sufate									
0	71.6 ± 2.0	71.6 ± 2.0	71.6 ± 2.0	123.3 ± 1.3	123.3 ± 1.3	123.3 ± 1.3	85.3 ± 4.4	85.3 ± 4.4	85.3 ± 4.4
633	74.8 ± 4.8	70.3 ± 1.9	73.7 ± 1.9	117.5 ± 1.8	128.3 ± 0.9	132.0 ± 0.4	94.3 ± 8.7	99.7 ± 10.3	93.2 ± 10.1
1039	68.1 ± 2.8	73.3 ± 1.7	72.9 ± 1.1	122.5 ± 2.7	124.5 ± 1.9	133.0 ± 0.8	85.7 ± 6.3	96.2 ± 4.3	99.2 ± 5.2
1386	71.6 ± 2.6	72.0 ± 2.1	76.3 ± 0.8	126.3 ± 1.8	123.5 ± 0.3	124.3 ± 1.3	98.9 ± 5.5	100.0 ± 5.1	85.9 ± 2.6
Anova (P values)									
	Site 1			Site 2			Site 3		
Zn_{rate}	0.71			< 0.10			< 0.10		
Zn_{source}	0.50			0.91			0.21		
Zn_{timing}	0.34			< 0.10			0.18		
$Zn_{rate*source}$	0.85			0.36			0.78		
$Zn_{rate*timing}$	0.39			< 0.10			0.10		
$Zn_{source*timing}$	0.70			< 0.10			0.40		
$Zn_{rate*source*timing}$	0.13			< 0.10			0.83		

Error bars represent a 95% confidence level. Averages were compared by the LSD (Zn sources and timing) and the Regression test (Zn rates) using an of $P \leq 0.1$

98%), with the highest leaf Zn content obtained at a dose of 895 g ha^{-1} (Fig. 3).

The C format promoted higher leaf Zn status in site 1, while in site 2 this performance was observed at the rate of 1386 g ha^{-1}, indicating that there was a variation in Zn status with formats of Zn applications (Fig. 3). All averages of leaf Zn in the treatments can be found in Supplementary Table 2S.

Carbonic Anhydrase Activity

Zn doses linearly increased CAA, with triple interaction at sites 1 and 2, and an isolated effect at site 3 (Fig. 4). Linear models explained more than 60% of the association between Zn doses and CAA at all the sites indicating a clear response of Zn doses to promote the the activity of CAA. In site 1, there was an exception requesting a quadratic model to explain the Zn doses in CAA (R2: 99%) with the optimal dose at 760 g ha^{-1} for $ZnSO_4$ applied at 1.0 m (Fig. 4).

With respect to controls at each site, CAA was lower at site 1 (224.8 UE g^{-1}), followed by sites 2 (249.8 UE g^{-1}) and Site 3 (397.3 UE g^{-1}). CAA ranged from 224.8 to 742.1 UE g^{-1}, representing a 70% increase with foliar application of Zn. All averages of leaf Zn in the treatments can be found in Supplementary Table 3S.

Plant Diseases

In sites 1 and 2, orange rust severity declined with Zn application by an average of 3.0 to 0.1% with a linear reduction represented by an R^2 of 54% (Site 1) and 40% (Site 2) (Fig. 5). While at site 3, there was a double interaction between Zn doses and application times: Zn doses displayed a linear response at 0.5 and 1.0 m with an R^2 higher than 83%. However, split Zn doses exhibited a quadratic response (R2: 96%), with the smallest decrease at 1250 g ha^{-1} (Fig. 5).

At Site 2, (isolated) micronized Zn oxide application at 0.5 m produced the least severe orange rust. While $ZnSO_4$ promoted a higher reduction in orange rust severity compared with ZnO (Fig. 5). All averages of severe orange rust in the treatments can be found in Supplementary Table 4S.

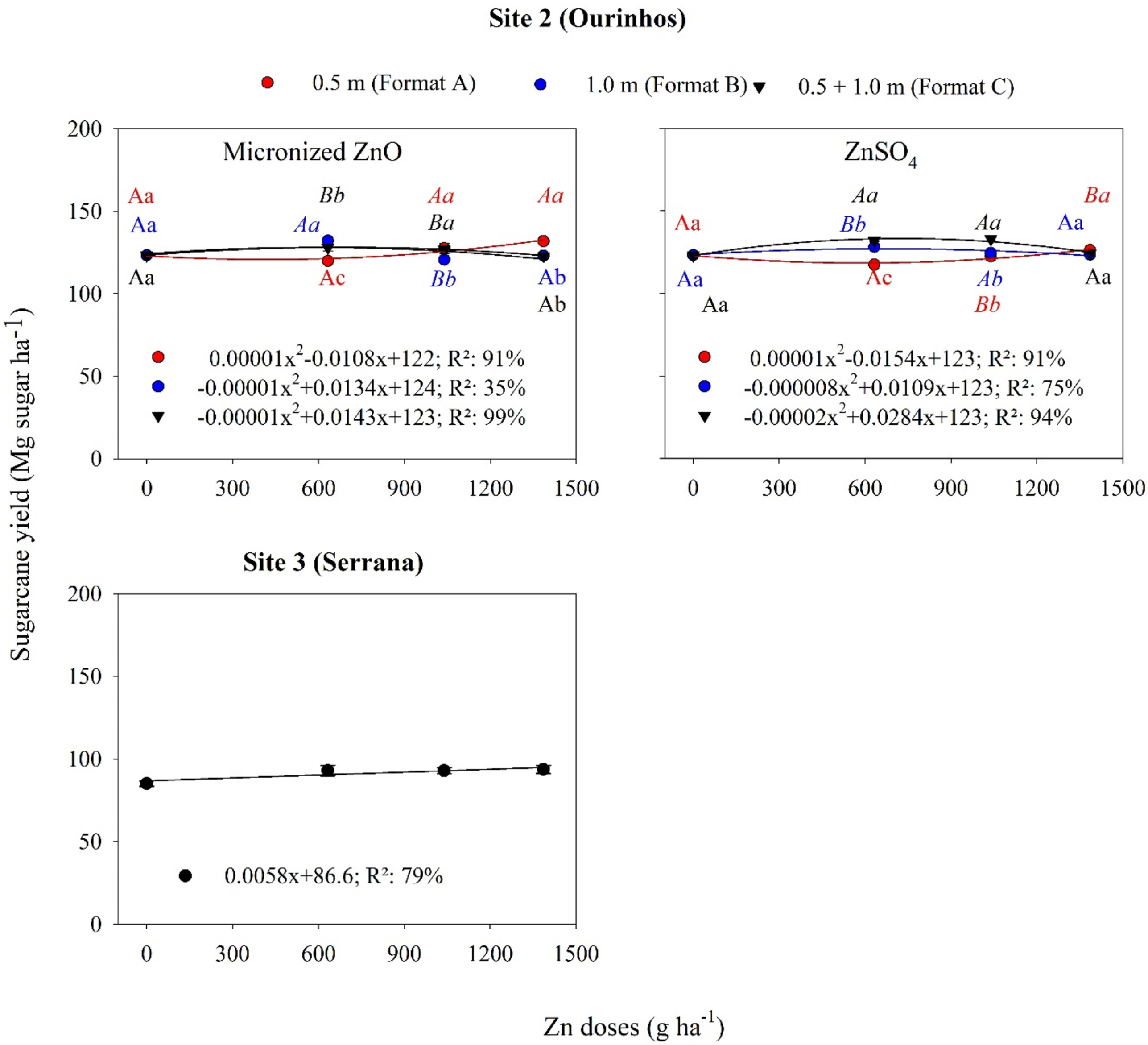


Fig. 2 Yields of sugarcane (Mg ha^{-1}) and sugar (Mg sugar ha^{-1}) with the application of Zn doses (0; 639; 1039; or 1386 g ha^{-1}) and sources (Zn sulphate or micronized Zn oxide), in 3 application times (application on the plant with height of 0.5 (100%; A Formatat), 1.0 (100%; B Formatat), or 0.5 (50%) and 1.0 m (50%) (C Formatat)) in Assis (Site 1), Ourinhos (Site 2), and Serrana (Site 3), Brazil. Error bars represent a 95% confidence level. Means were compared by the LSD (Zn sources and application times) and the Regression test (Zn doses) using a $P \leq 0.1$. Uppercase (Zn sources) and lowercase letters (application times) when different represent the difference between them

Red rot was not influenced by Zn management at any of the sites, with average internode and orifice damage ranging from 1.2 to 20.0% (Supplementary Table 5S).

Cycle Graph

In the cycle graph, the Zn inputs increased sugarcane (r: 0.11; $P \leq 0.05$) indicating a significant rise in field profitability (up to 2%). Moreover, Zn also increased leaf Zn content (r: 0.36; $P \leq 0.05$) and CAA (r: 0.55; $P \leq 0.05$), Fig. 6.

There was a decline in the incidence of orange rust (down to 13%) with a significant correlation with Zn application (r: – 0.55; $P \leq 0.05$), indicating that the Zn promoted the health of plants. However, this effect was not observed on red rot which was not influenced by Zn applications (r: – 0.06; $P \geq 0.05$), Fig. 6.

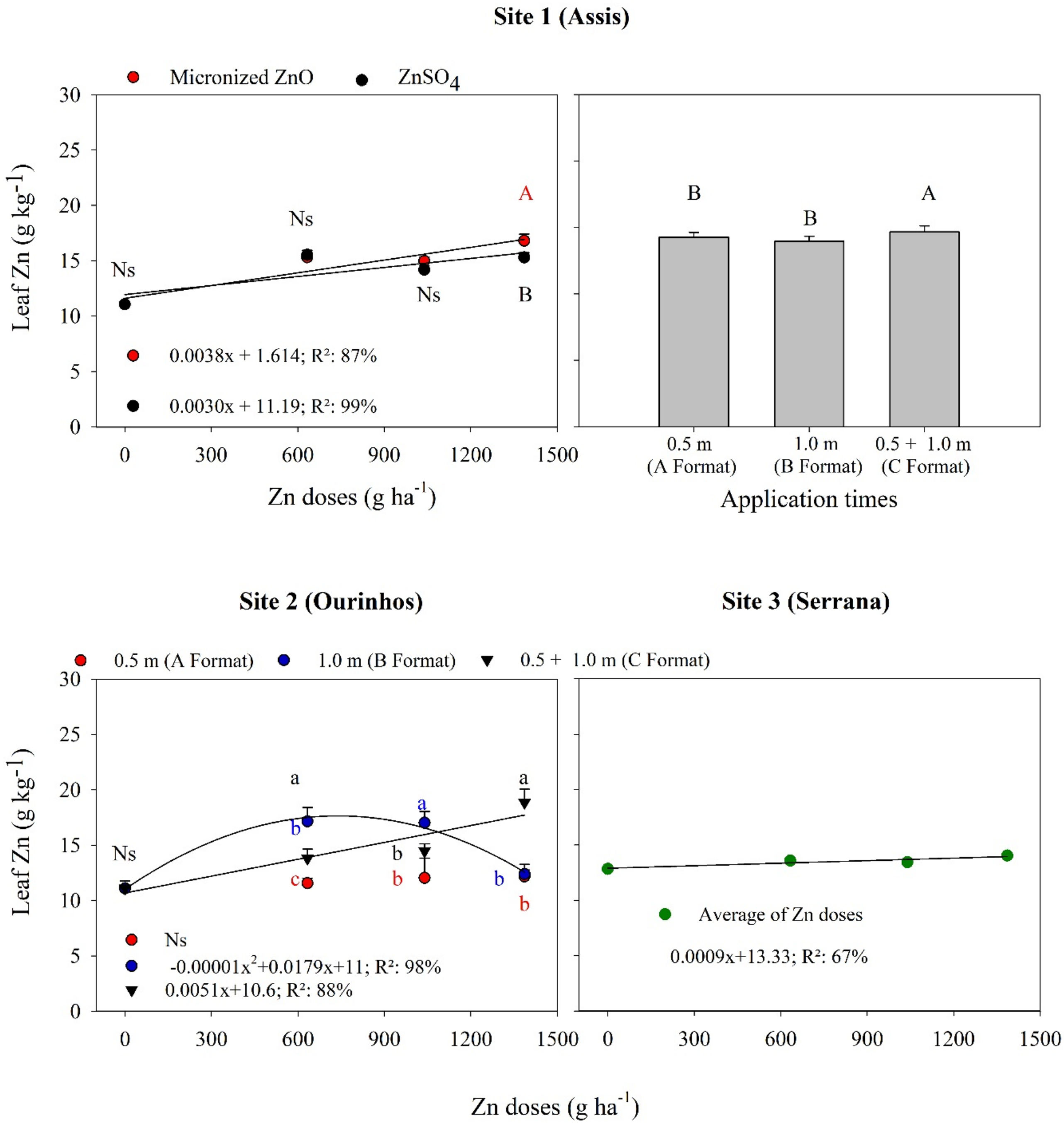
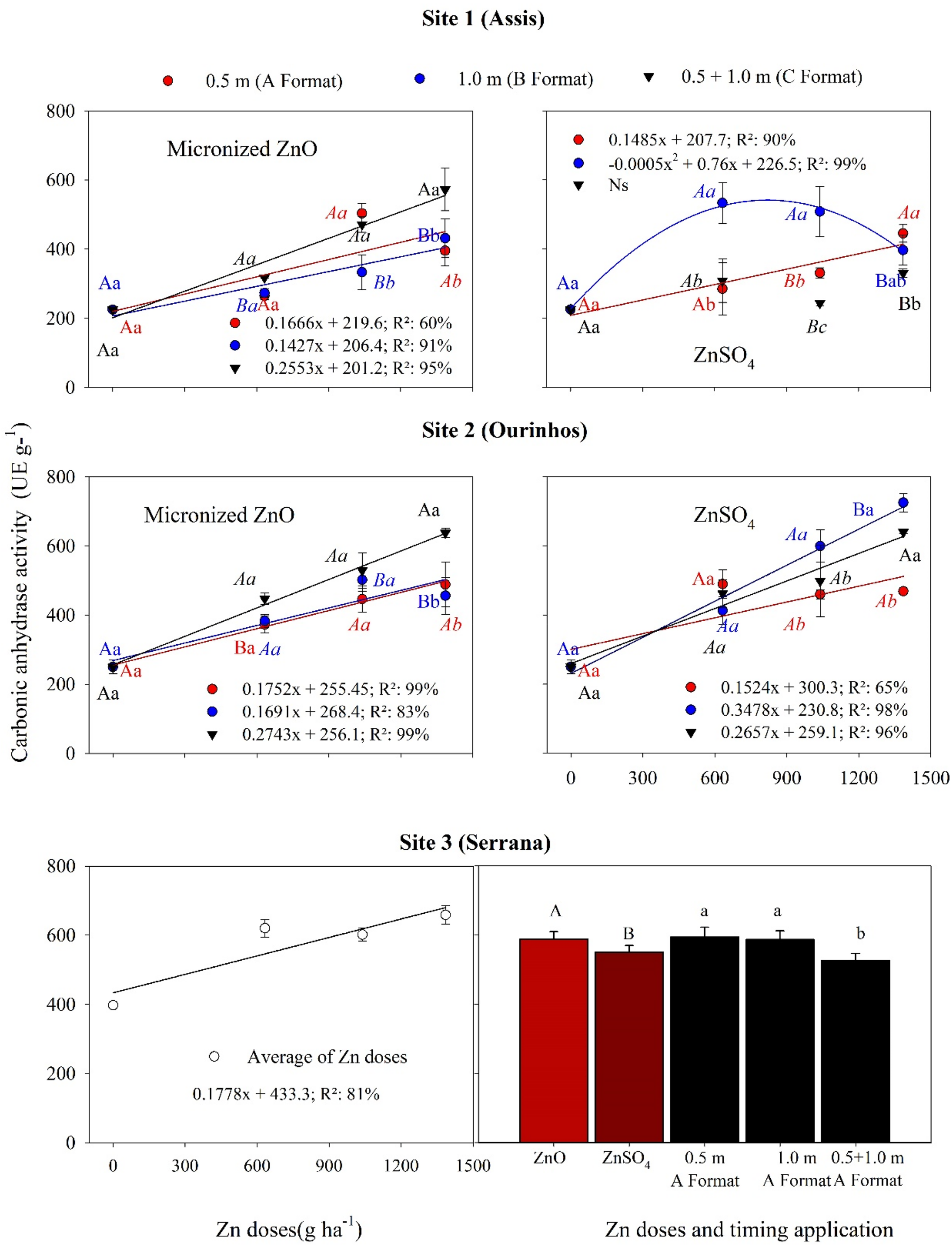


Fig. 3 Leaf Zn (g kg^{-1}) with the application of Zn doses (0; 639; 1039; or 1386 g ha^{-1}) and sources (Zn sulphate or Micronized Zn oxide), in 3 application times (application on the plant with height of 0.5 (100%; A Format), 1.0 (100%; B Format), or 0.5 (50%) and 1.0 m (50%) (C Format)) in Assis (Site 1), Ourinhos (Site 2), and Serrana (Site 3), Brazil. Error bars represent a 95% confidence level. Means were compared by the LSD (Zn sources and application times) and the Regression test (Zn doses) using a P ≤ 0.1. Uppercase (Zn sources) and lowercase letters (application times) when different represent the difference between them

Discussion

The foliar application of Zn sulfate or micronized zinc oxide increased sugarcane yield indicating that Zn foliar spraying is an adequate nutritional management option for sugarcane. The positive effect of Zn sulfate as a nutrient source due to the greater water solubility of Zn in $ZnSO_4$ is widely reported in the literature (Doolette et al. 2018). However, studies involving foliar application of micronized Zn oxide remain scarce, especially in sugarcane, with doubts



Site 1 (Assis)
0.5 m (A Format)
1.0 m (B Format)
0.5 + 1.0 m (C Format)
Micronized ZnO
0.1666x + 219.6; R²: 60%
0.1427x + 206.4; R²: 91%
0.2553x + 201.2; R²: 95%
ZnSO4
0.1485x + 207.7; R²: 90%
-0.0005x2 + 0.76x + 226.5; R²: 99%
Ns
Site 2 (Ourinhos)
Carbonic anhydrase activity (UE g-1)
Micronized ZnO
0.1752x + 255.45; R²: 99%
0.1691x + 268.4; R²: 83%
0.2743x + 256.1; R²: 99%
ZnSO4
0.1524x + 300.3; R²: 65%
0.3478x + 230.8; R²: 98%
0.2657x + 259.1; R²: 96%
Site 3 (Serrana)
Average of Zn doses
0.1778x + 433.3; R²: 81%
ZnO
ZnSO4
0.5 m A Format
1.0 m A Format
0.5+1.0 m A Format
Zn doses(g ha-1)
Zn doses and timing application

◂**Fig. 4** Carbonic anhydrase activity (UE g^{-1}) with the application of Zn doses (0; 639; 1039; or 1386 g ha^{-1}) and sources (Zn sulphate or Micronized Zn oxide), in 3 application times (application on the plant with height of 0.5 (100%; A Format), 1.0 (100%; B Format), or 0.5 (50%) and 1.0 m (50%) (C Format)) in Assis (Site 1), Ourinhos (Site 2), and Serrana (Site 3), Brazil. Error bars represent a 95% confidence level. Means were compared by the LSD (Zn sources and application times) and the Regression test (Zn doses) using a $P \leq 0.1$. Uppercase (Zn sources) and lowercase letters (application times) when different represent the difference between them

about its effectiveness. Queiroz et al. (2004) reported that 675 g ha^{-1} of Zn (foliar spraying of suspended micronized oxides) in sandy soil did not influence leaf contents of macro and micronutrients, stalk yield, or the technological attributes of sugarcane. Mahdieh et al. (2018) compared Zn foliar spraying in different crops (pepper, soybean, and bean) and found that micronized Zn oxide was as efficient as $ZnSO_4$. Rossi et al. (2019) demonstrated the inferior performance of ZnO as a nanoparticle due to the slow release of Zn^{2+} from ZnO. Previous studies suggest that ZnO dissolves relatively slowly in water, with only about 2% of Zn dissolving from ZnO in 24 h (Reed et al. 2012). However, controlled release micronized zinc oxide nanoparticles have been adhered to sugarcane leaf surfaces to provide a long-term source of Zn (Rossi et al. 2019). This performance of micronized zinc oxide explains the similar result obtained in our study for Zn sulfate.

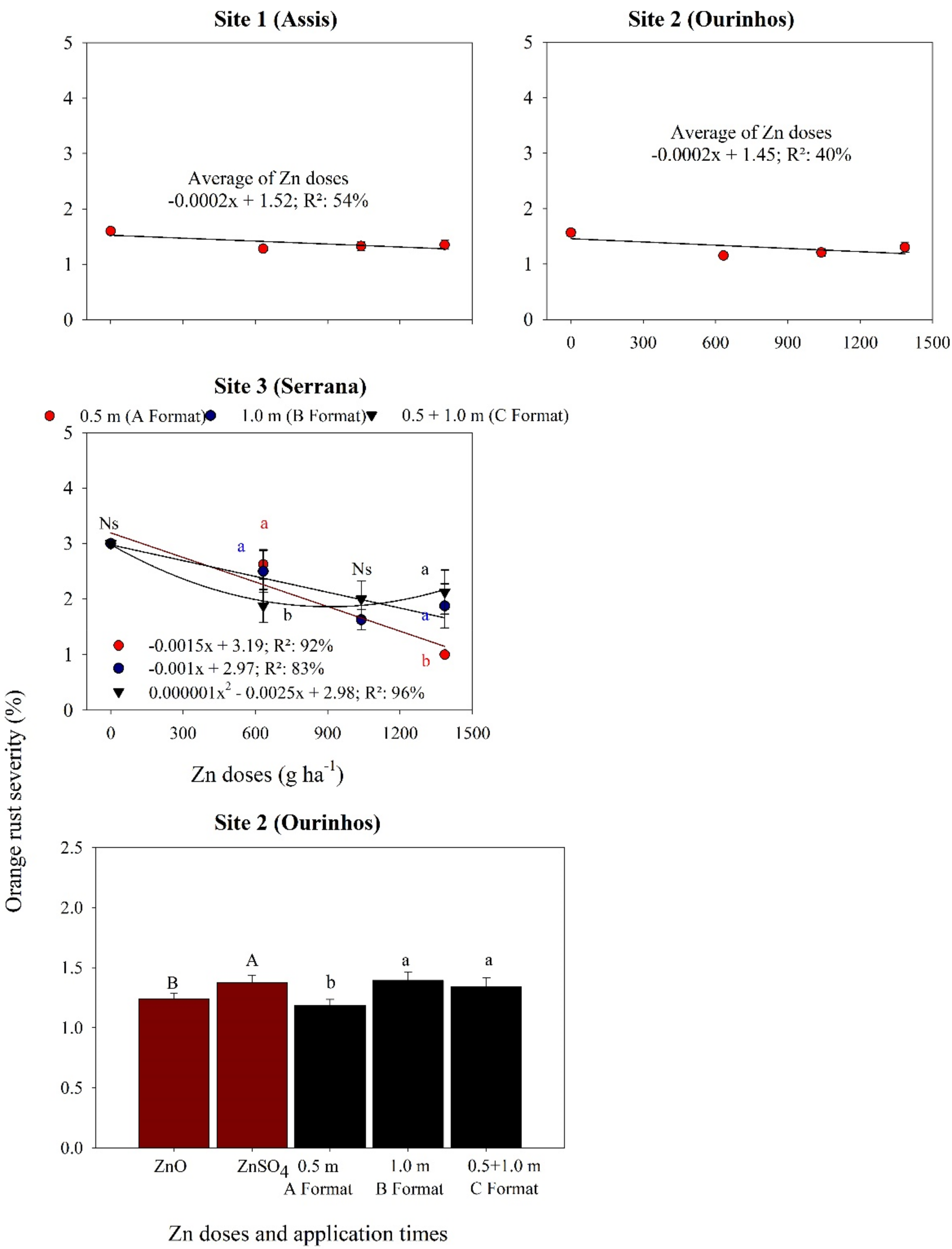
Based on the responses observed at each site, we found that sugarcane yield increased at sites 2 (Ourinhos) and 3 (Serrana), where soil Zn levels were high (above 1.0 mg dm^{-3}), according to Cantarella et al. (2022). In Assis (site 1), soil Zn content was low (< 0.5 mg dm^{-3}) with a sandy texture, and leaf Zn content and CAA increased. Mellis et al. (2016) evaluated the effect of 10 kg ha^{-1} of Zn ($ZnSO_4$) as a base dressing at 11 locations with sandy soils and observed an average yield increase of 16% in relation to the control treatment. Studies conducted in alkaline soils in India, Pakistan, and the United States have shown that a combination of soil and foliar Zn application is the best strategy to improve sugarcane yield and industrial quality. Wang et al. (2005), tested soil (4.4–33.8 kg ha^{-1}) and leaf application (1.3 kg ha^{-1}; $ZnSO_4$) of different Zn doses in acidic and limestone soils in the United States and observed increased sugarcane production at soil-applied doses of 4.4 and 8.9 kg ha^{-1}. Jamro et al. (2002) found that foliar Zn (1.5 kg ha^{-1}; $ZnSO_4$) increased internode size, plant height, and tillering in Pakistan.

The Zn requirement for sugarcane production is 0.59 kg 100 $tonnes^{-1}$ of stalks, with a relative exportation of 0.35 kg 100 $tonnes^{-1}$ for stalks and 0.24 kg 100 $tonnes^{-1}$ for leaves. In Brazil, Zn recommendations for sugarcane range from 2 to 10 kg ha^{-1} of Zn as base dressing, using sulfate or oxy sulfate (Cantarella et al. 2022). Results reported in the literature demonstrate that foliar fertilization may be more effective than its solid counterpart due to low fertilizer losses (Bindraban et al. 2015; Niu et al. 2021; Silva et al. 2022). Based on our findings and others reported in the literature, it can be inferred that foliar Zn application optimizes sugarcane yield in soil with Zn content higher than 0.5 mg dm^{-3}.

Leaf Zn content increased linearly at all the sites studied, with an average of 18 mg kg^{-1}, above the lower limit of the range considered adequate (15–50 mg kg^{-1} of Zn; Cantarella et al. 2022). In general, the Zn doses studied were associated with high sugarcane yield and leaf Zn contents at all the sites where Zn was applied. This demonstrates that micronized Zn oxide and Zn sulfate had a similar effect in terms of increasing leaf Zn content. Foliar diagnosis via chemical digestion is widely used in crop nutritional assessment. However, the effectiveness of this technology, especially for evaluating micronutrient fertilization efficiency, has been questioned by the scientific community. In the case of sugarcane, some authors report little sensitivity to nutritional variations, as well as differences in yield, with no difference in leaf content. This is due to the dilution effect and the difficulty in determining the optimal harvesting time. As such, the use of biological indicators has been widely recommended in recent years, particularly in scientific experiments.

Carbonic anhydrase activity is presented as an optimal biological indicator of zinc status in sugarcane. An increase in CAA from 224.8 to 742.1 UE g^{-1} (70%) was observed in leaf Zn content, with the highest CAA recorded at a Zn dose of 1386 g ha^{-1}. Our results are corroborated by Singh et al. (2019b). Carbonic anhydrase is a metalloenzyme involved in several biological processes, such as pH regulation, photosynthetic CO2 fixation, ion exchange, respiration, and stomatal closure (Bhat et al. 2017; Escudero-Almanza et al. 2012). The enzyme also contributes directly to the photosynthetic capacity of sugarcane (C4 plants). Zn-deficient plants exhibit low CAA and, consequently, a decline in the photosynthetic rate (Wei-Hong et al. 2014). In the present study, CAA increased with Zn application and linear models explained more than 60% of the relationship between Zn doses and CAA at all the sites investigated. Singh et al. (2019b) also found that both soil and foliar Zn applications increased CAA and plant Zn concentration in six wheat genotypes. In the present study, we tested three sugarcane varieties (RB 867515; CTC 9003; and RB 855156) and three soil types/sites (two sandy textured Oxisols and one clay textured Entisol), and the results demonstrated that anhydrase was an excellent indicator of Zn efficiency in sugarcane under all the conditions tested.

Additionally, there was a higher correlation between Zn inputs and CAA (0.55; $P \leq 0.05$) when compared to Zn inputs and leaf Zn content (r: 0.36; $P \leq 0.05$), indicating that CAA was more sensitive in terms of detecting Zn changes in plants. Our study also demonstrated that foliar



Site 1 (Assis)
Average of Zn doses
-0.0002x + 1.52; R²: 54%
Site 2 (Ourinhos)
Average of Zn doses
-0.0002x + 1.45; R²: 40%
Site 3 (Serrana)
0.5 m (A Format)
1.0 m (B Format)
0.5 + 1.0 m (C Format)
Ns
a
a
Ns
a
b
a
b
-0.0015x + 3.19; R²: 92%
-0.001x + 2.97; R²: 83%
0.000001x2 - 0.0025x + 2.98; R²: 96%
Zn doses (g ha-1)
Orange rust severity (%)
Site 2 (Ourinhos)
B
A
b
a
a
ZnO
ZnSO4
0.5 m A Format
1.0 m B Format
0.5+1.0 m C Format
Zn doses and application times

◂**Fig. 5** Severity levels of Orange rust (*Puccinia kuenhii*) in sugarcane with the application of Zn doses (0; 639; 1039; or 1386 g ha^{-1}) and sources (Zn sulphate or Micronized Zn oxide), in 3 application times (application on the plant with height of 0.5 (100%; A Format), 1.0 (100%; B Format), or 0.5 (50%) and 1.0 m (50%) (C Format)) in Assis (Site 1), Ourinhos (Site 2), and Serrana (Site 3), Brazil. Error bars represent a 95% confidence level. Means were compared by the LSD (Zn sources and application times) and the Regression test (Zn doses) using a $P \leq 0.1$. Uppercase (Zn sources) and lowercase letters (application times) when different represent the difference between them

Zn application can be used to increase sugarcane resistance to orange rust. This is an important outcome given the yield losses caused by orange rust (r: −0.14; $P \leq 0.05$). Interestingly, although all the varieties studied are genetically more resistant to orange rust, there was a significant reduction in orange rust incidence. It was expected that Zn would also influence red rot incidence, which reduced sugarcane yield (r: −0.30; $P \leq 0.05$); however, there was no clear evidence of this in the present study. This result is highly significant and demonstrates that Zn not only has a well-known effect on sugarcane metabolism (already known), but also on plant resistance. Some authors have associated the disease incidence (i.e., orange rust and red-rot) with Zn deficiency. Singh et al. (2014) showed that applying sulfur and Zn alone and in combination improved lentil yield (*Lens culunaris* L.) can mitigate the effects of drought stress due to the increase in endogenous hormones (auxins, gibberellins, and melatonin) and minimize the incidence of powdery mildew (*Erysiphe trifolii*), which improves aquaporin and antioxidant activity (Hassan et al. 2020). Thus, balanced Zn management played a significant role in determining the degree of plant resistance to biotic stress, especially diseases.

Conclusion

Foliar application of Zn sulfate or micronized zinc oxide increased sugarcane yield. Zn foliar spraying of the first ratoon crop increased carbonic anhydrase activity and leaf Zn content in sugarcane, with higher yields obtained by splitting applications into two 815 g ha^{-1} doses. Carbonic anhydrase activity is presented as an optimal biological indicator of zinc status in sugarcane. Zn doses in sugarcane reduce the severity of orange rust, improving sugarcane health and proving to be an optimal agronomical option for disease management in sugarcane fields. Foliar spraying of Zn is efficient in the first ratoon cane crop in Brazilian tropical soils only in soil Zn levels of around 1.0 mg dm^{-3}. Based on the results obtained, it can be concluded that soil fertilization with Zn sulfate or micronized zinc oxide improves the Zn status of sugarcane plants, thereby contributing to reducing Zn deficiency and diseases in plants and humans.

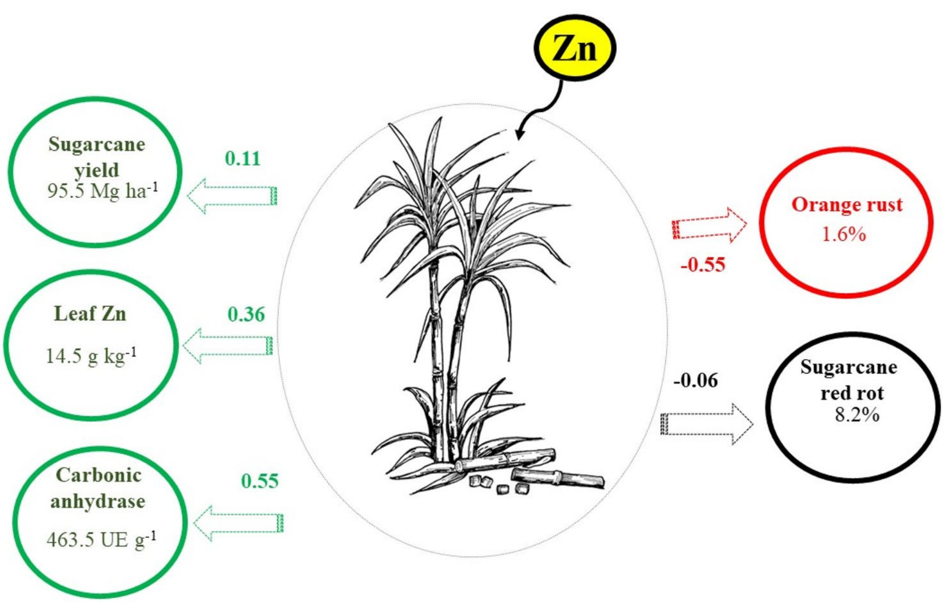


Fig. 6 Cycle graph was divided into two layers: (i) root layer with data of Zn inputs; (ii) layer 1 with yields of sugarcane (Mg ha^{-1}), sugar (Mg sugar ha^{-1}), Leaf Zn (g kg^{-1}), Carbonic anhydrase activity (UE g^{-1}), severity levels of orange rust (%), and sugarcane red rot (%). The k-apex represents the correlation between Zn inputs and sugarcane measurements demonstrated by green (positive), red (negative), and black (no correlation). Variables were linearly correlated by the Pearson test ($P \leq 0.05$)

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Declarations

Conflict of interest The authors declare no conflict of interest.

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