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OSVALDO ARAUJO JUNIOR

NUTRIENTS AND AMINO ACIDS FOLIAR APPLICATION TO ALLEVIATE SUGARCANE DROUGHT STRESS

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TÍTULO DA TESE: NUTRIENTS AND AMINO ACIDS FOLIAR APPLICATION TO ALLEVIATE SUGARCANE DROUGHT STRESS

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To all my family,

All people who believe in the research,

All those who somehow supported me, I dedicate.

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ABSTRACT

Sugarcane is commonly exposed to extreme weather patterns, such as drought, which is the main abiotic factor responsible for limiting productivity in the crop. The effects of drought have as a consequence the production of reactive oxygen species, due to cellular oxidative stress, severely affecting plant metabolism. Foliar applications of nutritional complexes combined with amino acids can be used as an alternative to attenuate the negative responses of plants caused by drought conditions. However, all its effects such as foliar application in sugarcane fields, exposed to water stress, which allow increases in plant metabolism and productivity gains of stalks and sugar, and extraction of a better quality broth, have not yet been explored. The study aimed to evaluate the effectiveness of foliar application of two fertilizers of different nutritional values, associated with different amino acids in the composition, in June, under the influence of the driest period of the year, in ratoon cane, in four different regions. The treatments consisted of: control (without application of the complex) and with application of the drought protection complex (ASPC) in the cities of Pradópolis-SP and Motuca-SP. In the regions of Pereira Barreto and Nova Independência, the treatments were control and with application of the protective foliar complex (PFC). The hypothesis of Chapter 1 and Chapter 2, that the application of two nutritional complexes with different amino acids, in 4 different regions, could mitigate water stress in the sugarcane crop, and increase the production of antioxidant enzymes, accumulation of sucrose, enabling gains in stalk and sugar productivity and higher quality of the juice extracted, was confirmed in this work. The strategy of applying nutritional complexes associated with certain amino acids in the composition proved to be effective in lieving water stress and, at the same time, improving crop development, stalk yield, sugar production and biomass.

Keywords: *Saccharum spp.*; oxigen reactive species; antioxidant metabolism; drought stress; stress management strategies.

RESUMO

A cana-de-açúcar é comumente exposta às condições extremas de padrões climáticos, como a seca, que é o principal fator abiótico responsável por limitar a produtividade na cultura. Os efeitos da seca têm como consequência a produção de espécies reativas de oxigênio, devido ao estresse oxidativo celular, afetando severamente o metabolismo vegetal. Aplicações foliares de complexos nutricionais aliados à aminoácidos, podem ser empregados como alternativa para atenuar as respostas negativas das plantas causadas pelas condições de seca. No entanto, ainda não foram explorados todos seus efeitos como aplicação foliar em canaviais, expostos ao estresse hídrico, que possibilitam aumentos no metabolismo da planta e ganhos de produtividade de colmos e açúcar, e extração de um caldo de melhor qualidade. O estudo teve como objetivo avaliar a eficácia da aplicação foliar de dois fertilizantes de diferentes valores nutricionais, associados à diferentes aminoácidos na composição, no mês de junho, sob a influência do período mais seco do ano, em cana soca, em quatro regiões distintas. Os tratamentos consistiram de: controle (sem aplicação do complexo) e com aplicação do complexo protetor contra à seca (ASPC) nos municípios de Pradópolis-SP e Motuca-SP. Nas regiões de Pereira Barreto e Nova Independência, os tratamentos foram controle e com aplicação do complexo foliar protetor (PFC). A hipótese do Capítulo 1 e do Capítulo 2, de que a aplicação de dois complexos nutricionais com aminoácidos distintos, em 4 regiões diferentes, poderia mitigar o estresse hídrico na cultura da cana-de-açúcar, e elevar a produção de enzimas antioxidante, acúmulo de sacarose, possibilitando ganhos em produtividade de colmo e açúcar e maior qualidade do caldo extraído, foi confirmada neste trabalho. A estratégia da aplicação de complexos nutricionais associados à certos aminoácidos na composição, mostrou ser efetiva para aliviar o estresse hídrico e, ao mesmo tempo, melhorar o desenvolvimento da cultura, o rendimento do colmo, a produção de açúcar e a biomassa.

Palavras-chave: *Saccharum spp.*; espécies reativas de oxigênio; metabolismo antioxidante; estresse por seca; estratégias de gerenciamento de estresse.

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SUMMARY

GENERAL INTRODUCTION

Brazil is the world's largest producer of sugarcane, responsible for the production of about 630.7 million tons estimated in the 2020/2021 harvest, and 8.4 million hectares, corresponding to an average productivity of stalks of 75.02 Mg ha^{-1} (CONAB, 2020). Although this production is considered satisfactory, productivity is well below the crop's potential, exceeding 200 Mg ha^{-1} (ADORNA; CRUSCIOL; ROSSATO, 2013).

The great difficulty in achieving this productive potential is related to the different production environments (DIAS, 1997), as well as soil and climate interaction, crop management, the selected cultivar (CESAR *et al.*, 1987), water availability (REICHARDT, 1996), between others indicating the need for practices to increase productivity and profitability.

The occurrence of seasonal droughts in several producing regions has been one of the limiting factors in the production and longevity of sugarcane fields. According to the Union of sugarcane industries (UNICA), in the 2018/2019 harvest there was a 6.05% drop in sugarcane production due to water scarcity. This number is equivalent to 36 million tons of sugarcane that were no longer produced, matching the entire production of the state of Paraná, the fifth largest producer of sugarcane in the country (CONAB, 2020).

Sugarcane has C4 metabolism, characterized by being more efficient than C3 plants in fixing CO_2, more efficient use of water and transpiration rates to the environment, however, weather conditions are the main causes of the impact of the potential productive. Sugarcane requires favorable environmental conditions, such as water availability between 1200 and 2500 mm (DOORENBOS; KASSAM, 1979; SINGH; SHUKLA; BHATNAGAR, 2007) and temperature between 25°C and 35°C during the growth phase of the stalks (MANHÃES *et al.*, 2015).

The phenological cycle of sugarcane encompasses the tillering and intense growth phase of the stalks, also known as formation phases (60 to 150 days after planting), being identified as the most critical due to water demand (RAMESH, 2000; MACHADO *et al.*, 2009), mainly because in these phases about 70 to 80% of sugarcane productivity is defined (SINGH; RAO, 1987; SMIT; SINGELS, 2006; ZHAO; GLAZ; COMSTOCK, 2010).

The expansion of sugarcane cultivation in Brazil to non-traditional cultivation areas was accompanied by increased exposure to biotic and abiotic stresses, more often exposing the crop to stressful conditions. Stress is defined as intense pressure from some unfavorable factor, which tends to prevent the normal functioning of the plant's metabolism (JONES, 1990).

Intense climatic conditions, especially in unfavorable production environments, can lead to overproduction of reactive oxygen species (ROS), increasing to concentrations above the cell's antioxidant capacity, causing a redox imbalance, ceasing the electron transport chain and triggering oxidative stress (GRATÃO *et al.*, 2005). As plant responses to these stresses, we can mention changes in the content of chlorophyll pigments (BANERJEE; ROYCHOUDHURY, 2019; CHA-UM *et al.*, 2012); cellular osmotic adjustment (PATADE; BHARGAVA; SUPRASANNA, 2011); early closure of stomata (SILVA *et al.*, 2013; VAN HEERDEN *et al.*, 2004; ZHAO; GLAZ; COMSTOCK, 2013) and decreased photosynthetic capacity (BANERJEE; ROYCHOUDHURY, 2019; TAKAHASHI; BADGER, 2011).

ROS are molecules that cause severe damage to plant cells, causing damage to proteins, nucleic acids, photosynthetic pigments, in addition to activating programmed cell death and causing lipid peroxidation of cell membranes (CHOUDHURY *et al.*, 2017). Plants can fight these molecules through efficient defense systems, such as antioxidant systems in plant cells (PANDY *et al.*, 2016), enzymatic and non-enzymatic (MARQUEZ-GARCIA *et al.*, 2011; AZEVEDO *et al.*, 2012; GALLEGO *et al.*, 2012).

In the enzymatic defense system, catalases, superoxide dismutase (SOD), ascorbate peroxidase (APX) and antioxidant molecules (eg, ascorbate and glutathione) stand out (TAVANTI *et al.*, 2021). The non-enzymatic defense system encompasses compounds such as glutathione (GSH), ascorbate (AsA), alkaloids, phenols, tocopherols, carotenoids and proline (GRATÃO *et al.*, 2005).

Three classes of SOD's can be found in plants and classified according to their metallic cofactor : manganese (Mn), copper/zinc (Cu/Zn) or iron (Fe) (ALSCHER; ERTURK; HEATH, 2002).

Catalase (CAT) is an enzyme that catalyzes the conversion of H_2O_2 to water and O_2 released during the transformation of glycolate to glyoxalate during photorespiration (IGAMBERDIEV; LEA, 2002) or during the β-oxidation of fatty acids (HOLTMAN *et al.*, 1994). Ascorbate peroxidase (APX) is one of the most widely

distributed antioxidant enzymes in plant cells and can be found in chloroplasts, cytosol, mitochondria and peroxisomes (ASADA, 1999), which, like CAT, also converts H_2O_2 into water and oxygen (CAO *et al.*, 2017).

Glutathione is a tripeptide that exists in the body in its reduced (GSH) and oxidized (GSSG) forms, acting directly or indirectly in protein synthesis, metabolism and cell protection (MEISTER *et al.*, 1983). Glutathione reductase (GR2) is a flavoprotein that catalyzes the reduction of GSSG to GSH, in a NADPH-dependent process. Under stress conditions, amino acid metabolism is greatly altered, with protein synthesis decreased and proteolysis increased (SODEK, 2004).

Plants, when exposed to water stress, may present accumulation of some amino acids, mainly proline, which may be correlated with drought tolerance (MOLINARI *et al.*, 2007), being widely used as a physiological-biochemical indicator of water stress (SHAO *et al.*, 2006).

The effectiveness of foliar fertilization with micronutrients can contribute to important metabolic processes, either as a substrate (organic compost) or as activators of enzymatic processes (MALAVOLTA; VITTI; OLIVEIRA, 1997). These metabolic functions can be part of the structure of some vital organic compound for the plant; enzyme constituent, participating in a specific structure, prosthetic/active group of enzymes; enzyme activator, affecting the speed of many reactions in plant metabolism.

Knowledge of the many metabolic and structural functions of nutrients and their requirements during the crop cycle can facilitate management and provide improvements in crop quality and productivity (EPSTEIN; BLOOM, 2005; MARSCHNER, 2012).

Adequate supply of macro and micronutrients at the right time is one of the most critical management practices for achieving satisfactory yields and sustainable income during all crop cycles (CRUSCIOL *et al.*, 2020). Foliar fertilizers can have a positive effect in situations of water stress, playing an important role in cellular activities, promoting both the control of stomatal opening and closing and osmotic adjustment of tissues, as well as acting on enzymatic activities (DOMINGOS; LIMA; BRACCINI, 2015; MANTOVANI *et al.*, 2017), minimizing the deleterious effects of water stress.

Macronutrients such as nitrogen, calcium, potassium and magnesium present a positive correlation with the increase in SOD, CAT and POD concentrations, while the micronutrients zinc, copper, manganese and iron act as cofactors of SOD in different

cellular compartments (DINAKAR; DJILIANOV; BARTELS, 2012; WARAICH *et al.*, 2012).

Amino acids are constituents of proteins, and precursors of several substances that regulate plant metabolism (FLOSS; FLOSS, 2007), which can increase production and resistance to stresses caused by high temperatures and water stress (RUSSO; BERLYN, 1991). The use of amino acids via foliar is an interesting strategy to minimize the damage caused by water stress in order to improve the physiological characteristics of the plant, acting directly on the plant's metabolism, saving energy expenditure and assisting in the production of enzymes, proteins and hormones (COLLAÇO JUNIOR, 2019).

Amino acids act as biostimulants, attenuating the effects resulting from abiotic stresses, providing positive effects on plant growth and development, directly reflecting on productivity (HILDEBRANDT, 2018; KHAN *et al.*, 2019).

Much is discussed about the isomerism of amino acids and its effects on plant metabolism. In general, D-amino acids (D-AAs) and L-amino acids (L-AAs) stereoisomers (enantiomers) have the same chemical and physical properties, with the exception of rotating the plane of polarized light in different directions, i.e. left or right. right (FISCHER, 1909). Studies show that the types are synthesized by living organisms and have specific functions in plant metabolism, however D-AAs have more toxic tendencies to plant metabolism (KOLUKISAOGLU; SUAREZ, 2017).

Essential amino acids (leucine, isoleucine, methionine, phenylalanine, arginine, histidine, tryptophan, valine, threonine and lysine) are synthesized only by plants, while non-essential amino acids (alanine, asparagine, cysteine, glutamine, aspartic acid, glycine, proline, serine and tyrosine) are synthesized by plants and also by humans (KUMAR *et al.*, 2017). Amino acid synthesis is generated through substrates from three main pathways: glycolysis, the citric acid cycle, and the pentose phosphate pathway (TAIZ *et al.*, 2017).

The hypothesis that the application of these nutritional complexes (ASPC/PFC) may influence the plant's enzymatic antioxidant system as a response to alleviate water stress, improving biometric parameters, quality of raw material, yield of stalks and sugars, in addition to the production of biomass were accepted in Chapter 1 and 2 of this work.

The perception of isolated factors of nutrients and amino acids is known, however the idea of this work was to evaluate them together. Therefore, the objective

of this work is to attenuate the effects of water stress in the sugarcane crop, using nutritional complexes composed of mineral nutrients and amino acids (ASPC/PFC), applied via foliar.

CHAPTER 1

FOLIAR APPLICATION OF A DROUGHT PROTECTION SUPPLEMENT MITIGATES SUGARCANE WATER STRESS

ABSTRACT

Extended periods of drought negatively affect sugarcane crop production. Water deficits limit plant growth and lead to undesirable morphological and physiological changes, most notably overproduction of reactive oxygen species (ROS). When applied to sugarcane plants, foliar supplements containing specific nutrients and/or organic molecules such as amino acids can increase plant metabolism, stalk and sugar yields, and the quality of the extracted juice. However, the effects of such foliar supplements on sugarcane plants exposed to water stress have not been comprehensively explored. To address this gap, the present study evaluated the effectiveness of foliar application of an abiotic stress protection complement (ASPC) composed of 18 amino acids and 5 macronutrients (N, P_2O_5, K_2O, S and Mg) during the driest period of the year, between June and August. The experiments were carried out in fields cultivated with fifth ratoon sugarcane at Pradópolis-SP (site 1) in the 2019 agricultural year and third ratoon sugarcane at Motuca-SP (site 2) in the 2020 agricultural year. The sugarcane variety was SP80-3280 at both sites. The experimental design was randomized complete block (RCB) with two treatments and twelve replicates. The two treatments were foliar application of ASPC and no application of ASPC (control) and were applied at the beginning of the dry season in June at both sites. ASPC was applied at a dose of 6 L ha^{-1} in a water volume of 100 L ha^{-1}. The foliar application of ASPC mitigated the negative effects of drought by increasing the levels of antioxidant enzymes [superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)] and reducing the activity of polyphenol oxidase (PPO). The Trolox equivalent antioxidant capacity increased by up to 45% in the treated plants compared with the control plants, reflecting higher antioxidant enzyme activity and lower malondialdehyde (MDA) levels. The level of H_2O_2 was 11.27 nM g^{-1} protein in plants treated with ASPC but 23.71 nM g^{-1} protein in control plants. Moreover, compared with the control plants, the application of ASPC increased productivity to 11.55 Mg ha^{-1} and sucrose accumulation to 2.26 kg Mg^{-1}, thus increasing the quality of the raw material. By positively stabilizing the cellular redox balance of the plants, ASPC application resulted in greater biomass production and, in turn, greater energy generation. Therefore, applying ASPC is an effective strategy for relieving water stress while improving crop development, stalk yield, sugar accumulation, and biomass.

Keywords: *Saccharum* spp.; oxidative stress; antioxidant enzymes; water deficit.

1.1 INTRODUCTION

Many regions of Brazil are susceptible to seasonal droughts, which limit sugarcane production and longevity. Drought promotes changes in plant metabolism, morphology, and physiology (DIAS *et al.*, 2012; SILVA *et al.*, 2011; VIEIRA *et al.*, 2014). Exposure of sugarcane to water stress can induce the production of reactive oxygen species (ROS) at higher concentrations than the cell's antioxidant capacity, leading to redox imbalance, disruption of the flow of the electron transport chain, and oxidative stress due to the toxic effects of ROS (GRATÃO *et al.*, 2005). ROS include superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and singlet oxygen ($^1O_2$). ROS can cause oxidative damage to various cellular constituents such as proteins, DNA, RNA and lipids (FOYER, 2018). In the absence of stress, ROS can act as signalers at low concentrations, but at high concentrations, they are highly oxidizing agents and harmful to plants (FOYER *et al.*, 2020). In addition to drought stress, plant production of ROS may intensify upon exposure to abiotic stresses such as high temperatures (GAVELIENĖ; NOVICKIENĖ; PAKALNIŠKYTĖ, 2013), heavy metals (GRATÃO *et al.*, 2012), water deficit (JEYARAMRAJA; THUSHARA, 2013), high salt concentrations (ELLOUZI *et al.*, 2011), and herbicide application (ZABALZA *et al.*, 2007; ZOBIOLE *et al.*, 2010). Under drought conditions, plants activate stomatal closure to reduce plant transpiration, resulting in lower CO_2 fixation and changes in photosynthetic activity that accentuate ROS production (GUPTA; RICO-MEDINA; CAÑO-DELGADO, 2020).

Plants have developed defense systems to mitigate the damage from ROS, including enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) (TAIZ *et al.*, 2017). In addition to these endogenous systems, foliar application of low concentrations of organic compounds such as amino acids can minimize the oxidative damage caused by water stress. Amino acids are constituents of proteins and precursors of several regulators of plant metabolism (FLOSS; FLOSS, 2007) and can activate plant metabolism to increase the plant's ability to tolerate biotic and abiotic stresses, especially under unfavorable conditions for development, such as root asphyxia, drought, and extreme temperatures (VAN OOSTEN *et al.*, 2017; RUSSO; BERLYN, 1991).

In addition to organic compounds, mineral nutrition plays an important role in plant stress tolerance via effects on antioxidant enzyme activation, osmotic adjustment, stomatal opening/closing and amino acid synthesis (CRUSCIOL *et al.*, 2013; FAGAN *et al.*, 2016). Nutrients such as potassium, magnesium and phosphorus can increase SOD, CAT, POD and APX activity in plants and play important roles in cellular activities such as stomatal control, osmotic adjustment and enzyme activation (MANTOVANI *et al.*, 2017; RODRIGUES *et al.*, 2018). The nutritional requirements of crops for macronutrients are mainly met by applying fertilizers to the soil due to the high absorption of nutrients by roots. However, foliar fertilization can be a more effective and viable form of supplementation for plants (FAGERIA *et al.*, 2009).

Although the roles of these organic molecules and mineral nutrients in increasing essential metabolites to maintain plant metabolism are well known, more research is needed to clarify the specific mechanisms by which these molecules mitigate stress in plants (NGOROYEMOTO *et al.*, 2019; SILVA *et al.*, 2020). Knowledge of the metabolic and structural functions of organic nutrients and the plant's requirements for these compounds during different phenological stages can enable crop management and improvements in crop quality and productivity (EPSTEIN; BLOOM, 2005; MARSCHNER, 2012). According to Castro and Carvalho (2014), all plants produce amino acids; however, this production is reduced under stress conditions, resulting in insufficient concentrations to mitigate the impacts of stress. Thus, nutritional supplements that contain amino acids, such as proline, glycine, arginine (ANJUM *et al.*, 2012), glutamate (PHILIPPE *et al.*, 2019), and alanine, are designed to provide the necessary amount to improve plant development conditions under biotic and abiotic stress (MÓGOR, 2015) and minimize increases in energy expenditure (FAGERIA *et al.*, 2009). These bioactivating substances can mitigate the effects of exposure to extreme weather events (VAN OOSTEN *et al.*, 2017). Although specific studies of the activities of these molecules in plants are limited, their ability to increase metabolites that are essential for maintaining plant physiology during periods of stress is well known (NGOROYEMOTO *et al.*, 2019; SILVA *et al.*, 2020).

This study evaluated the impact of foliar application of abiotic stress protection complement (ASPC) on the productivity of sugarcane under water stress. It was hypothesized that ASPC application would positively influence antioxidant system activity, biometric and technological parameters, biomass and sugarcane productivity.

1.2 MATERIAL AND METHODS

1.2.1 Experimental area

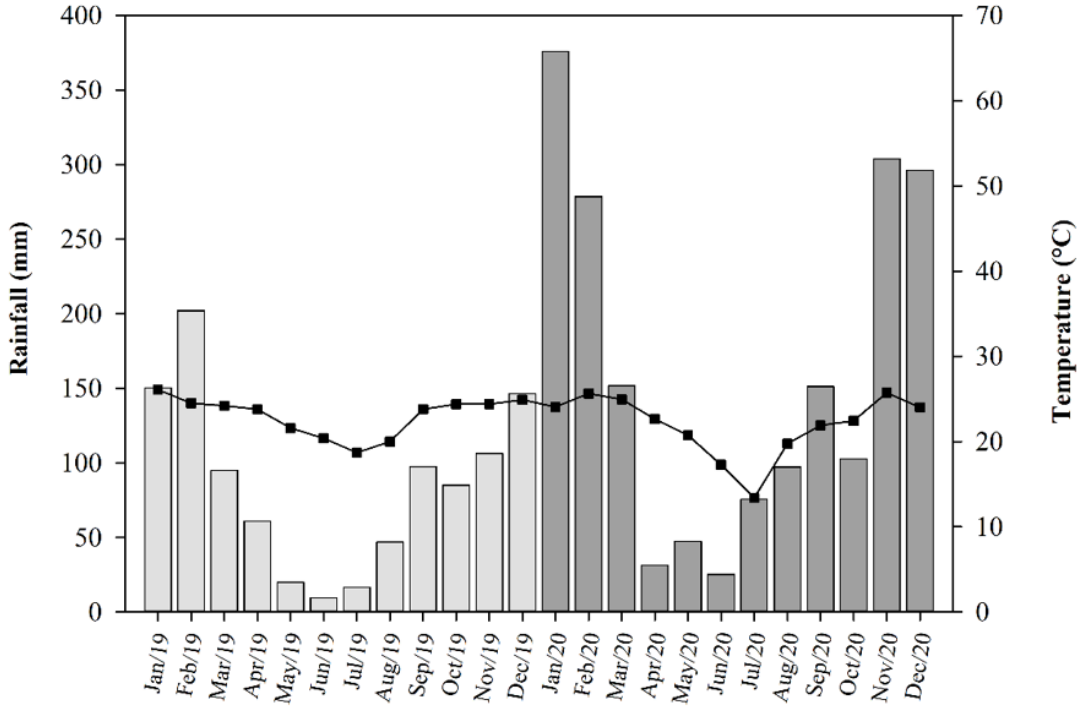
The experiments were performed at two different sites during the driest period of the year, between June and August, in 2019 and 2020. The experiments were carried out on sugarcane (*Saccharum* spp. hybrid) fifth ratoon (site 1, 2019) and third ratoon (site 2, 2020). The variety SP80-3280 was selected for both sites based on its high sucrose content and ratoon yield, moderate tillering, large ratoon regrowth and medium to late ripening. Both sites are located in São Paulo state in areas belonging to Usina São Martinho. Site 1 is located in the municipality of Pradópolis (21°.21'.34" S, 48°.03'.56" W, average altitude 538 m), while site 2 is located in the municipality of Motuca (21°.30'.30" S, 48°.09'.12" W, average altitude 538 m). Sites 1 and 2 have a tropical savanna climate (Aw) according to the Köppen-Geiger climate classification system (ALVARES *et al.*, 2013). The average annual temperature is 23.4 and 21.6°C at sites 1 and 2, respectively, and the average precipitation is 1419 and 1344 mm (Figure 1). The soil is classified as Rhodic Eutrudox at site 1 and Hapludox Rhodic at site 2 (SOIL SURVEY STAFF, 2014). The chemical characteristics of the soil were determined before the installation of the field experiments. Ten soil subsamples were collected between the ratoon rows at each site and pooled to form a composite sample. The chemical characteristics of the soil in the experimental area are presented in Table 1.

Table 1 - Soil classification and chemical characteristics of experiments at each site

Location	Experience (site)	Only classification	Depth	pH $CaCl_2$	MO g dm^{-3}	$P_{(resina)}$ mg dm^{-3}	S	Al^{+3}	$H+Al^{+3}$	K	Ca	Mg	SB	CTC	V%
									---------- mmol $_{c}$ dm^{-3} ----------						%
Pradópolis (SP) 2019	1	Rhodic Eutrudox	0,00-0,20	5.1	26	19	9	0	40	2.6	42	15	60	100	60
			0.20-0.40	5.1	23	16	29	0	42	1.7	33	15	49	91	53
Motuca (SP) 2020	2	Hapludox rhodic	0.00-0.20	5.3	17	55	27	0	21	2.6	27	11	41	61	67
			0,20-0,40	5.0	14	34	33	1	25	0.8	17	6	24	49	49

Source: by the author

Figure 1 - Monthly rainfall and mean temperatures during the experimental period at site 1 and site 2

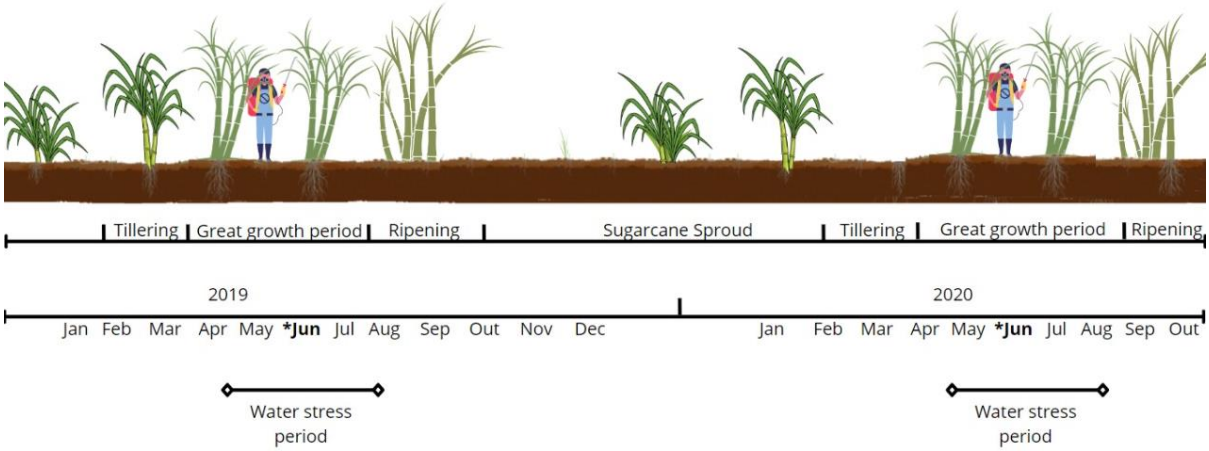


Source: by the author.

1.2.2 Experimental design and description of treatments

The experimental design was a randomized complete block (RCB) with two treatments and twelve replicates, giving a total of 24 experimental plots. Each plot had 8 10-m-long rows with an inter-row spacing of 1.5 m. The treatments consisted of 1) control: no application of ASPC and 2) ASPC: application of ASPC. At both sites, the treatments were applied in June, and the sugarcane was harvested in October (Figure 2). ASPC was applied at the full manufacturer recommended dose of 6 L a.i. ha^{-1} in a water volume of 100 L ha^{-1}. The concentrations of the components of ASPC are given as % (p/p) (Tables 2 and 3). ASPC is classified as a mixed mineral foliar fertilizer and has an average density of 1.28 g/ml at 20°C. The applications followed the manufacturer's specifications and were performed under adequate environmental conditions with the help of a pressurized tank (CO_2) equipped with a single 1/4KLC-9 brass fieldjet coupled to a 2.6-m-long rod, which allowed simultaneous and homogeneous application to four rows of plants (application range of 7.5 m) at a pressure of 344 kPa for each 100 L ha^{-1}. There were no problems with pests, weeds or diseases at the experimental sites. Thus, crop management was carried out according to the recommendation for each site following the factory's calendar for sugarcane cultivation practices.

Figure 2 - Schematic experimental schedule with description of the conduct of the experimente and application of the drought protection complex (ASPC) each year (*)



Source: by the author.

Table 2 - Concentrations of total organic carbon and macronutrients in the application of the drought protective complex (ASPC)

	Macronu*trie*nts					
	Mg	K_2O	P_2O_5	N	S	COT
Dose 6 L ha^{-1} (g)	153.6	460.8	460.8	76.8	245.76	122.8
Concentration (%)	2.0	6.0	6.0	1.0	3.2	1.6

Source: by the author

Table 3 - Concentrations of amino acids present in the concentration of total organic carbon in the application of the drought protective complex (ASPC)

	Amino Acids																	
	Gly	Glu	Hys	Ala	Tau	Pro	Asp	Lys	Arg	Leu	Ser	The	Tir	Val	Fen	Met	Ile	Cis
Dose 6 L.ha^{-1} (g)	180.5	139.8	135.2	113.7	104.5	87.6	86.8	69.9	68.4	57.6	44.5	40.7	37.6	37.6	29.9	26.9	23.8	10.8
Concentration (%)	2.35	1.82	1.76	1.48	1.36	1.14	1.13	0.91	0.89	0.75	0.58	0.53	0.49	0.49	0.39	0.35	0.31	0.14

*Gly – glicine; Glu-glutamine; Hys-histidine; Ala-alanine; Tau-taurine; Pro-proline; Asp-aparagine; Lys - lysine; Arg – arginine; Leu-leucine; Ser-serine; Thr – threonine; Tir-tirosine; Val – Valine; Fen-Fenilanine; Met – methionine; Ile –isoleucine; Cys – cysteine.

Source: by the author

1.2.3 Leaf collection for enzyme sampling

For enzymatic sampling, the TVD (Top Visible Dewlap) or leaf +1 were considered, according to Van Dlllewijn (1952), discarding the apex and the base of the leaf, using only the middle third to posteriorly evaluate the contents of enzymes superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6) , peroxidase (POD; EC 1.11.1.7) and polyphenol oxidase (PPO; EC 1.10.3.1). The samplings were performed within 9:00 and 10:00 am at 90 days after ASPC application. For enzymatic analysis, the samples were quickly frozen in liquid nitrogen and stored at - 80 °C, placed in falcon tubes..

1.2.4 Enzymatic analysis of metabolites in sugarcane leaf

Twenty leaves were collected and dried in a forced-air oven at 65°C for 72 hours. The material was then ground in a Wiley mill inside a sieve with a mesh diameter of 1 mm. The total sugars, soluble sugars, starch and sucrose in the samples were measured, and the results were expressed in (%) for each 100 g of sample (NELSON, 1944; SOMOGYI, 1945).

1.2.5 Oxidative stress and antioxidant enzymes

Before enzymatic analysis, the total protein level was determined using the methodology described by Bradford (1976). Lipid peroxidation was evaluated based on malondialdehyde (MDA) levels following a previously reported method (HEATH; PACKER, 1968) using a coefficient of 155 mM L^{-1}. The results were expressed in nanomoles of MDA per gram of fresh weight. H_2O_2 was determined according to a reference calibration curve and expressed in mol g^{-1} of fresh mass.

SOD activity was evaluated as described previously (GIANNOPOLITIS; RIES, 1977), and the results were expressed in units (U) of SOD per gram of protein. CAT activity was assessed as described previously (HAVIR; MCHALE, 1987), and the results were expressed in nanomoles per minute per milligram of protein. POD activity was evaluated according to ALLAIN *et al.* (1974), and the results were expressed in micromoles of H_2O_2 per minute per gram of fresh weight. Finally, PPO activity was

evaluated according to the literature (KAR; MISHRA, 1976), and the results were expressed in micromoles of catechol per minute per gram of protein.

1.2.6 Sugarcane biometric evaluations

For the biometric evaluations, 20 sugarcane plants were randomly collected from each plot. The stalk height of each plant was measured as the distance from the ground to the auricular region of the +1 leaf and expressed in meters; the stalk diameter was evaluated with the aid of a digital caliper (MARAFON, 2012). The biometric evaluations were performed prior to harvest at the phenological stage of maturation.

1.2.7 Sugarcane quality evaluations

The stalks used for the biometric evaluations were also used for technological evaluations. The stalks were cleaned, defoliated and sent to the payment for cane by sucrose content (PCTS) laboratory of the mill to determine the following parameters: sucrose concentration according to Fernandes (2011), % fiber (water insoluble dry matter in sugarcane), % purity (percentage of sucrose in total solids content in sugarcane juice), total reducing sugars (TRS; all forms of reducing or inverted sugars in sugarcane), reducing sugars (RS %; reducing substances in cane and sugar products calculated as invert sugar, predominantly hexoses).

1.2.8 Stalk and sugar yield

Prior to harvest, two rows were defined within the useful area of the plot, and the plants contained within a 4-m length of each of these rows were used to evaluate stalk mass. The stalks were weighed, and the value was extrapolated to obtain the stalk yield in Mg ha^{-1}. Then, the sugar yield (Mg ha^{-1}) was calculated by multiplying the stalk yield (Mg ha^{-1}) by the TRS and dividing by 100.

1.2.9 Biomass and energy production

To calculate bagasse at 50% moisture, the results for fiber and stalk productivity were used. Trash was calculated by considering 140 kg of bagasse per Mg of stalks

according to Hassuani, Leal e Macedo (2005). Energy was calculated considering that 1 Mg of trash has 4.96 MWh of primary energy and 1 Mg of bagasse has 4.94 MWh of primary energy (1 MWh = 3,600.00 MJ) (HASSUANI; LEAL; MACEDO, 2005).

1.2.9.1 Statistical Analysis

For statistical analysis, all measured variables were analyzed by one-way ANOVA, and means were compared using Fisher's protected least significant difference (LSD) test at $p \leq 0.05$ for each site.

1.3 RESULTS

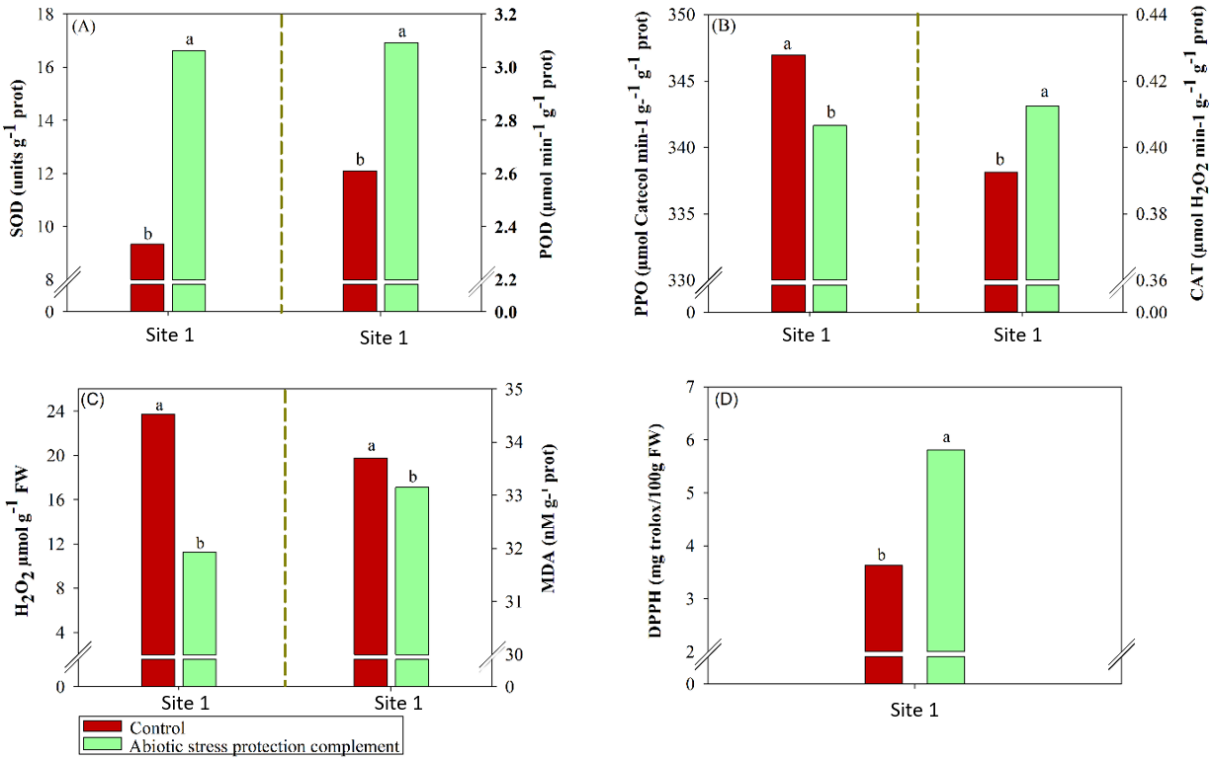
1.3.1 ASPC application improves antioxidant enzyme activity

Leaf enzymatic metabolite concentrations were influenced by the application of ASPC at site 1 (Figure 3). In addition, the activities of the antioxidant enzymes SOD, CAT and POD were significantly ($p < 0.10$) higher in the ASPC treatment than in the control at site 1 (Figure 3). ASPC application increased SOD and POD activity by 44% and 15.5% compared with the control, respectively, corresponding to 9.3 units g^{-1} protein and 2.61 µmol min^{-1} g^{-1} protein, (Figure 3A). ASPC application increased CAT activity by 4.88% compared with the control (0.39 µmol H_2O_2 min^{-1} mg^{-1} protein) at site 1 (Figure 3B).

As expected, ASPC application reduced the activity of PPO, an oxidizing enzyme that causes samples to darken in the presence of H_2O_2, at site 1. PPO activity decreased by 1.56% compared with the control (346.95 µmol catechol min^{-1} g^{-1} protein) (Figure 3B). The concentration of MDA, a marker of oxidative stress, was lower in the ASPC treatment at site 1: 33.15 nM g $^{-1}$ protein versus 33.7 nM g^{-1} protein in the control (Figure 3C). Consistent with the decrease in MDA, ASPC application increased the Trolox-equivalent antioxidant capacity (TEAC), which measures the antioxidant capacity of a given plant tissue sample. Compared with the control (3.38 mg TE g^{-1}), ASPC application significantly ($p < 0.10$) increased the TEAC (DPPH) by 45.57% at site 1 (Figure 3D).

In line with the increases in SOD, CAT, and POD activity in the ASPC treatment, H_2O_2 levels decreased significantly ($p < 0.10$) by 11.27 µmol g $^{-1}$ to 23.71 µmol g $^{-1}$ in the ASPC treatment compared with the control at site 1 (Figure 3C).

Figure 3 - Drought protection complex (ASPC) application on sugarcane antioxidant enzyme parameters at harvest: (A) superoxide dismutase (SOD) e peroxidase (POD), (B) polyphenol oxidase (PPO) and catalase (CAT), (C) Hydrogen peroxide (H_2O_2) e Malondialdehyde (MDA) and (D) Trolox equivalent antioxidant capacity (DPPH) of sugarcane (site 1). The treatments were as follows: Control, no ASPC application; ASPC: application of the ASPC at both sites in June, in the beginning of the drought season. Averages followed by the same letters do not differ by the LSD test ($p < 0.10$)



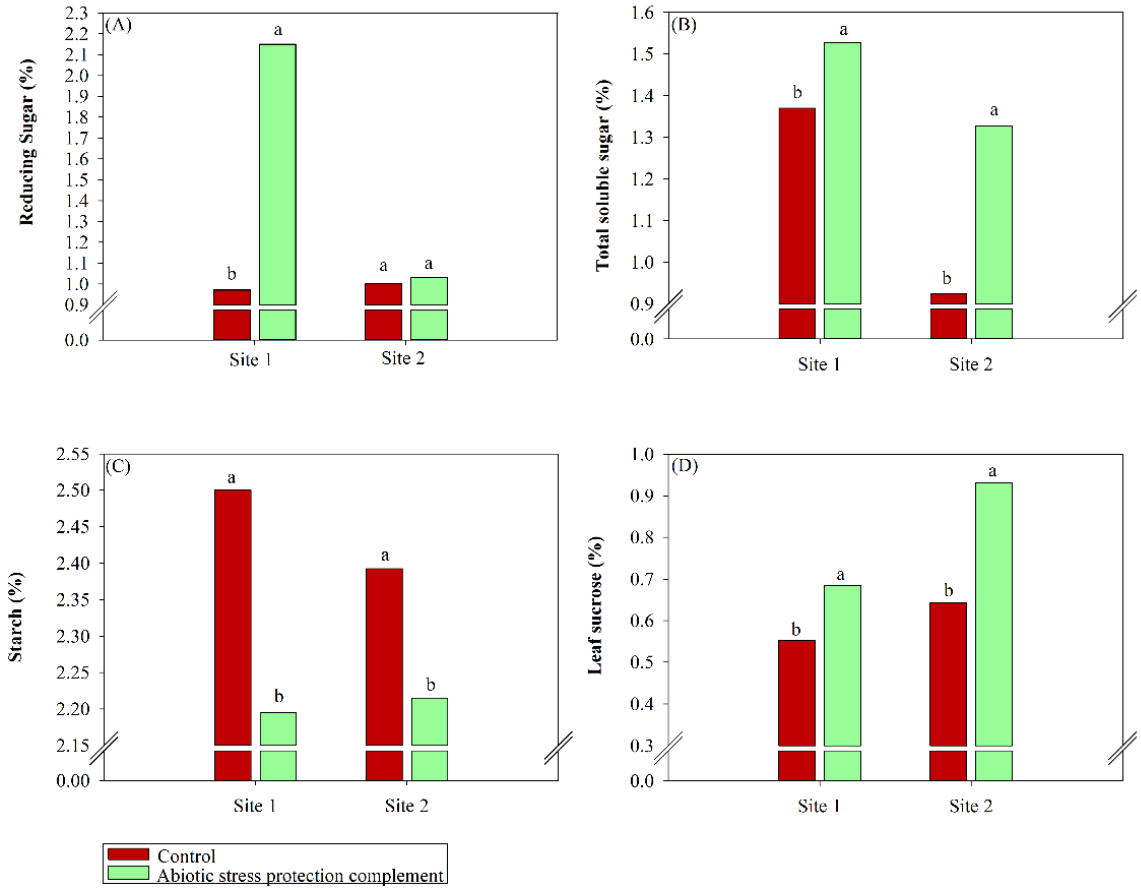
Source: by the author.

1.3.2 ASPC application improves sugarcane leaf quality

The leaf quality of sugarcane was significantly higher ($p < 0.10$) in the ASPC treatment. At site 1, leaf reducing sugars, glucose and fructose increased by an average of 54.88% in the ASPC treatment compared with the control (0.97%), and at sites 1 and 2, total soluble sugars were higher in the ASPC treatment (10.46% and 30.83%, respectively) than in the control (1.37% and 0.92%, respectively) (Figures 4A and 4B). In addition, leaf sucrose increased by 20.29% and 31.18% in the ASPC

treatment at sites 1 and 2, respectively, compared with the control (0.55% and 0.64%) (Figure 4D). In contrast to the results for sucrose, leaf starch content was higher in the control treatment (2.5% at site 1 and 2.39% at site 2) than in the ASPC treatment (2.20% at site 1 and 2.21% at site 2) (Figure 4C).

Figure 4 - Sugarcane leaf metabolic parameters: Reducing sugars (%); Total soluble sugars (%); Starch (%); Foliar sucrose (%), in the harvest affected by the ASPC application strategy. The treatments are as follows: Control: no ASPC application; Nutritional complex: application of ASPC, applied at the beginning of the local dry season 1 and 2 (June). Means followed by the same letters do not differ by the LSD test ($p < 0.10$)



Source: by the author.

1.3.3 ASPC application improves sugarcane quality

ASPC application significantly ($p < 0.10$) increased the sucrose concentration (%) in the stalk by 1.23% and 2.64% compared with the control, with values of 15.26% and 16.23% at sites 1 and 2, respectively (Figure 5A). Due to the increase in sucrose

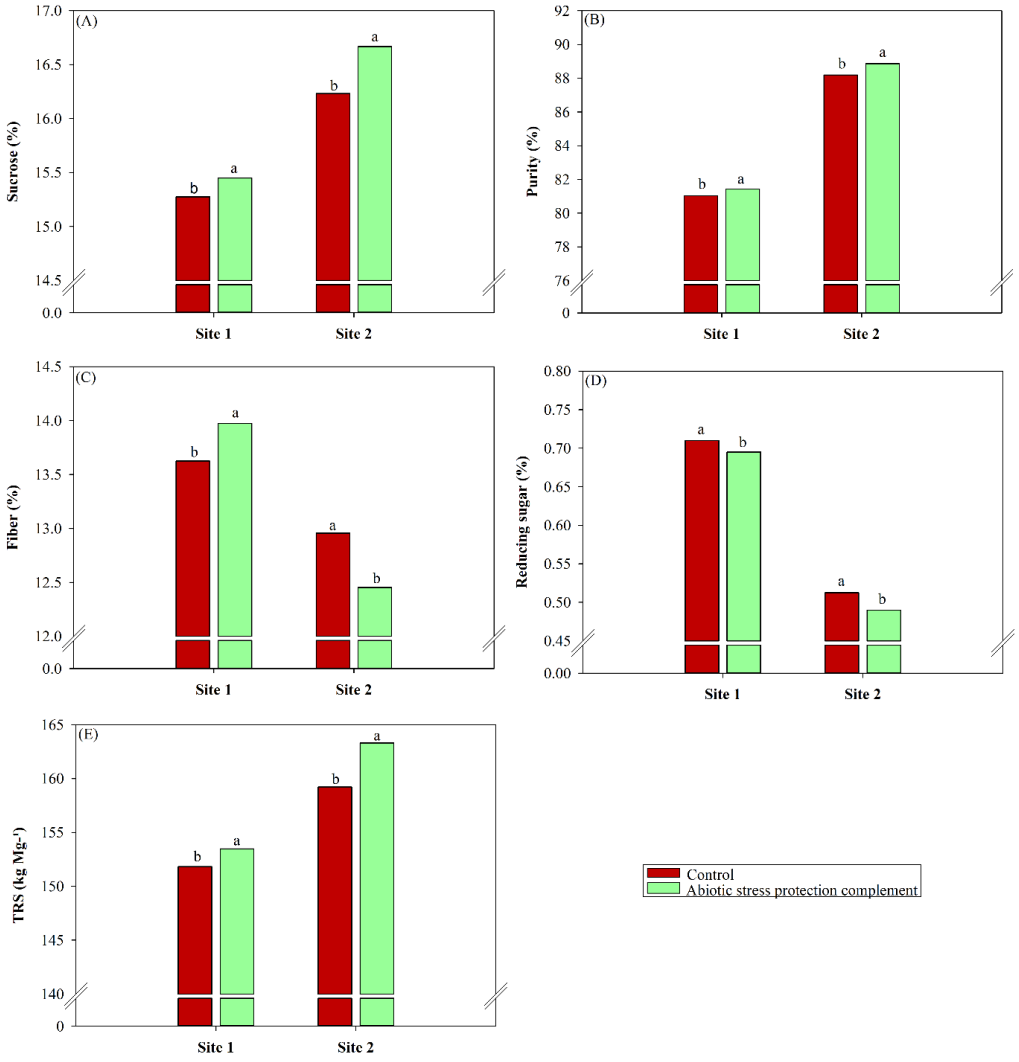
content, the levels of reducing sugars decreased in the ASPC treatment at both sites (Figure 5D).

Sugarcane purity (%) was higher in the ASPC treatment at both sites (Figure 5B). Thus, ASPC improved the quality of the raw material for industrial use, with average juice purity values above 80% at sites 1 and 2, representing gains of 0.5% and 0.77% compared with the control (81.02% and 88.19%, respectively).

In contrast to the results for sucrose concentration and purity, fiber content decreased by 4.1% in the ASPC treatment compared with the control (12.96%) at site 2. At site 1, fiber content increased by 2.58% in the ASPC treatment compared with the control (13.62%) (Figure 5C).

The sucrose concentration and reducing sugar levels were used to calculate TRS. Since ASPC application significantly ($p < 0.10$) increased the sucrose concentration at sites 1 and 2, TRS increased by 1.08% and 2.5% compared with the control (151.82 and 159.2 kg of Mg^{-1}), respectively (Figure 5E).

Figure 5 - Effects of ASPC application on the technological parameters of sugarcane at harvest: (A) sucrose concentration (%), (B) purity (%), (C) fiber (%), (D) reducing sugars (%) and (E) total reducing sugars (Kg Mg^{-1}). The treatments were as follows: Control, without ASPC application; ASPC: application of Drought protective complex at the beginning of the dry season, in June, at both sites. Means followed by the same letters do not differ by the LSD test ($p < 0.10$)



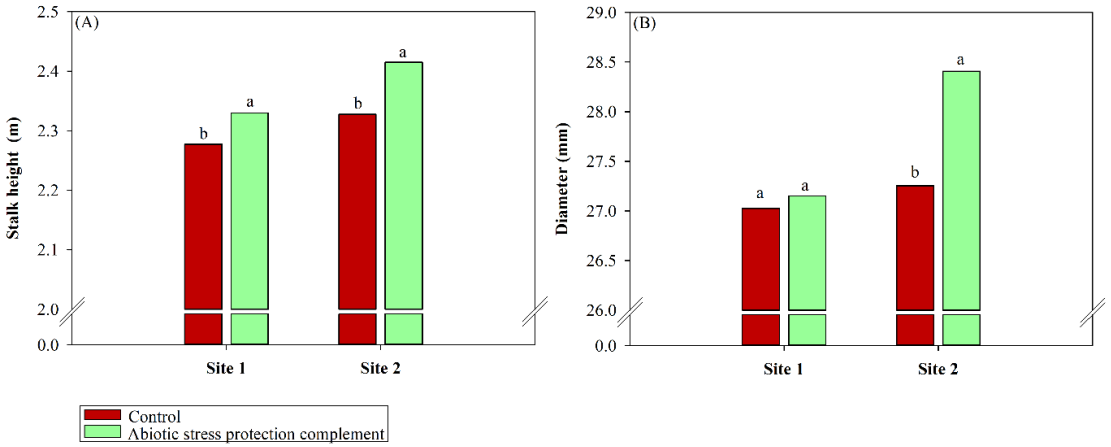
Source: by the author.

1.3.4 ASPC application improves sugarcane biometric parameters

Among the biometric parameters, ASPC positively affected ($p < 0.10$) stalk height (Figure 6A) at both sites, with average gains of 2.15% at site 1 and 3.72% at site 2 compared with the control (2.28 and 2.33 m, respectively). In addition, ASPC application increased stalk diameter by 4.01% compared with the control (27.26 mm) at site 2. At site 1, there was no significant difference in stalk diameter between the

treatments (Figure 6B).

Figure 6 - Drought protection complex (ASPC) effects on sugarcane biometric parameters at harvest: (A) stalk height (m), (B) diameter (mm). The treatments were as follows: Control, no ASPC application; ASPC: application of the ASPC at both sites in June, in the beginning of the drought season. Averages followed by the same letters do not differ by the LSD test ($p < 0.10$)

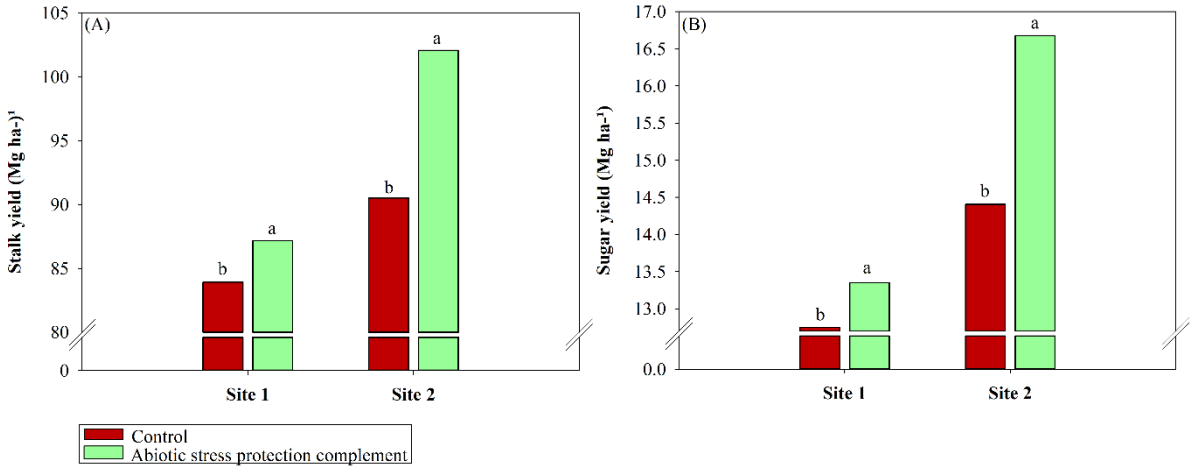


Source: by the author.

1.3.5 ASPC application improves stalk and sugar yields

ASPC application significantly increased stalk yield, with average gains of 3.73% site 1 and 11.32% at site 2 compared with the corresponding controls (83.93 Mg ha^{-1} and 90.51 Mg ha^{-1}) (Figure 7A). Because sugar yield is calculated from the product of TRS and stalk yield, increases in these two parameters will increase sugar yield. Sugar yield increased by averages of 4.78% and 13.56% at sites 1 and 2 compared with the control (12.74 Mg ha^{-1} and 14.41 Mg ha^{-1}) (Figure 7B).

Figure 7 - Drought protection complex (ASPC) effects on sugarcane yield parameters at harvest: (A) stalk yield (Mg ha^{-1}), (B) sugar yield (Mg ha^{-1}). The treatments were as follows: Control, no ASPC application; ASPC: application of the ASPC at both sites in June, in the beginning of the drought season. Averages followed by the same letters do not differ by the LSD test ($p < 0.10$)

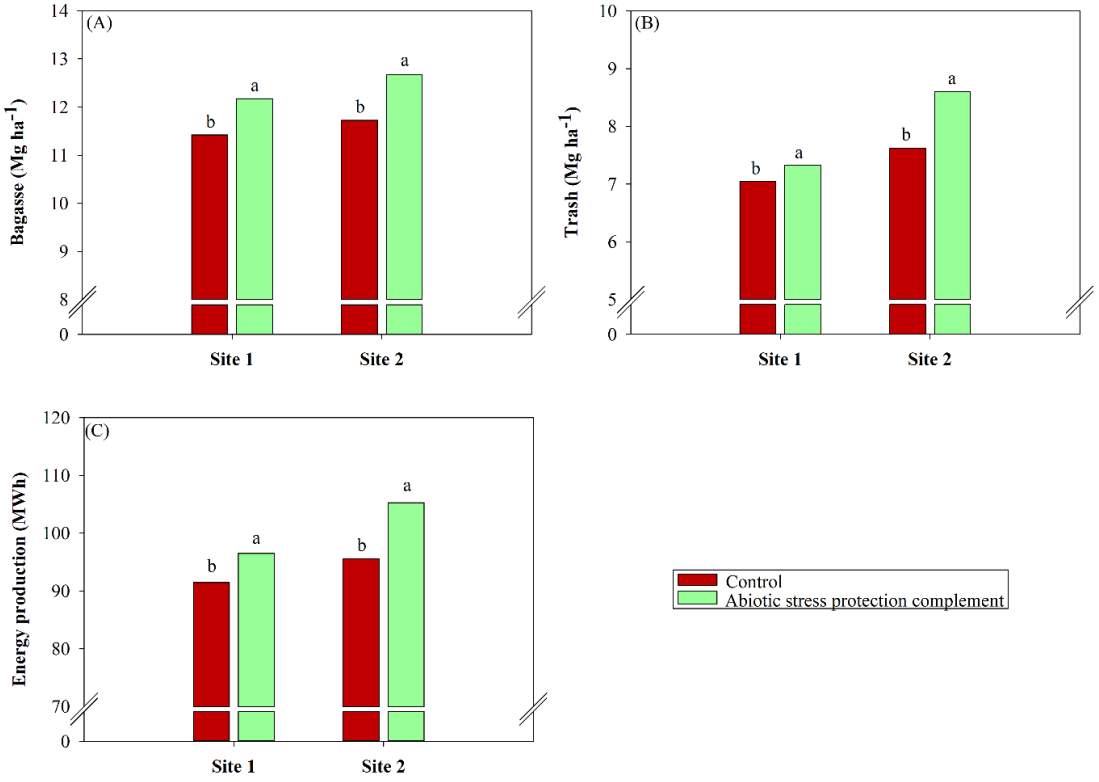


Source: by the author.

1.3.6 ASPC application improves biomass and energy production

In general, ASPC application increased biomass production (bagasse and straw) at both sites (Figure 8). Bagasse and straw production were highest in the ASPC treatment at site 2, with gains of 7.87% and 11.63% compared with the control (11.7 and 7.6 Mg ha^{-1}), respectively (Figures 8A and 8B). Consistent with the increase in biomass production, ASPC application increased energy production at both sites, with average gains of 5.18% and 9.21% at sites 1 and 2, respectively, compared with the control (91.5 and 95.6 MWh) (Figure 8C).

Figure 8 - Drought protection complex (ASPC) effects on sugarcane biomass and energy parameters at harvest: (A) bagasse (Mg ha^{-1}); (B) trash (Mg ha^{-1}) and (C) energy production (MWh). The treatments were as follows: Control, no ASPC application; ASPC: application; ASPC: application of the ASPC at both sites in June, in the beginning of the drought season. Averages followed by the same letters do not differ by the LSD test ($p < 0.10$)



Source: by the author.

1.4 DISCUSSION

Water deficiency directly affects plant metabolism and physiology, such as gas exchange and water and nutrient absorption, resulting in low growth rates, reduced photosynthesis and photoassimilate production, and, consequently, lower plant biomass (ANJUM *et al.*, 2012). In this work, we evaluated the ability of ASPC, which contains amino acid-based organic compounds as the main ingredient, to mitigate water stress in sugarcane plants.

At site 1, ASPC application favorably influenced all evaluated parameters, even though the sugarcane plants were fifth ratoon cane and the soil had relatively low levels of P_2O_5 (Table 1). ASPC includes phosphorus, a nutrient that is required in several chemical reactions and is fundamental for storing energy as ADP and ATP and for

assimilating CO_2 (CASTRO, 2016). The inclusion of phosphorus may have contributed to the effectiveness of ASPC in mitigating water stress.

Some of the amino acids present in ASPC, such as glycine, proline, threonine, alanine, glutamate, and tryptophan, can act as plant signals against abiotic stresses such as drought and function as excellent ROS suppressors (ARNAO; HERNÁNDEZ-RUIZ, 2015; FORD; LEA, 2007; JOSHI *et al.*, 2010; MUTHURAMALINGAM *et al.*, 2018; PARTHASARATHY *et al.*, 2018). In *Arabidopsis thaliana L*., proline accumulation is the main response to abiotic stress (KAUR; ASTHIR, 2017). Arginine is a precursor of proline and cytokinin, two fundamental molecules in the fight against oxidative stress (WINTER *et al.*, 2015). Amino acids such as glycine can improve the stability of cell membranes and protect them from peroxidation during conditions of environmental stress (MOHAMMADIPOUR; SOURI, 2019).

The higher activities of SOD, POD, and CAT (Figures 3A and 3B) in sugarcane that received foliar application of ASPC indicate that the plant antioxidant system was stimulated to combat ROS. SOD is the first line of defense against ROS in plant cells. SOD dismutates $O_2^{-\bullet}$ to O_2 and H_2O_2. The latter is subsequently dismutated to water and oxygen by CAT (MITTLER, 2002; SHARMA *et al.*, 2012). In addition, total antioxidant capacity as measured by Trolox-DPPH was higher in the ASPC treatment than in the control (Figure 3D), indicating that ASPC activated the antioxidant system to eliminate ROS in leaves.

In contrast to the effects of ASPC application on SOD, POD and CAT activity, PPO activity decreased in ASPC-treated plants (Figure 3B). PPO catalyzes the formation of quinones and acts on phenolic compounds in the presence of oxygen, resulting in the oxidation and hydroxylation of phenols, which leads to the production of melanoidins, brown pigments that cause tissue darkening (VALERO *et al.*, 1989). According to Hammer (1993), PPO can be present inside plastids and in the cellular cytoplasm under tissue degeneration conditions. The higher PPO activity in the control plants implies greater levels of oxidative damage and lipid peroxidation in cells (Figure 3B). The reduction in PPO activity in the ASPC treatment also improved the quality of the final product, as dark-colored sugarcane juice due to the presence of PPO is undesirable in the sugar industry (NAIKOO *et al.*, 2019; VICKERS *et al.*, 2005).

ASPC application also decreased the level of MDA, the main product of lipid peroxidation in response to water deficit. This metabolite is formed by the action of ROS on cell membranes, which results in the oxidation of polyunsaturated fatty acids

and causes irreversible cell damage (CARMODY *et al.*, 2020; SHUKLA *et al.*, 2021). As water stress increases, plants increase ROS levels and, consequently, lipid peroxidation. The effects of ROS are particularly severe in organelles such as chloroplasts and mitochondria, which have extensive oxidative metabolism (MANO, 2012). Foliar application of ASPC likely promoted the metabolic activation of the plant's antioxidant system, reducing oxidative damage from water stress (TAIZ *et al.*, 2017).

The reduction in foliar starch content (Figure 4C) reflected the attenuation of plant stress by the application of ASPC. The demand for metabolic energy in ASPC-treated plants triggered the degradation of starch into complex carbohydrates, which accumulated in the form of sucrose (KAUR; ASTHIR, 2017). As a result, the photosynthetic activity of the plant was reestablished, leading to a decrease in the free energy of cellular water due to the higher concentration of solutes inside the cell and allowing stomatal opening (TAIZ *et al.*, 2017).

When plants close their stomata, CO_2 fixation decreases, and the photosynthetic process is blocked, dramatically increasing the production of ROS (GUPTA; RICO-MEDINA; CAÑO-DELGADO, 2020). Nutrients in ASPC such as potassium, phosphorus, and magnesium may have influenced the concentrations of solutes and stomatal adjustment to keep the plants more hydrated and more photosynthetically active. In addition, amino acids such as proline and glycine reduce the energy expenditure required to overcome water stress (GUPTA; RICO-MEDINA; CAÑO-DELGADO, 2020; GURGEL; GHEYI; OLIVEIRA, 2010).

Amino acids in ASPC such as proline, glycine, arginine and glutamic acid provide the input for the activation and synthesis of enzymes and proteins that mobilize starch into reducing sugars and total soluble sugars (Figures 4A and 4B). These amino acids may have mitigated the deleterious effects of water deficit by reducing damage to cell structures (MERWAD; DESOKY; RADY, 2018) and promoting regulation of gas exchange (BEKKA *et al.*, 2018) and osmotic adjustment with the environment (DAWOOD *et al.*, 2014).

The greater accumulation of total soluble sugars (sucrose, glucose, fructose) in sugarcane treated with ASPC (Figure 4B) also reflects the attenuation of water stress. The conversion of starch into hexoses reduced the free energy of water and increased its retention inside cells, providing better osmotic adjustment (SOUZA *et al.*, 2020). The increase in sucrose synthesis may have been due to activation of the enzyme sucrose phosphate synthase (SPS) (DONG; BECKLES, 2019).

The increase in the leaf sucrose concentration in the ASPC treatment (Figure 4D) was probably due to degradation of the starch present in the leaves (Figure 4C) and the activation of acid and/or neutral invertases to release reducing sugars (BATTA *et al.*, 2011), which raised the sugar content in the source tissues and consequently increased the total soluble sugars (Figures 4A, 4B and 4D) (ZEEMAN; KOSSMANN; SMITH, 2010).

The potassium and magnesium present in ASPC acted as physiological activators of the partitioning of carbohydrates, which is directly related to the accumulation of sucrose and starch in leaves (CAKMAK; KIRKBY, 2008). Magnesium is linked to the loading of sugar in phloem, and potassium fosters the source-sink relationship. Therefore, the absence of these elements would result in sucrose and starch accumulation in leaves (Figures 4C-4D).

In addition, the presence of sucrose and hexoses maintains the osmoprotective balance of plant cells and helps mitigate the loss of water to the extracellular medium (SILVA *et al.*, 2021). This osmoregulation is controlled not only by K^+ ions in stomatal opening and closing but also by sucrose production and degradation in guard cells (PACHECO; LAZZARINI; ALVARENGA, 2021). The high activity levels of SPS and sucrose synthase (Susy) in guard cells compared with other cell types in leaves suggest a possible role of sucrose in stomatal osmoregulation (WINTER *et al.*, 2015).

Plants that received foliar ASPC had high sucrose content (Figure 4D). When absorbed by plant leaves, amino acids provide benefits either by mitigating abiotic stresses or providing energy more efficiently and economically to plants (TAIZ *et al.*, 2017). The conversion of starch to sucrose by SPS is involved in primary and secondary metabolism and leads to the synthesis of several compounds that influence product quality, such as POL (apparent sucrose), purity, TRS in the cane, reducing sugar content and fiber percentage (ZHANG *et al.*, 2020).

The main value associated with sugarcane cultivation is directly related to the accumulation of sucrose in the stalks (Figure 5A). A higher concentration of sucrose will result in a higher evaluation of the technological parameters of sugarcane, such as the higher purity obtained in this study (Figure 5B). The lower concentration of sucrose in the control stalks under water deficit (Figure 5A) was due to the lower assimilation of CO_2 by the canopy, which reduced the supply of photoassimilates.

The reductions in the percentages of glucose, fructose and fiber (sites 1 and 2) resulted in higher TRS. Bioactivators can increase production, leading to higher

sucrose content and sugarcane productivity (SILVA *et al.*, 2021; SOUZA *et al.*, 2020). One possible explanation for the greater accumulation of sucrose in the ASPC treatment compared with the control is that the increase in the activity of enzymes related to the synthesis and transport of sucrose in the stalk enabled more efficient regulation of sucrose concentration levels at the end of the sugarcane harvest due to the reduction of starch content (WU *et al.*, 2021; ZHANG *et al.*, 2020).

Fiber consists of cellulose, hemicellulose, lignin, pectins, proteins, and phenolic compounds and provides biomass for energy production (GE *et al.*, 2018). The increase in fiber content in the ASPC treatment (Figure 5C) did not reduce the sucrose concentration. In addition, the fiber content met the levels considered adequate for the industry, i.e., between 12 and 13%.

The stalk content of reducing sugars (Figure 5D) was lower in the ASPC treatment than the control, indicating that the nutrients and amino acids may have activated acid and/or neutral invertases. In mature cane, increased neutral invertase activity and low acid invertase activity are frequently observed and are correlated with the effective accumulation of sucrose in the stalks (BATTA *et al.*, 2011; JAIN; CHANDRA; SOLOMON, 2013).

The magnesium and potassium present in ASPC may have promoted the partitioning and transport of carbohydrates, resulting in increased sucrose content compared with the control. The amino acids present in ASPC, such as glycine, enable the accumulation of carbohydrates (sucrose, glucose, fructose), thereby providing a source of energy and carbon rings for the biosynthesis of polyphenols, which are generally related to plant defense responses (LIU *et al.*, 2016).

Stalk height and diameter were directly affected by ASPC application (Figures 6A and 6B). When water stress occurs in a critical period of sugarcane development, the rate of growth typically decreases (ZHANG *et al.*, 2020). The amino acids present in ASPC, especially proline, favor greater protection of the plant against lack of water. Glycine can improve cell membrane stability and protect the membrane from peroxidation during the plant development cycle and/or conditions of environmental stress (SOURI; HATAMIAN, 2019).

The smaller stalk diameter in the control treatment can be explained by the greater competition for photoassimilates between the leaves and stalk of the plant. There is a direct interaction of amino acids with plant nutrition, as amino acids increase the efficiency of the absorption, transport and assimilation of nutrients. The application

of ASPC may have promoted the more efficient use of water in the plant and the production of proteins required for chlorophyll synthesis, thereby increasing the photosynthetic rate and the transport of sugars to increase stalk growth and diameter (CASTRO, 2016; YARONSKAYA *et al.*, 2006).

Stalk and sugar yields were highest in the ASPC treatment. The application of amino acids reduced plant energy expenditure throughout the season, increased the activity of the antioxidant system, and improved the efficiency of protection against ROS. In addition, the plants developed well even under conditions of water stress, probably due to a more efficient primary metabolism, with direct positive responses of stalk and sugar yields (CASTRO, 2016; TAIZ *et al.*, 2017).

Bagasse and straw are directly affected by plant vegetative variations. The accumulation of dry mass in the culms may be associated with greater demand for carbon by the culms. In fact, the culm is a sink of high importance in the fate of photoassimilates (PAMMENTER; ALLISON, 2002), and the activity of sinks in sugarcane regulates the level of activity of the source (INMAN-BAMBER; JACKSON; HEWITT, 2011).

The stalk and fiber yields were used to calculate bagasse at 50% moisture, while straw was calculated considering 140 kg of straw per Mg of stalk (HASSUANI; LEAL; MACEDO, 2005). These parameters were clearly influenced by the application of ASPC. In addition, the higher biomass productivity of the ASPC-treated plants generated a direct response in the energy potential of the plants due to the increase in vegetative parameters, even under drought conditions.

The use of ASPS as a management practice to mitigate water stress in sugarcane crops resulted in better productivity even under adverse environmental conditions. ASPS acts directly on plant physiology, mobilizes defense enzymes, creates adequate conditions for metabolic functions, and promotes the synthesis of essential carbohydrates for full crop growth and development.

1.5 CONCLUSION

We present that the product under study based on macronutrients with amino acids, applied to sugarcane, is effective in the quality and production of stalks under water stress. Management of stress mitigation by ASPC application improves sugarcane stalk and sugar yields. Still, the ASPC action represents an alternative for the physiological and enzymatic response that can increase the assimilation of carbon processes and the metabolization. In addition to saving energy from plants, it acts positively on the plant's antioxidant system and on the synthesis of which, in turn, ASPC, carbohydrates, the concentration of reducing sugars and fibers, in addition to increasing the purity of the juice.

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CHAPTER 2

APPLICATION OF A PROTECTIVE FOLIAR COMPLEX TO ALLEVIATE SUGARCANE DROUGHT STRESS

ABSTRACT

Water stress negatively impacts plant morphology and physiology and leads to cellular damage due to metabolic oxidative stress. The application of nutrients and organic molecules, such as amino acids, may mitigate stress effects caused by drought. The aim of the present study was to evaluate the effects of a protective foliar complex (PFC) on late-harvest sugarcane (dry season). Two experiments were carried out in commercial areas of sugarcane production in the central-south region of Brazil in 2019 in fourth and second ratoons of the sugarcane variety CTC4. The treatments consisted of foliar application or no application of PFC at the beginning of the dry period in June at both sites. PFC was applied at a dose of 10 L ha^{-1} in a water volume of 100 L ha^{-1}. In PFC-treated plants, the activities of antioxidant enzymes [superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT)] increased, whereas the activity of polyphenol oxidase (PPO) decreased. The levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) decreased in PFC-treated plants. PFC application increased sucrose accumulation by an average of 2.26 Mg ha^{-1} and thus improved the quality of the raw material. The PFC also mitigated the damage caused by drought, with an average increase in stalk productivity of 10.7 Mg ha^{-1}. The positive effects of the PFC on plant antioxidant enzymes enabled greater accumulation of biomass and, in turn, energy cogeneration.

Keywords: *Saccharum* spp, oxigen reactive species, antioxidant metabolism, crop protection, stress management strategies

2.1 INTRODUCTION

Continued climate change has negatively impacted the productivity of most crops in the last decade (FAO, 2016) due to drastic reductions in arable areas and crop yields and increasing exposure to biotic and abiotic stresses. Stress is defined as excessive pressure from unfavorable factors that inhibit normal functioning of plant metabolism and restrict crop development and productivity. Abiotic stresses include drought, high temperatures, salinity, mineral deficiency and low soil pH (JONES, 1990; ASHRAF; FOOLAD, 2007).

The intensity and frequency of water stress or drought conditions are increasing globally (EFEOĞLU *et al.,* 2009), challenging agriculture in regions in which rainfall is

poorly distributed throughout the year. Such water deficits are particularly problematic for sugarcane production (RIBEIRO *et al.,* 2013; VIEIRA *et al.,* 2015). In Brazil, the expansion of sugarcane cultivation to less favorable production environments has further accentuated crop exposure to stressful conditions, impacting the entire industry sector (VIANNA; SENTELHAS, 2014).

The responses of plants, including sugarcane, to water deficit include both morphological and physiological changes. Morphological responses include inhibition of leaf and stalk growth, reduced stalk diameter and biomass accumulation, senescence, and smaller leaf area (MACHADO *et al.,* 2009; INMAN-BAMBER; LAKSHMANAN; PARK, 2012; JANGPROMMA *et al.,* 2012).

Physiological responses occur at the cellular level and include changes in the content of chlorophyll pigments (CHA-UM *et al.,* 2012; BANERJEE; ROYCHOUDHURY, 2019), cellular osmotic adjustment, early stomatal closure, and decreased quantum efficiency of photosystem II (TAKAHASHI; BADGER, 2011; BANERJEE; ROYCHOUDHURY, 2019).

Stress conditions also increase reactive oxygen species (ROS) (FOYER; SHIGEOKA, 2011), which weaken cellular redox homeostasis in favor of oxidizing molecules in what is known as oxidative stress (PINCIROLI *et al.,* 2018). ROS include superoxide anion (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-).

ROS are produced in the mitochondria, chloroplasts and peroxisomes due to the O_2 dependence of the metabolic processes of aerobic respiration, photosynthesis and photorespiration, respectively (BARBOSA *et al.,* 2014). Crop defense systems include antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX) and glutathione reductase (GR) (TAIZ *et al.*; 2017).

Mineral nutrients play an important role in regulating plant stress tolerance. These nutrients regulate the osmotic potential of plant cells, amino acid synthesis, mechanical strength, cell wall structure, stomatal opening and closure, and enzyme activation, including antioxidant enzymes (MARSCHNER, 2012; WARAICH *et al.,* 2012; CRUSCIOL *et al.,* 2013; FAGAN *et al.,* 2016).

The demand for these nutrients in plants is usually met by applying fertilizers to the soil for absorption by the plant through the roots. However, for applying amino acids and nutrients, such as P_2O_5, K_2O, Mg, B, Cu, Mn, Co, Mo, Zn, Fe foliar fertilization may

be more economically viable and effective (FAGERIA *et al.,* 2009), particularly under drought conditions, since the reduced water content in the soil reduces the absorption of water and nutrients (TISDALE; NELSON; BEATON, 1985).

Other strategies for limiting the effect of abiotic stresses are related to amino acids (HILDEBRANDT, 2018). Amino acids are precursors and constituents of proteins that are important for stimulating cell growth (RAI, 2002). Thanks to their acidic and basic groups, amino acids also function as buffers to help maintain favorable pH within the plant cell (WATERLOW, 1984; SADAK; ABDELHAMID; SCHMIDHALTER*,* 2015). Therefore, amino acids in general have known biostimulant activities that can attenuate the effects of abiotic stress, positively impact plant growth and development, and directly improve crop productivity (HILDEBRANDT, 2018; KHAN *et al.,* 2019).

Appropriate nutrition also attenuates the deleterious effects of stress by stimulating antioxidant enzymes in the defense system. Nitrogen (N), calcium (Ca), potassium (K) and magnesium (Mg) increase concentrations of SOD, CAT and POD, and zinc (Zn), copper (Cu), manganese (Mn) and iron (Fe) are SOD cofactors in different cell compartments (DINAKAR; DJILIANOV; BARTELS*,* 2012; WARAICH *et al.,* 2012).

It was hypothesized that PFC application would influence the plant antioxidant enzyme system to alleviate drought stress and improve biometric parameters, raw material quality, stalk and sugar yields, and biomass production.The aim of the present work was to evaluate the ability of a protective foliar complex (PFC) comprising mineral nutrients and amino acids to mitigate the effects of abiotic stress, particularly water stress, in sugarcane when applied to the leaves. The PFC was applied before the appearance of stress symptoms with the goal of preserving sugarcane yield and the industrial quality of the raw material, even under adverse abiotic conditions.

2.2 MATERIAL AND METHODS

2.2.1 Experimental area description

The experiments were carried out in fourth ratoon (Site 1) and second ratoon (Site 2) sugarcane (*Saccharum* spp. hybrids) of variety CTC 4, which is characterized by high stalk yield, excellent tillering, high sucrose content, medium to late ripening, harvest between June and September, medium demand for soil fertility and tolerance

to drought conditions. The experiments were installed in 2019 in two different localities in the state of São Paulo, Brazil, between June and August, the driest period of the year.

Site 1 is located at the Santa Adélia mill in Pereira Barreto – SP, Brazil (20º.38'.17” S, 51º.06'.09” W and average elevation of 335 m). Site 2 is located at the Ipê mill (Pedra agroindustrial group) in Nova Independência – SP, Brazil (21º.06'.14" S, 51º.29'.24" W and average elevation of 316 m). Soil classification and chemical characteristics were determined before the installation of the field experiments. Ten soil subsamples were collected from the experimental area between the sugarcane rows and combined into one composite soil sample for each site. Soil data from the experimental areas are shown in Table 1.

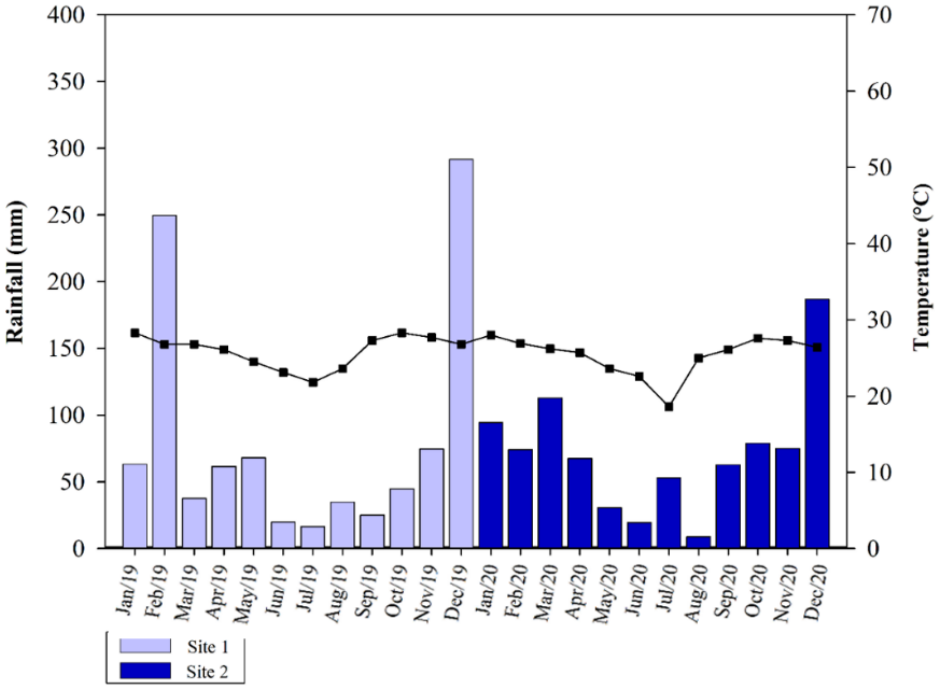
Both sites have a tropical savannah climate (Aw) according to the Köppen-Geiger climate classification system, with a defined dry season in winter. The annual averages of temperature and precipitation are 21.6 °C and 1237 mm at site 1 and 21.9 °C and 1250 mm at site 2 (CENTRO DE PESQUISAS METEOROLÓGICAS E CLIMÁTICAS APLICADAS À AGRICULTURA (CEPAGRI/UNICAMP), 2021). Climatological data for the study period were collected from meteorological stations located in Pereira Barreto, SP – Site 1, and Nova Independência, SP - Site 2 (AGRITEMPO, 2021) (Figure 1).

Table 1 - Soil classification and chemical characteristics at each experimental site

Location	Experimental Site	Soil classification	Depth (m)	pH $CaCl_2$	M.O. g dm^{-3}	$P_{(resin)}$ mg dm^{-3}	S	Al^{+3}	$H+Al^{+3}$	K	Ca	Mg	SB	CTC	Fe	Cu	Mn	Zn	B	V%
								----------		mmol$_c$ dm^{-3}			----------		----------		mg dm^{-3}	----------	%
Pereira Barreto (SP)	1	Rhodic Eutrudaf	0.0-0.2	4.9	13	7	6	1	17	2.65	15	5	23	40	29	0.2	8.2	0.4	0.8	58
			0.2-0.4	4.6	8	4	10	1	17	3.10	12	4	19	36	16	0.2	5.2	0.2	0.3	53
Nova Indepêndencia (SP)	2	Rhodic Eutrudox	0.0-0.2	4.7	9	6	9	1	18	0.27	13	4	17	36	8	0.4	7.4	0.5	0.6	49
			0.2-0.4	4.5	6	2	4	1	16	0.42	13	3	16	32	3	0.7	9.6	0.3	0.6	50

Source: by the author.

Figure 1 - Monthly rainfall and mean temperatures during the experimental period at site 1 and site 2



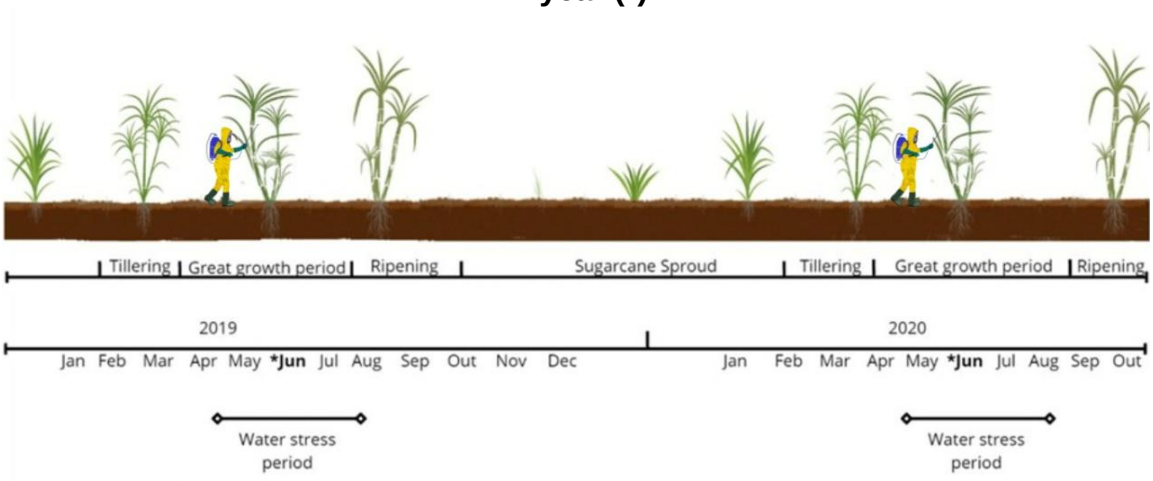
Source: by the author.

2.2.2 Experimental design and treatments

The experimental design followed a randomized blocks coordinate (RBC) design involving two treatments and twelve replications. Each plot consisted of eight rows with a length of 10 m and spacing between planting rows of 1.5 m. The two treatments were (1) control, no protective foliar complex application (PFC); and (2) PFC application. The PFC was applied in June 2019 (site 1) and in June 2020 (site 2). The sugarcane was harvested in October at both sites (Figure 2).

The PFC was applied at the full recommended dose of 10 L a.i. ha^{-1}. According to the technical recommendations of the manufacturer, the dose range for a water volume of 100 L ha^{-1} is between 5 and 15 L ha^{-1}. The concentrations of amino acids and nutrients in the PFC are reported as % (p/p) in Table 2. The PFC is classified as a mixed mineral foliar fertilizer. The PFC contains a multisaline aqueous solution, superconcentrated plant nutrients, and emollients, thickeners, moisturizers and vegetable oil to produce a stable suspension with an average density of 1.14 g/ml at 20°C and pH of 5.2 ± 0.4.

Figure 2 - Schematic experimental schedule with description of the conduct of the experimente and application of the protective foliar complex (PFC) each year (*)



Source: by the author.

Table 2 - Concentrations of amino acids and macro and micronutrients in the protective foliar complex (PFC)

	Macronutrients			Micronutrients							*Amino Acids								
	Mg	K_2O	P_2O_5	Zn	Mo	Mn	Fe	Cu	Co	B	Lys	Met	Met + Cys	Thr	Trp	Ile	Val	Arg	COT
Dose 10 L ha^{-1} (g)	46	285	205	205	57	34	30	46	4.6	57	13.68	5.13	6.84	9.12	3.42	10.26	10.26	15.96	912
Concentration (%)	0.4	2.5	1.8	1.8	0.5	0.3	0.26	0.4	0.04	0.5	0.12	0.045	0.06	0.08	0.03	0.09	0.09	0.14	8

*Lys - lysine; Met – methionine; Cys – cysteine; Thr – threonine; Trp – tryptophan; Ile –isoleucine; Val – Valine; Arg – arginine.
Source: by the author.

All applications were carried out under suitable environmental conditions in the early morning using pressurized spraying equipment (CO_2) with a single 1/4KLC-9 brass fieldjet application tip coupled to a 2.6-m-long rod, which enabled simultaneous and homogeneous application in four rows of plants with an application range of 7.5 m. The working pressure was 344 kPa for a water volume of 100 L ha^{-1}. The application and dosage of the products followed the recommendations of the manufacturers.

There were no problems with pests, weeds or diseases on the sugarcane at either site, and thus the management of the experimental fields was carried out according to the recommendations for each site following the mill's calendar for cultivation practices.

2.2.3 Leaf sampling for enzymatic analysis

For enzymatic sampling, the TVD (top visible dewlap) or leaf +1 was considered according to Van Dlllewijn (1952). The apex and the base of the leaf were discarded, and only the middle third was used to evaluate the contents of superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), peroxidase (POD; EC 1.11.1.7) and polyphenol oxidase (PPO; EC 1.10.3.1). Leaves were sampled between 9:00 and 10:00 am at 90 days after PFC application. For enzymatic analysis, the samples were quickly frozen in liquid nitrogen and stored at -80 °C in Falcon tubes.

2.2.4 Oxidative stress and antioxidant enzymes

Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) levels according to the method of Heath and Packer (1968) using a coefficient of 155 mM L^{-1}. The results were expressed in nanomoles of MDA per gram of fresh weight. Before enzymatic analysis, the total protein level was determined using the methodology described by Bradford (1976). According to Alexieva *et al.* (2001), the H_2O_2 content was determined by reference to a calibration curve and expressed in µmol g^{-1} of fresh weight (FW).

SOD activity was evaluated as described by Giannopolitis and Ries (1977) and expressed in units (U) of SOD per gram of protein. CAT activity was assessed as described by Havir and Mchale (1987) and expressed in nanomoles per minute per milligram of protein. POD activity was evaluated according to Allain *et al.* (1974) and

expressed in micromoles of H_2O_2 per minute per gram of fresh weight. Finally, PPO activity was evaluated according to Kar and Mishra (1976) and expressed in μmol catechol min^{-1} g^{-1} protein.

2.2.5 Sugarcane biometric evaluations

All biometric evaluations were performed prior to harvest at the ripening stage, 90 days after PFC application, using 20 random stalks collected from each plot. Two parameters were measured: (1) plant height (m) measured from the distance from the ground to the auricular region of the leaf +1 and (2) stalk diameter (mm) measured in the third sugarcane stalk internode (using a digital caliper) (MARAFON, 2012).

2.2.6 Sugarcane quality evaluations

At the same time as the biometric evaluations, the stalks were cleared at the height of the apical bud, defoliated and forwarded to the mill's PCTS laboratory to analyze the following characteristics: sucrose (%) (sucrose concentration in the stalk fresh weight (FW)), fiber % (dry water-insoluble matter in the sugarcane), purity % (sucrose present in the total solids content in cane juice), total reducing sugar (TRS) (all forms of sugars in sugarcane in the form of reducing or inverted sugars), reducing sugars (RS %) (reducing substances in cane and sugar products calculated as invert sugar—predominantly hexoses). The analyses were performed according to the methodology defined in the Sucrose Content-Based Sugarcane Payment System in accordance with Consecana's semiannual updates for the technological evaluations described by Fernandes (2011).

2.2.7 Stalk and sugar yields

At the same time as the biometric evaluations, two rows were defined within the useful area of each plot, and the stalk mass was evaluated in a 4-m length of each row. The stalks in these portions of the rows were weighed, and the result was extrapolated to obtain the stalk yield in Mg ha^{-1}. The sugar yield (Mg ha^{-1}) was then calculated by multiplying the values of stalk yield (Mg ha^{-1}) by the TRS divided by 100.

2.2.8 Biomass and energy production

Fiber and stalk yield were used to calculate bagasse at 50% moisture, and crop residue yield was calculated considering 140 kg of crop residue per Mg of stalks and 60% collection from the soil surface (HASSUANI; LEAL; MACEDO, 2005). Energy production was calculated considering that 1 Mg of crop residue has 4.96 MWh of primary energy and 1 Mg of bagasse has 4.94 MWh of primary energy (1 MWh = 3,600.00 MJ) (HASSUANI; LEAL; MACEDO, 2005).

2.2.9 Statistical analysis

For all data, normality was assessed using the Shapiro-Wilk test, and homoscedasticity was checked with Levene's test. Mean data were then submitted to individual analysis of variance (ANOVA) by the F-test ($p < 0.10$) and analyzed using Fisher's protected least significant difference (LSD) at ($p < 0.10$) when significant.

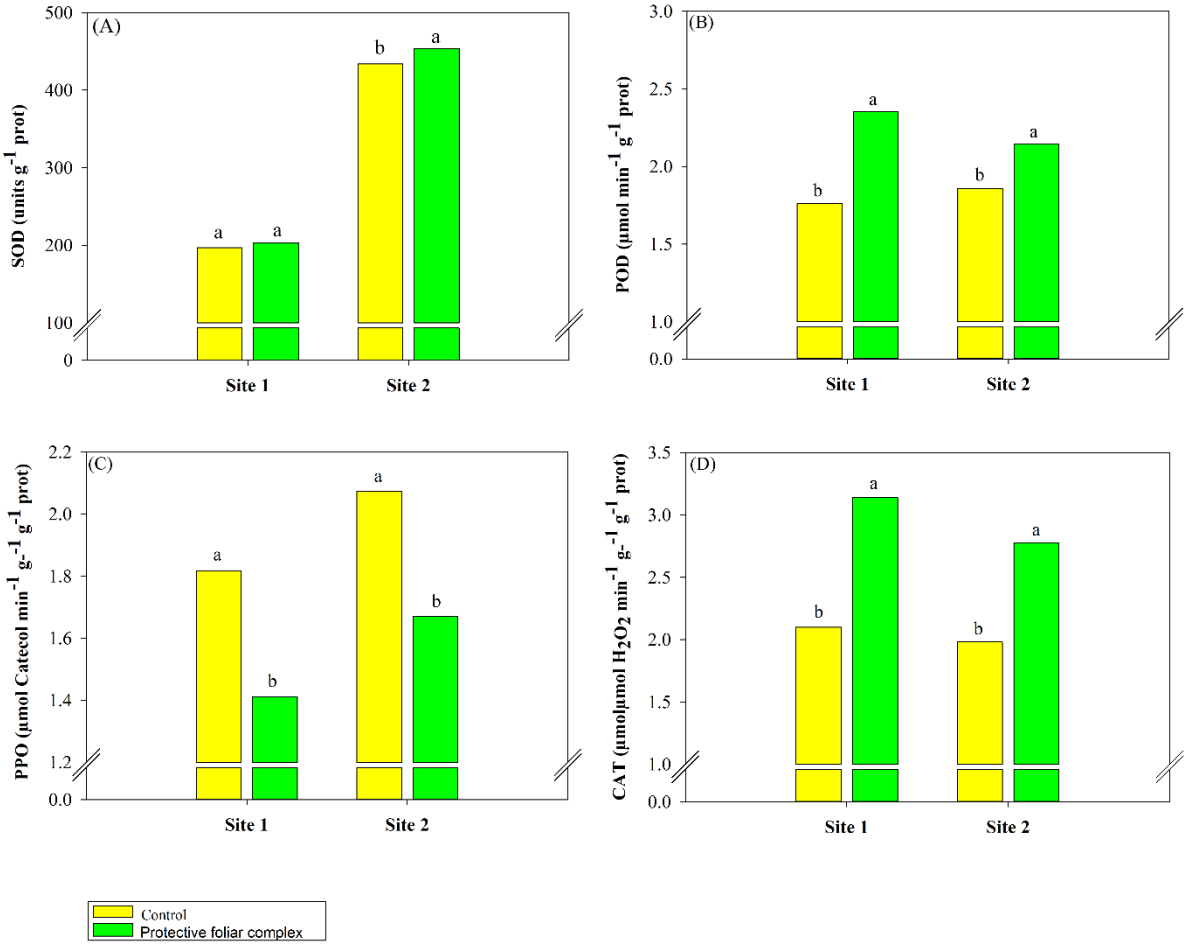
2.3 RESULTS

2.3.1 PFC application improves antioxidant enzyme activity

The enzymatic parameters were influenced by the application of the PFC (Figure 3A), except for SOD at site 1, which did not differ between the treatments. PFC application increased POD and CAT activity at both sites. At site 1, PFC application increased POD and CAT activity by 33.5% and 49.5%, respectively, compared with the control (1.76 µmol min^{-1} g^{-1} protein and 2.10 µmol H_2O_2 min^{-1} mg^{-1} protein, respectively) (Figures 3B and 3D). At site 2, PFC application increased SOD, POD, and CAT activity by 4.5%, 15.7% and 39.9% compared with the control (434 units g^{-1} protein, 1.85 µmol min^{-1} g^{-1} protein, and 1.98 µmol H_2O_2 min^{-1} mg^{-1} protein, respectively) (Figures 3A, 3B and 3D).

PPO activity was inversely proportional to SOD, POD, and CAT activity, and thus PFC application decreased PPO activity at both sites (Figure 3C). PPO activity decreased by 22% at site 1 and 19.3% at site 2 compared with the control (1.82 and 2.07 µmol catechol min^{-1} g^{-1} protein at sites 1 and 2).

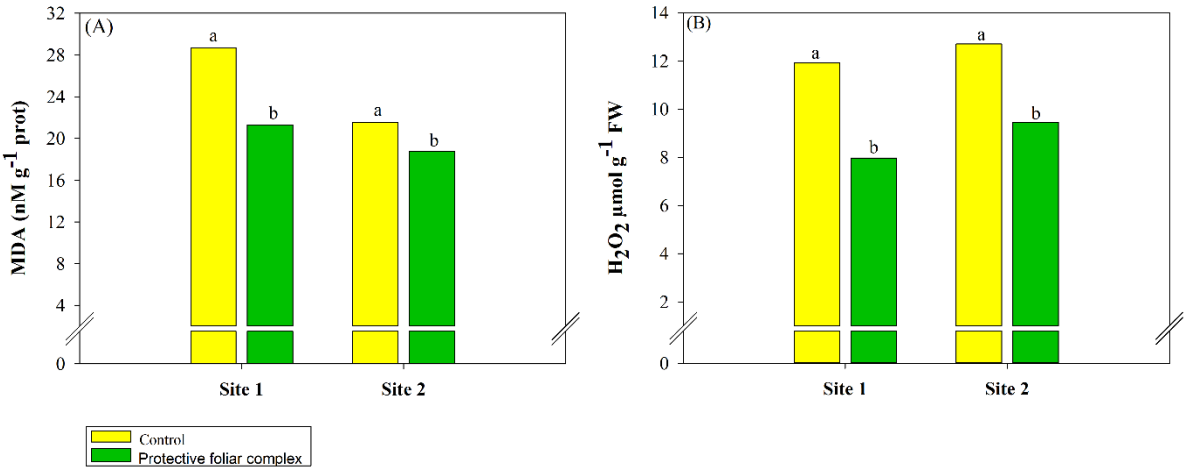
Figure 3 - Effects of protective foliar complex (PFC) application on sugarcane antioxidant enzyme parameters at harvest: (A) superoxide dismutase (SOD), (B) peroxidase (POD), (C) polyphenol oxidase (PPO), and (D) catalase (CAT). The treatments were as follows: Control, no PFC application; PFC: application of the PFC at both sites in June, in the beginning of the drought season. Averages followed by the same letters do not differ by the LSD test ($p < 0.10$)



Source: by the author.

The reactive potential of MDA was lower in the PFC treatment than in the control at both sites, with values of 21.29 and 18.78 nM g^{-1} protein in the PFC treatment and 28.67 and 21.54 nM g^{-1} protein in the control at sites 1 and 2, respectively (Figure 4A). Following the same trend, leaf H_2O_2 levels were also lower in the PFC treatment, with decreases of 33.0% and 25.6% compared with the control values of 11.91 and 12.71 µmol g^{-1} FW at sites 1 and 2, respectively (Figure 4B).

Figure 4 - Protective foliar complex (PFC) effects on lipid peroxidation parameters at harvest: (A) Malondialdehyde (MDA) and (B) Hydrogen peroxide (H_2O_2) of sugarcane. The treatments were as follows: Control, no PFC application; PFC: application of the PFC at both sites in June, in the beginning of the drought season. Averages followed by the same letters do not differ by the LSD test ($p < 0.10$)



Source: by the author.

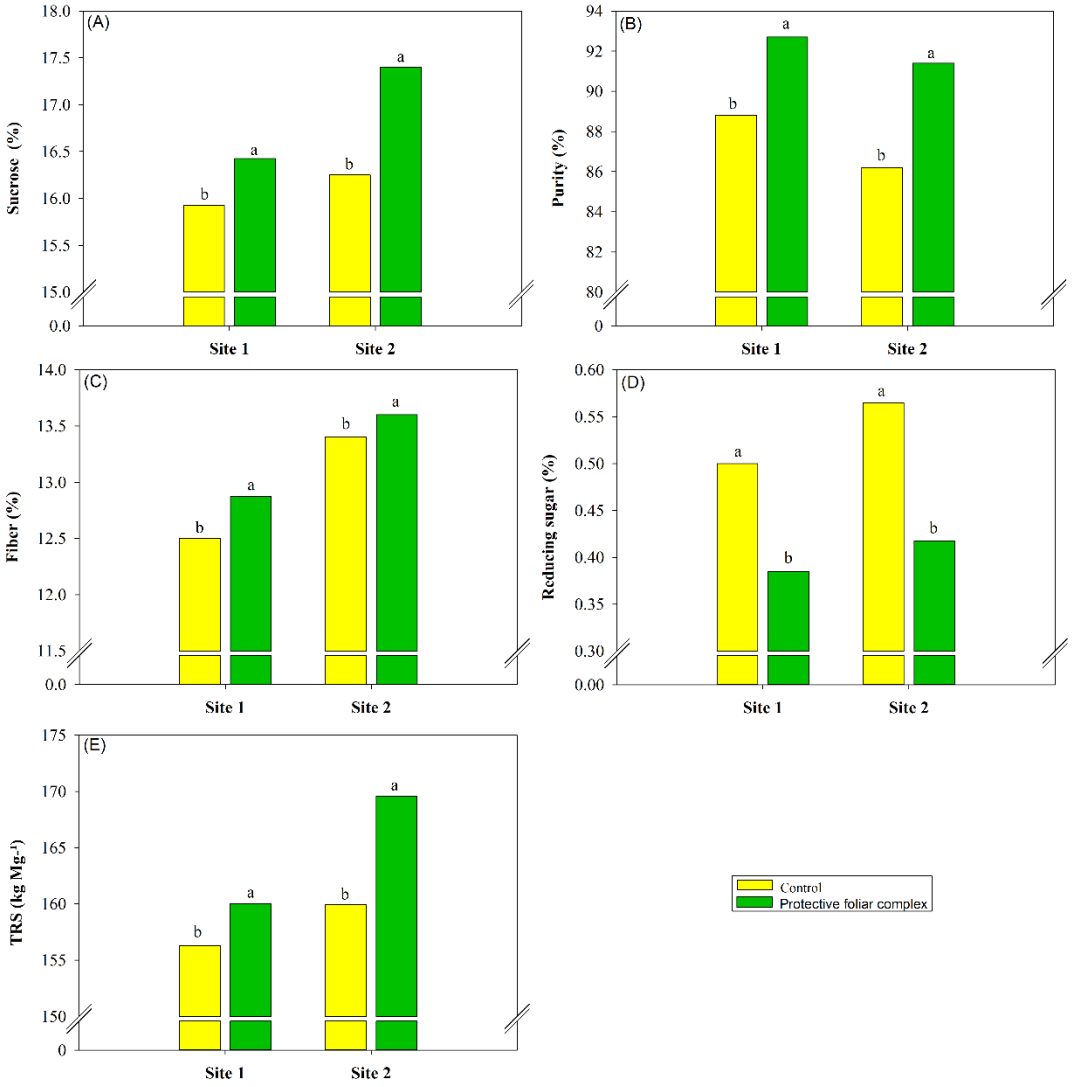
2.3.2 PFC application improves sugarcane quality

The sucrose concentration was 3.1% and 7.4% higher in sugarcane treated with the PFC than in the control treatment at both sites. The average sucrose concentration in the control treatment was 15.9 and 16.2% at sites 1 and 2, respectively (Figure 5A). In contrast to the sucrose concentration, the levels of reducing sugars decreased in sugarcane treated with the PFC at both sites (Figure 5D).

PFC application improved the quality of the raw material for industrial use, with a juice purity above 80% at both sites. The juice purity increased to 92.7% at site 1 and 91.3% at site 2 (Figure 5B). Similarly, fiber was increased in sugarcane treated with the PFC. The highest fiber content was obtained at site 1, where the fiber content was approximately 2.4% higher than the value of 12.5% in the control treatment (Figure 5C).

TRS is calculated from the concentrations of sucrose and RS (Figure 5E). As both parameters were influenced by the application of the PFC, TRS increased at both sites, particularly at site 2, where it increased by 6.1% compared with the value of 159.9 kg Mg^{-1} in the control treatment.

Figure 5 - Protective foliar complex (PFC) effects on sugarcane parameters of quality at harvest: (A) sucrose concentration (%), (B) purity (%), (C) fiber (%), (D) reducing sugars (%) and (E) total reducing sugars (kg Mg^{-1}). The treatments were as follows: Control, no PFC application; PFC: application of the PFC at both sites in June, in the beginning of the drought season. Averages followed by the same letters do not differ by the LSD test ($p < 0.10$)



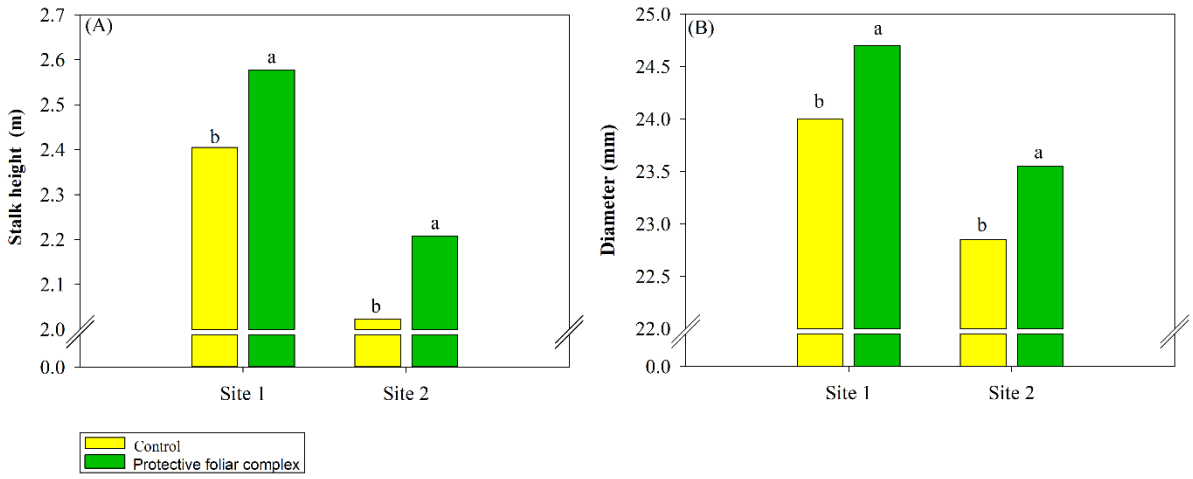
Source: by the author.

2.3.3 PFC application improves sugarcane biometric parameters

PFC application influenced biometric parameters at sites 1 and 2 (Figure 6A). Plant height increased by an average of 7.5% and 8.9% compared with the control values of 2.40 m and 2.02 m at sites 1 and 2, respectively. PFC application increased stalk diameter (Figure 6B) from 24.00 mm (control) to 24.70 mm at site 1 and from 22.86 mm (control) to 23.56 mm at site 2. It is noted in site 2 that despite being a

second ratoon sugarcane and the soil having low fertility, it influenced the growth of sugarcane with the application of PFC.

Figure 6 - Protective foliar complex (PFC) effects on sugarcane biometric parameters at harvest: (A) stalk height (m), (B) diameter (mm). The treatments were as follows: Control, no PFC application; PFC: application of the PFC at both sites in June, in the beginning of the drought season. Averages followed by the same letters do not differ by the LSD test ($p < 0.10$)

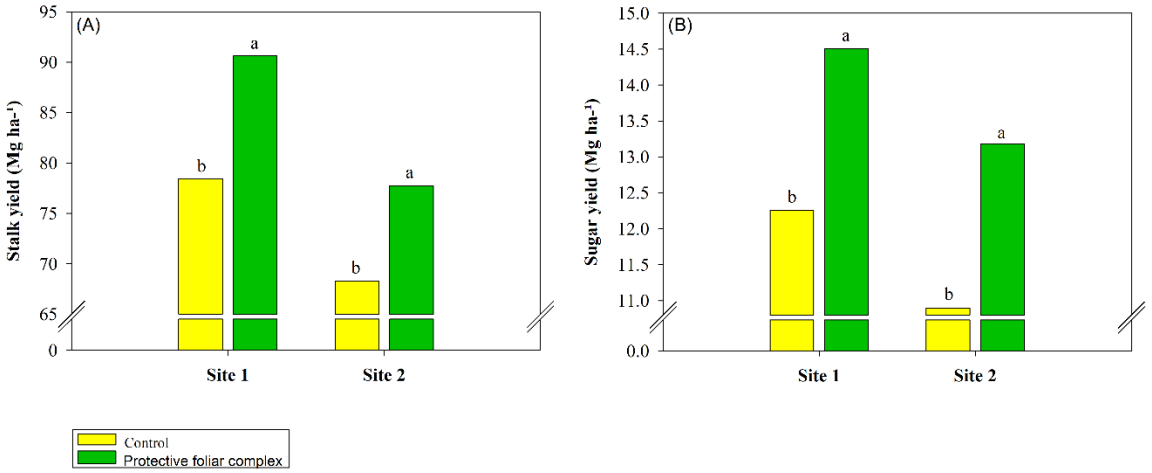


Source: by the author.

2.3.4 PFC application improves stalk and sugar yields

PFC application significantly increased stalk yield at both sites (Figure 7A), with average increases of 15.5% and 13.7% at sites 1 and 2 relative to the control (78.44 Mg ha^{-1} and 68.33 Mg ha^{-1}, respectively). Sugar yield is calculated as the product of TRS and stalk yield, so the increase in sugar yield observed in this study was directly related to the gain in stalk yield (Figure 7B). PFC application increased sugar yield by 18.2% and 21% compared with the control values of 12.26 Mg ha^{-1} and 10.89 Mg ha^{-1}, respectively, at sites 1 and 2.

Figure 7 - Protective foliar complex (PFC) effects on sugarcane yield parameters at harvest: (A) stalk yield (Mg ha^{-1}), (B) sugar yield (Mg ha^{-1}). The treatments were as follows: Control, no PFC application; PFC: application of the PFC at both sites in June, in the beginning of the drought season. Averages followed by the same letters do not differ by the LSD test ($p < 0.10$)

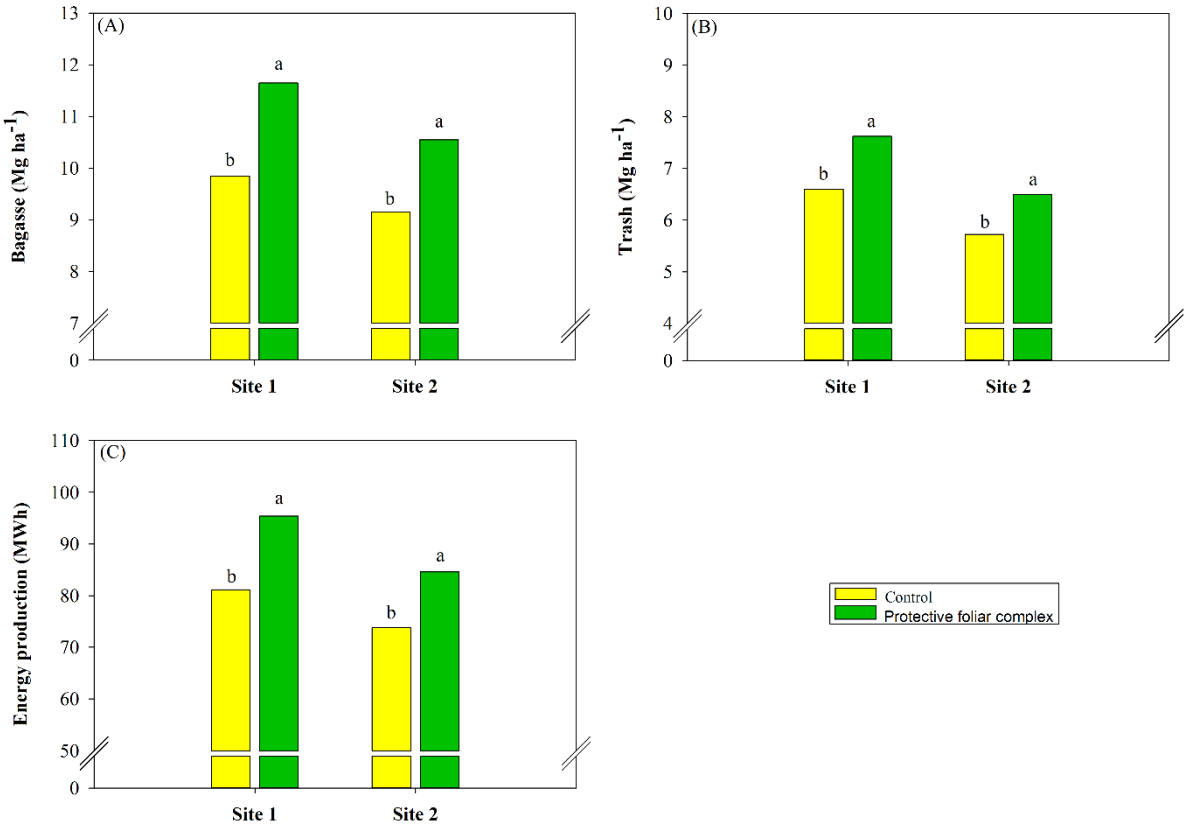


Source: by the author.

2.3.5 PFC application improves biomass and energy production

The application of PFC to sugarcane increased biomass production (bagasse and crop residue) at both sites (Figures 8A and 8B). At site 1, PFC application increased bagasse and crop residue production by 19.3% and 15.15% compared with the control values of 9.8 Mg ha^{-1} and 6.6 Mg ha^{-1}. A similar pattern was observed for energy production (Figure 8C); PFC application increased energy production by 17.4% and 14.6% compared with the control values of 81.2 MWh and 73.8 MWh at site 1 and site 2, respectively.

Figure 8 - Protective foliar complex (PFC) effects on sugarcane biomass and energy parameters at harvest: (A) bagasse (Mg ha^{-1}); (B) trash (Mg ha^{-1}) and (C) energy production (MWh). The treatments were as follows: Control, no PFC application; PFC: application of the PFC at both sites in June, in the beginning of the drought season. Averages followed by the same letters do not differ by the LSD test ($p < 0.10$)



Source: by the author.

2.4 DISCUSSION

This study focused on the morphophysiological strategies of plants in response to stress induced by drought conditions. Water stress is further compounded by oxidative stress induced by the overproduction of ROS, especially in chloroplasts, mitochondria and peroxisomes. These factors directly affect the efficiency of photosynthesis and inhibit sugarcane growth, resulting in lower crop yields (BARBOSA *et al.,* 2014; MAIA JÚNIOR *et al.,* 2019). Factors such as the severity, duration and combination of stresses determine the ability of plants to respond positively to the stressful environment. The plant response is dependent on phenological plasticity, which can be negated by excessive environmental patterns of drought, cold, and heat

(HAUVERMALE; SANAD, 2019). In this study, we evaluated the ability of a protective foliar complex (PFC) to mitigate the effects of water stress.

In site 2, despite being a second ratoon cane and the soil presenting very low levels of K_2O and Fe (Table 1), it is noted that the application of PFC positively influenced the evaluations carried out in this work. K_2O and Fe supplied via foliar PFC may have acted respectively in the regulation of stomatal opening and closing in guard cells, controlling water loss in plants (DECHEN; NACHTIGALL, 2007; CASTRO, 2016), and in electron transport in the photosynthetic apparatus, through the enzymatic activation of ferredoxins (CASTRO, 2016).

Improvements in water use efficiency by plants may be linked to the action of the enzymatic antioxidant system. As shown in Figure 1, the negative action of water stress on the redox balance of the plant favors ROS accumulation (WARAICH *et al.*, 2011; WARAICH *et al.*, 2012). This response was probably mitigated by the action of the PFC, as it enhanced the activity of antioxidant enzymes, such as SOD, CAT and POD.

Foliar application of the micronutrients B, Cu, Mn, Mo, Zn and Fe found in the PFC induces antioxidant responses and carbohydrate metabolism. According to Tavanti *et al.* (2021), foliar application of fertilizers containing B, Cu, Fe, Mn, Mo, Ni, Se and Zn at low concentrations has the ability to elicit and activate antioxidative enzymes, non-oxidizing metabolism, as well as sugar metabolism to mitigate damage by oxidative stress. Plants treated with micronutrients show higher tolerance to abiotic stress and better nutritional status.

These cellular-level responses work together to provide better protection against oxidative damage. In addition, the presence of amino acids reduces oxidative stress by decreasing harmful ROS concentrations in plants (SOURI, 2016). The dual benefits of amino acids and micronutrients in mitigating water stress have led to increases in their use in agriculture. In general, amino acids act as chelators to facilitate the absorption of nutrients, which act in primary metabolism to improve photosynthesis (SADAK; ABDELHAMID; SCHMIDHALTER, 2015; SOURI, 2016).

ROS formation is a plant response to unfavorable environmental conditions, including water stress (GUPTA; RICO-MEDINA; CAÑO-DELGADO, 2020). Under these conditions, plants alter their metabolism, physiology and morphology as part of their defense mechanisms. These changes have the greatest impact on the

photosynthetic mechanism, resulting in the production of large amounts of ROS (ALI *et al.*, 2020).

Several plant nutrients, especially micronutrients (Cu, B, Fe, Mn, Zn, Mo and Ni), have important roles in controlling oxidative stress. They activate different enzymes that function in the antioxidant defense mechanism of the plant, including SOD, CAT and POD (TAVANTI *et al.*, 2021). Once the activity of antioxidant enzymes increases (Figure 3), SOD dismutates O_2^- into H_2O_2, which CAT and POD convert to H_2O (WARAICH *et al.*, 2011). These activities relieve plant stress and promote metabolic function even under unfavorable conditions, thereby maintaining plant productivity. Moreover, by reducing H_2O_2 levels, these enzymes reduce cellular damage caused by lipid peroxidation, as indicated by the decrease in levels of MDA, an important biomarker of oxidative stress in plants (FERREIRA *et al.*, 2017; PERVEEN *et al.*, 2019; CONCEIÇÃO *et al.*, 2021).

As shown in Figure 4, the increased tolerance of PFC-treated sugarcane to water stress may have increased the accumulation of sucrose in stalks. Higher sucrose concentrations are directly linked to lower glucose and fructose concentrations (RS), and the juice purity of PFC-treated sugarcane was greater than 80%, which is recommended for raw material milling. However, PFC application did not decrease fiber content.

These results can be explained by the effects of phosphorus (P) and Mg absorbed by leaves on the metabolism and transport of sucrose. In addition, P increases the availability of metabolic energy via adenosine triphosphate (ATP), and its inorganic form (Pi) moves triose phosphate molecules from the chloroplast to the cytosol of cells. In this way, Pi acts as a signal to begin sucrose synthesis, since lower Pi levels indicate a predominance of starch synthesis, which occurs in chloroplasts (WANG; NING, 2019; ZHANG *et al.*, 2020; WU *et al.*, 2021). Mg is involved in the loading of carbohydrates into phloem and promotes the activation of enzymes linked to the synthesis of sucrose in the cytosol (TAIZ *et al.*; 2017; GARCIA *et al.*, 2020).

The attenuation of water stress might also be related to the presence of K_2O, which benefits osmoregulation, photosynthesis, protein synthesis, cation-anion balance, energy transfer, solute translocation and drought tolerance (MARSCHNER, 2012). Potassium impacts drought tolerance due to its involvement in the activation of the rubisco enzyme and in the opening and closing of stomata (OOSTERHUIS; LOKA; RAPER, 2013).

The results of this study also suggest that the greater accumulation of sugars in PFC treated sugarcane was due to the potentiated effect of nutrients together with amino acids present in the product. Previous studies in other plant species have shown that amino acids such as tryptophan, increase the total levels of carbohydrates and polysaccharides in stressed and non-stressed plants (AZIZ; MAZHER; FARAHAT, 2010; SADAK; ABDELHAMID; SCHMIDHALTER, 2015).

In addition, the accumulation of amino acids helps balance the opening and closing of stomata to maintain stable cellular water balance (KAMRAN *et al.*, 2009). Thus, amino acids improve the fixation of carbon, a substrate for carbohydrate synthesis. The application of PFC increased the vegetative parameters of sugarcane, particularly stalk height and diameter (Figure 6).

In corn, the application of amino acids threonine, aspartic, serine, glutamic, proline, glycine, alanine and valine was reported to increase plant height and diameter, with greater increases in plant height when amino acids were combined with zinc (EL-GHAREIB *et al.*, 2014). Jamro *et al.* (2002), working with foliar applications in sugarcane, verified that low doses of zinc and copper promoted greater plant heights, stem weight, number and length of internodes. They also observed that the higher rates of B and Mn increased all growth characteristics compared to their lower rates.

Other studies suggest that applying amino acids to plants can increase chlorophyll pigment content, which is directly related to the rate of photosynthesis. Improvements in primary metabolism lead to increases in secondary components such as sugars and proteins, which are necessary for cell formation and provide substrates for fresh and dry matter production (ABO BASHA; EL-AILA, 2015; SADAK; ABDELHAMID; SCHMIDHALTER, 2015). The presence of micronutrients such as Fe, Cu, and Mn also contributes to increasing photosynthetic pigments (KIRKBY; ROMHELD, 2004).

PFC application enhanced stalk and sugar yields ($p < 0.10$) in the present study (Figure 7), probably by activating the antioxidant system and improving water stress.by ameliorating water stress. The role of amino acids in plants as biostimulants is well documented, and amino acids strongly influence crop yield and growth by helping to mitigate abiotic stress in general (KOWALCZYK; ZIELONY; GAJEWSKI, 2008).

In fact, the combined application of amino acids and micronutrients creates a chelating effect in which the amino acids improve nutrient absorption by acting directly on ion transport and enhancing membrane permeability (MARSCHNER, 2012). Once

absorbed, amino acids act directly in the amination process, which promotes crop yield and dry matter production (SHEKARI; JAVANMARDI, 2017) by increasing the total content of amino acids, fats, carbohydrates and cellulose (OSUJI *et al.*, 2011).

In addition, the application of amino acids can promote ammonia fixation and influence C4 plant metabolism, which is linked to the biosynthesis of important cell components, such as flavonoids, isoflavonoids, stilbenes, aurones, cutin, suberin, sporopollenin, catechins, proanthocyanidins, lignans, lignins, phenylpropenes, acylated polyamines and many other alkaloid derivatives (FRASER; CHAPPLE, 2011). In general, the increased yields of stalk and sugar of PFC-treated sugarcane may be explained by the greater availability of substances required for growth, which generate substrates for protein synthesis in cellular tissues, and higher cell concentrations of osmoregulatory molecules (SADAK; ABDELHAMID; SCHMIDHALTER, 2015), which relieve water stress in the plant.

The phytotonic effect of the PFC was evident in the increases in crop biomass, bagasse (Figure 8) and crop residues, which are the result of gains in stalk yield and fiber (Figures 6 and 7). Bagasse volume is calculated using stalk yield and fiber at 50% moisture considering a crop residue estimate of 140 kg Mg^{-1} of crop residue per ton of stalk yield (HASSUANI; LEAL; MACEDO, 2005). This vegetative gain can be burned for use in electrical energy cogeneration. Thus, treating sugarcane with the PFC increased the energy potential of the plants (Figure 8), even under water stress conditions.

In summary, the PFC evaluated in this study is an important tool for mitigating water stress in sugarcane. As a result, lower sugarcane yields losses can be obtained even under abiotic stress. The nutrients and amino acids in the PFC helped maintain metabolic processes in sugarcane to promote positive responses of plant physiology and the antioxidant enzyme system. In addition, the PFC promoted the production of primary metabolites that are essential to the synthesis of metabolic energy and substrates for plant growth, resulting in increased biomass and sucrose accumulation.

2.5 CONCLUSION

This study demonstrated that applying a protective foliar complex comprising macronutrients, micronutrients and amino acids to sugarcane efficiently increased quality, stalk and sugar yields, and tolerance to drought stress. In addition, the application of the PFC positively stimulated the plant's physiological response and antioxidant system, which enhanced carbon fixation and decreased lipid peroxidation. As a result, sucrose accumulation and sugarcane juice purity increased, despite an increase in fiber content. Moreover, biometric parameters improved, including plant height and stalk diameter, resulting in greater biomass for use as a substrate in the cogeneration of electricity. Future work should examine the long-term effectiveness of the protective foliar complex in a complete crop cycle of sugarcane.

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FINAL CONSIDERATIONS

This study investigated during 2 sugarcane harvests in 4 different regions the effects of water stress at the end of the sugarcane cycle (ratoon) and the relationship between the effects of applying 2 water protectors after different against water stress . Both products at the sites can mitigate water stress, representing gains in productivity of stalk and sugar, biomass and energy, increased levels of antioxidant enzymes, as well as a higher quality of the juice of the final industrializable product.

In addition, the drought protection of both complexes is a new option for managing sugarcane under stress, improving biometric parameters such as taller and thicker stalks and, consequently, higher yields of stalks and sugars and a plant metabolically stronger. Relevant questions about isolating the synergistic or antagonistic effect of certain nutrients and amino acids that make up a fertilizer, as well as concentrations and doses throughout a complete sugarcane cycle and in long-term cultivation, deserve further investigation.

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