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MULTI-ELEMENT FOLIAR FERTILIZATION ASSOCIATED TO RIPENER IN SUGARCANE: ENZIMATIC ACTIVITY AND SUGAR AND ENERGY YIELD

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MULTI-ELEMENT FOLIAR FERTILIZATION ASSOCIATED TO RIPENER IN SUGARCANE: ENZIMATIC ACTIVITY AND SUGAR AND ENERGY YIELD

Thesis presented to São Paulo State University - School of Agriculture, to obtain Doctor degree in Agronomy (Agriculture)

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TÍTULO DA TESE: MULTI-ELEMENT FOLIAR FERTILIZATION ASSOCIATED TO RIPENER IN SUGARCANE: ENZIMATIC ACTIVITY AND SUGAR AND ENERGY YIELD

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“Whoever you are, whatever social position you have in life, the highest or the lowest, always aim for strength, a lot of determination and always do everything with a lot of love and a lot of faith in God, that someday you get there, somehow you get there”

Ayrton Senna.

ABSTRACT

Brazil is the world´s largest sugarcane producer and it has been one of the main agricultural crops grown in the country and it is extreme important for the national economic scenario. Foliar fertilizers application can provide productivity gains and technological quality for the crop. However, its use at the ideal time and the impacts of this management on the development of sugar cane are still little explored. Therefore, two studies were conducted over three subsequent harvest years, between 2017 and 2019, aiming to evaluate the effectiveness of multi-nutrient foliar applications at vegetative and maturation sugarcane stage, associated or not with ripener. In first study, 18 experiments were conducted at early harvest season, with foliar application occurring 120 days before harvest (vegetative stage) and 35 days before harvest (maturation stage), at different locations, measuring growth parameters, quality, energetic potential, and sucrose metabolism enzymes activity. In second study, 21 experiments were conducted at middle and late harvest sugarcane season, within application of multi-nutrients occurring 150 and 60 DBH, respectively, for each season at vegetative stage, and 30 DBH at maturation stage, evaluating stalk and sugar yield, estimate energy and metabolites in leaves. The studies were conducted at different locations in São Paulo and Minas Gerais state, and treatments were defined considering all applications possible, following a pattern for both studies, which were: (*1*) control with no applications of multi-nutrient foliar fertilizer and ripener (Control), (*2*) multi-nutrient foliar fertilizer applied at sugarcane vegetative stage (V), (*3*) multi-nutrient foliar fertilizer applied at sugarcane maturation stage (M), (*4*) ripener applied at maturation stage (R), (*5*) multi-nutrient foliar fertilizer applied both vegetative and maturation stages (VM), (*6*) multi-nutrient foliar fertilizer applied at vegetative stage plus ripener application at maturation stage (VR), (*7*) multi-nutrient foliar fertilizer applied at maturation stage plus ripener application also in maturation stage (MR), (*8*) multi-nutrient foliar fertilizer applied both vegetative and maturation stages plus ripener application at maturation stage (VMR). In summary, considering the two studies carried out, the multi-nutrient foliar application provided increases in plant growth parameters (height, diameter and stalk yield), with emphasis on treatments with two foliar applications (VM and VMR). In both studies was applied the same foliar fertilizer, which composition is N, K, Mg, S, B, Mn, Zn e Mo. Sulfometuron-methyl and bispiribaque-sódio were the ripener used. The ripener applied treatments increased sucrose, TRS

and sugar yield, and its association with fertilizers enhanced the observed gains, also observed in energy results. Considering growth and quality results, VMR treatment showed highest values in tons of sugar per hectare, through all years and seasons. It was concluded that multi-nutrient foliar fertilization contributed with higher sucrose synthesis and its association with ripener could lead to greater content of sugar in plant, consequently achieving higher productivity and quality of sugarcane.

Key words: *Saccharum spp.;* ripening; nutrient foliar application; sugar yield; metabolites; sucrose; enzymatic activity; sucrose phosphate synthase;

FERTILIZAÇÃO FOLIAR MULTI-ELEMENTAR ASSOCIADA À MATURADOR NA CANA-DE-AÇÚCAR: ATIVIDADE ENZIMÁTICA E PRODUTIVIDADE DE AÇÚCAR E ENERGIA

RESUMO

O Brasil é o maior produtor mundial de cana-de-açúcar, sendo esta uma das principais culturas agrícolas cultivadas no país e de extrema importância para o cenário econômico nacional. Dessa forma, a aplicação de fertilizantes via foliar pode proporcionar ganhos de produtividade e qualidade tecnológica para a cultura. No entanto, sua utilização no momento ideal e os impactos desse manejo no desenvolvimento da cana-de-açúcar ainda são pouco explorados. Para tanto, dois estudos foram desenvolvidos, ao longo de três safras subsequentes entre os anos de 2017 a 2019, com o objetivo de avaliar a eficácia da aplicação foliar de nutrientes na fase de crescimento vegetativo da planta e também na fase de maturação, associados, ou não, ao maturador. No primeiro estudo 18 experimentos foram conduzidos na época de início de safra, com a aplicação na fase vegetativa ocorrendo com 120 dias, em média, e na fase de maturação com 35 dias antes da colheita, em diferentes localidades, analisando os efeitos nos parâmetros de crescimento da planta, qualidade, potencial energético e atividade de enzimas que atuam no metabolismo da sacarose. No segundo estudo, 21 experimentos foram realizados nas épocas de meio e final de safra, com as aplicações na fase vegetativa ocorrendo com 150 e 60 dias antes da colheita, respectivamente, para cada época, e 30 dias na fase de maturação, avaliando produtividade de colmos, qualidade tecnológica, produção de energia e teor de metabolitos nas folhas. Os experimentos foram instalados em diferentes localidades, divididos entre os estados de São Paulo e Minas Gerais e os tratamentos foram definidos considerando todas as possibilidades de aplicação, padronizados para os dois estudos, sendo eles: (1) controle sem nenhuma aplicação (C), (2) fertilizante foliar aplicado no estágio vegetativo da cana-de-açúcar (V), (3) fertilizante foliar aplicado no estágio de maturação da cana (M), (4) maturador isolado aplicado no estágio de maturação (R), (5) fertilizante foliar aplicado nos estágios vegetativo e de maturação (VM), (6) fertilizante foliar aplicado no estágio vegetativo e maturador aplicado no estágio de maturação (VR), (7) fertilizante foliar aplicado no estágio de maturação juntamente com maturador (MR) e (8) fertilizante foliar aplicado

no estágio vegetativo e também no estagio de maturação, associado com maturador (VMR). Em ambos os trabalhos, utilizou-se o mesmo fertilizante foliar, composto pelos seguintes nutrientes: N, K, Mg, S, B, Mn, Zn e Mo. Os maturadores utilizados foram o sulfometuron-methyl e bispiribaque-sódio. Em síntese, considerando os dois estudos realizados, foi possível observar que a aplicação foliar de nutrientes proporcionou incremento nos parâmetros de crescimento da planta (altura, diâmetro e produtividade de colmos), com destaque para os tratamentos que receberam duas aplicações de nutrientes (VM e VMR). Os tratamentos com aplicação de maturador proporcionaram incremento nos teores de pol, ATR e produtividade de açúcar e a associação com o fertilizante foliar potencializou os ganhos observados, sendo este padrão também observado nos resultados de produção de energia. Considerando os resultados de produção e qualidade, o tratamento VMR apresentou os maiores resultados em toneladas de açúcar por hectare, ao longo dos anos e épocas. Assim, concluiu-se que a adubação foliar contribui com maior síntese de sacarose e sua associação com a aplicação do maturador proporciona maior acúmulo de açúcar na planta, consequentemente resultando em ganhos de produtividade e qualidade da matéria prima.

Palavras-chave: *Saccharum spp.*; maturação; adubação foliar; produtividade de açúcar; metabólitos; sacarose; atividade enzimática; sacarose fosfato sintase;

SUMMARY

INTRODUCTION

Brazil is the largest sugarcane (*Saccharum* spp. hybrids) producer in the world, global leader in the production of sugar and its derivatives, and will remain by 2030 (OECD; FAO, 2021). Sugarcane predominantly grows in tropical and subtropical regions with well-distributed rainfall and can be harvested after 12 to 18 months in the first harvest and cultivated up to five consecutive years in the same area. The crop products allow flexibility to produce sugar and ethanol in the industry, as well as molasses or thick juice and the residue from sugarcane crushing (bagasse) to supply energy production (raw material for cogeneration for electricity) (BOARETTO; MAZZAFERA, 2013). However, its productivity is below the genetic potential (WACLAWOVSKY et al., 2010), caused by biotic and abiotic stresses during the entire phenological sugarcane stages (CHEAVEGATTI-GIANOTTO et al., 2011; DINARDO-MIRANDA; VASCONCELOS; LANDELL, 2008).

In south-central region in Brazil, one of the most important sugarcane producer areas, the harvest season usually begins in March to April, and ends in November and December, period that presents high temperatures and rainfall, in which climatic conditions promote the vegetative growth of the plant as well as sucrose accumulation (CASTRO, 2016).

For the adequate development of sugarcane, two climatic conditions are necessary. The first type of climatic condition refers to the occurrence of a hot and humid season to guarantee germination, root growth, tillering and complete vegetative development, which occurs with the beginning summer, covering the early harvest season. The second climatic condition is with low temperatures and water deficit aiming to stimulate, through stress to the plant, the physiological process of natural maturation, occurring in winter and beginning of spring, a period that encompasses middle and end-harvest season (CAPUTO et al., 2008).

Sugarcane greatest vegetative growth period occurs under high light intensity, high temperature and water availability, conditions that allow greater formation of leaves, stems, and root system, due to the use of photoassimilates for plant development (HUMBERT, 1984; VIANA, 2007). Therefore, at early harvest season, climatic conditions are favorable to sugarcane vegetative growth, this being the higher production crop period, where nutritional management drives increase in yield gains. On the other hand, at this season, the conditions for natural maturation are critic, thus,

the use of ripeners at early harvest season has become a frequently used practice in sugarcane cultivation, seeking to anticipate the plant maturation, promote improvements in the quality of the raw material, optimizing industry results and assisting in harvest planning (CAPUTO et al., 2008).

Ripeners, or also known as plant regulators, are defined as substances whose physiological effects are similar to those of some known hormones, in which are chemical compounds that modify plant morphology and physiology, interrupting or delaying the plant growth, changing its metabolism and directing the sucrose synthesized for storage in the sugarcane stems (LEITE et al., 2011).

Under opposite conditions to vegetative growth (low temperatures and low water availability), the plant stops its growth, and a change occurs in its metabolism, which directs the sucrose synthesized in the leaves to accumulate in the vacuole of the parenchyma cells of the stem (ALEXANDER, 1973; DEUBER, 1978; ANDRADE; CARDOSO, 2004). In the mid-late sugarcane season in southeastern Brazil, the reduction of temperatures, drought and reduction nutrients uptake from the soil is frequent, thus impairing the activity of enzymes responsible for the photosynthetic process and consequently the growth and development (SCUDELETTI et al., 2021), requiring further studies during this harvest season.

New and advanced agricultural technologies and management need to be promoted and implemented to mitigate stress caused by environment and improve the production and quality of raw materials. Some studies highlight that micronutrient application is a great option to promote gains in crop productivity and quality (JAMRO et al., 2002; PROM-U-THAI et al., 2020; STEWART et al., 2020; ZEWAIL et al., 2020), but information on the use of foliar fertilization of a multi-nutrient complex is scarce in sugarcane.

Foliar fertilizer application has been used to protect the photosynthesis process through regulation of primary plant metabolism to increase productivity and quality gain, but this is a rule due to the limitations imposed by the stress of extreme heat, cold, drought and high incidence of light (WANG et al., 2003; HEFFERNAN, 2013; TEIXEIRA et al., 2013; SLAMA et al., 2015; SOLIMAN et al., 2019; ELSHEERY et al., 2020; GUPTA, RICO-MEDINA, CANO-DELGADO, 2020; SOLIMAN et al., 2020).

Knowing the many metabolic and structural functions of nutrients and their requirements throughout the crop cycle, as well as the right moment that plants need them most, can facilitate management and provide improvements in crop quality and

productivity (EPSTEIN; BLOOM, 2005; MARSCHNER, 2012). The maintenance of the photosynthetic apparatus and osmoprotector production and accumulation by the foliar fertilization of a multi-nutrient complex seems to be an interesting strategy to mitigate stresses and increase the productivity and quality of sugarcane.

In this scenario, the efficiency of applying foliar nutrients at vegetative and in the maturation crop stage through different harvest seasons, combined or not with ripener, looking for improvements in technological quality and productivity as well as its impact on plant metabolism, has not been widely recognized in sugarcane.

The hypothesis raised in study was that a multi-nutrient foliar fertilizer applied at final vegetative and maturation development stages of sugarcane can promote improvements in growth and quality yield; foliar fertilizer combined with ripener application may provide higher theoretical recuperable sugar and sugar yield than only ripener application; energy production must be greater with multi-nutrient foliar application; and foliar fertilization applied both vegetative and maturation stage could lead to higher growth parameters results.

In order to evaluate this hypothesis, as there are different agronomic managements for each harvest season, this thesis is composed by two chapters with several experiments applying nutrient and ripener to sugarcane in three harvest seasons (early, middle and late harvest seasons). Following similar management of nutrient and ripener, first chapter focused in sugarcane in the early harvest (March-April) that plant has high yields and most common cultivated; however, it is far from achieving the sugarcane potential yield production and still needs information about sugarcane metabolism. The second chapter aimed to evaluate the sugarcane response in the middle and late harvest seasons (Winter-Spring) that are lack of knowledge in technology of multi-nutrient foliar applications and in which stage of plant development could potentially provide greater results, especially because the sugarcane harvest at these two seasons has low yields and requires more studies in its metabolism improvements.

CHAPTER 1

EARLY-HARVEST SUGARCANE RESPONSE TO FOLIAR MULTINUTRIENT FERTILIZATION AND RIPENER IN MULTI-SITES

ABSTRACT

The innovative management of sugarcane production by applying foliar multinutrient fertilizers and ripeners may enhance the global production of renewable fuels, sugar, and energy. These chemical products promote plant growth, sucrose accumulation, and metabolic processes to increase the yields of sugarcane and its products per unit area. However, the effects of combining these practices and the most effective sugarcane development stages for application are unknown. This study evaluated the effects of applying foliar multinutrient (N, K, Mg, S, B, Mn, Zn and Mo) fertilizer and ripener alone or in combination on sugarcane plant development, quality parameters, product production, and enzymatic activities. Eighteen field experiments with early-harvest sugarcane were conducted during three growing seasons in south-central Brazil. Eight treatments were performed as completely randomized blocks with four replicates. The treatments comprised isolated or combined applications of foliar fertilizer (at the vegetative and/or maturation stages) and ripener (at the maturation stage). The biometric parameters of sugarcane were highest when the foliar multinutrient fertilizer was applied at the vegetative stage (V), at the vegetative and maturation stages (VM), at the vegetative stage combined with ripener at the maturation stage (VR), and at the vegetative and maturation stages combined with ripener at the maturation stage (VMR). Compared with the control, these treatments increased stalk yield by averages of 7.2, 10.5, 5.9 and 9.2 Mg ha^{-1}, respectively. Ripener application resulted in the highest theoretical recoverable sugar content, regardless of whether foliar multinutrient fertilizer was supplied (increases of 7.2 kg sugar Mg $stalk^{-1}$). Foliar multinutrient fertilization at both stages and ripener application increased sugar yield by an average of 2.1 Mg ha^{-1}. Energy production was highest in V, VR, VM, and VMR, with average increases of 7.8% and 5.68 kWt. Additionally, near sugarcane harvest, neutral invertase activity was highest and sucrose synthase activity was lowest in VMR. Our results indicate that combined management with foliar multinutrient fertilization at the vegetative and maturation stages and ripener application at the maturation stage is a feasible strategy to enhance sugarcane plant development, stalk and sugar yields, and metabolic enzyme activity.

Keywords: *Saccharum* spp., source-sink relationships, sucrose, plant growth regulators, maturation

1.1 INTRODUCTION

Brazil is the world's largest producer of sugarcane (*Saccharum* spp.). In the 2022/2023 growing season, total sugarcane production in Brazil was 678 million Mg—81 Mg ha^{-1} on approximately 8.5 million ha of cultivated area (CONAB, 2023). By 2030, Brazil is expected to produce 36% of global sugarcane (OECD, 2021), and the resulting ethanol will represent 21% of the global biofuel supply, making a substantial contribution to mitigating climate change and CO_2 emissions from automobiles (Moreira and Pacca, 2020; OECD, 2021). Sugarcane is also an important source of bagasse for bioenergy and 20% of global sugar production (OECD, 2021). To meet the high global demand for sugarcane without expanding its cultivated area, improvements are needed in yields, which remain far below the genetic and productive crop potential of 118–157 Mg ha^{-1} (Waclawovsky et al., 2010; Marin et al., 2016).

One option for improving sugarcane development and quality is the application of foliar multinutrient fertilizers and ripeners. To stimulate growth and productivity, foliar fertilizers are typically applied at the tillering and stalk growth stages, i.e., 40–120 and 120–270 days after planting, respectively (Jamro et al., 2002; Franco et al., 2011; Ghaffar et al., 2012; Ismail et al., 2016). These fertilizers provide nutrients that participate in the plant's physiological, metabolic, and morphological processes as components of plant structure or cofactors of the structure or activity of approximately one-third of plant enzymes (Arnon and Stout, 1939; Epstein and Bloom, 2005; Nelson and Cox, 2012; Buchanan et al., 2015). At the end of the stalk growth and maturation stages, these nutrients activate enzymes, including antioxidant enzymes; stimulate photosynthetic activity; minimize oxidative stress; and consequently reduce premature leaf senescence (Foyer, 2018; El-Mogy et al., 2019; Tavanti et al., 2021). Among the enzymatic processes affected directly and indirectly by nutrients, sucrose synthesis is one of the most important (Afzal et al., 2021; Vasantha et al., 2021). The main enzymes in carbohydrate metabolism are soluble acid invertase (SAI), neutral invertase (NI), sucrose synthase (SuSy), and sucrose phosphate synthase (SPS), which act in both the synthesis and breakdown of sucrose (Alexander, 1973; Leite et al., 2009, 2015).

In addition to applying foliar fertilizer in the vegetative and maturation stages, a common practice in sugarcane production is to apply growth regulators known as ripeners during the maturation stage. In south-central Brazil, the largest area of production in the country, the prevailing weather conditions in the early harvest season between March and June are high temperatures, high rainfall, and high solar radiation. These conditions favor vegetative development and delay natural maturation (Castro, 2006; Leite et al., 2015). Natural maturation

occurs under specific conditions that reduce stalk growth and direct the photosynthate produced in the leaves to accumulate in the storage parenchyma cells: low temperatures, rainfall, photoperiod, and thermal amplitude (Alexander, 1973; Castro, 2006; Marin et al., 2016). Ripeners are a necessary tool to delay vegetative development and stimulate the storage of the produced photosynthates (Leite et al., 2009). Ripener application also increases the flexibility of harvest management and planning and the quality of the raw material (Morgan et al., 2007; Caputo et al., 2008; de Almeida Silva and Caputo, 2012). The combined application of foliar fertilizers and ripener increases sucrose yields by 1.8-fold compared to the increase in sugarcane yield (Jackson et al., 2000; Inman-Bamber et al., 2008). Increasing sucrose production is important because it reduces the costs associated with harvesting, transporting, and grinding stalks.

The effectiveness of the foliar application of nutrients and ripeners is dependent on several factors, including management and timing of application (Férnandez and Brown, 2013; Férnandez et al., 2013). There have been few studies of the simultaneous use of foliar fertilizers and ripeners in the maturation stage, and conflicting effects of this practice on raw material quality have been reported (Pawar et al., 2003; Jain et al., 2013). To address this gap, this study examined the effects of foliar application of a multinutrient fertilizer in association with ripener on the quality and productivity of early-harvest sugarcane. The research was guided by the following hypotheses:

(*i*) The foliar application of nutrients in the vegetative stage of stalk growth and in the maturation stage will increase stalk productivity and stalk sucrose accumulation.

(*ii*) The application of ripeners in the maturation stage will improve the industrial quality of the raw material by increasing the theoretical recoverable sugar content.

(*iii*) Combining foliar fertilization in the final stage of stalk growth and in the maturation stage with ripener application will increase production in tons of stalks per hectare, sucrose per ton of stalks, and consequently sugar production per hectare.

1.2 MATERIALS AND METHODS

1.2.1 Site description

Eighteen field experiments were conducted at commercial sugarcane plantations in south-central Brazil at nine sites in São Paulo State (SP) and one site in Minas Gerais State (MG) for three crop years (2017/2018, 2018/2019 and 2019/2020). All experiments were performed in the early-harvest sugarcane season (autumn, April-June) and were distributed as follows: five experiments in 2017/2018 (experiments 1–5), eight in 2018/2019 (experiments 6–13), and five in 2019/2020 (experiments 14–18). Table 1 presents the data on foliar applications, harvest time, geographic coordinates, cultivars, and row spacing. The cultivar was chosen according to the characteristics of each site and recommendations for the early-harvest season (Daros et al., 2010; UDOP, 2018).

According to Köppen's classification, the climate is Aw (dry winters) at Pontal-SP, Indiaporã-SP, Barrinha-SP, Populina-SP, and Guará-SP (experiments 1, 2, 8, 9, 14, 15, and 16); Cfa (humid, hot summers) at Iturama-MG, Santa Maria da Serra-SP, and Iguaraçú do Tiête-SP (experiments 3, 4, 10, 11, and 17); and Cwa (hot summers and dry winters) at Luis Antônio-SP (experiments 5, 12, 13, and 18). Precipitation and temperature (mean minimum and maximum) data were obtained from meteorological stations near each site (Figure 1). The average temperature range for the sites was 20.8–23.5°C in 2017/2018, 21.0–23.1°C in 2018/2019, and 21.0–22.8°C in 2019/2020.

Soil classification was performed using the international system (Soil Survey Staff, 2014). Prior to the installation of the experiments, the soil characteristics at depths of 0.00–0.25 and 0.25–0.50 m were determined, as shown in Table 2, and soil correction and fertilization were carried out according to the recommendations of van Raij et al. (1997). None of the selected sites had a history of vinasse use and all phytosanitary and pest management was performed according to recommendations for sugarcane cultivation.

Table 1. Sugarcane cultivar, ratoon, and date of establishment and first and second application and harvest in each season.

Site	Experiment	Latitude	Longitude	Cultivar	Row Spacing	Ratoon	1st Application*	2nd Application	Harvest
Pontal (SP)	1	21°02'06.0"S	48°05'18.6"W	RB85-5456	0.90 x 1.60	3	Jan. 2017	May. 2017	Jun. 2017
Indiaporã (SP)	2	19°53'42.0"S	50°19'22.0"W	IACSP95-5000	1.50	2	Jan. 2017	Apr. 2017	May. 2017
Iturama (MG)	3	19°50'29.3"S	50°17'44.3"W	RB96-6928	1.50	2	Jan. 2017	Apr. 2017	May. 2017
Santa Maria da Serra (SP)	4	22°34'18.8"S	48°13'28.6"W	RB96-6928	0.90 x 1.50	1	Jan. 2017	Apr. 2017	May. 2017
Luís Antônio (SP)	5	21°33'03.2"S	47°49'48.4"W	RB85-5156	1.50	2	Jan. 2017	Mar. 2017	May. 2017
Pontal (SP)	6 and 7	21°02'06.0"S	48°05'18.6"W	RB85-5536	0.90 x 1.60	3	Dec. 2017	Mar. 2018	May. 2018
Indiaporã (SP)	8 and 9	19°53'42.0"S	50°19'22.0"W	IACSP95-5000	1.50	3	Jan. 2018	Apr. 2018	Jun. 2018
Igaraçú do Tiête (SP)	10 and 11	22°33’39.3” S	48°28’39.5” W	RB96-6928	0.90 x 1.50	1	Jan. 2018	Apr. 2018	May. 2018
Luís Antônio (SP)	12 and 13	21°33'03.2"S	47°49'48.4"W	RB85-5156	1.50	3	Jan. 2018	Apr. 2018	May. 2018
Guará (SP)	14	21°08’43.9”S	48°04’23.8"W	CTC 4	1.50	2	Dec. 2018	Mar. 2019	Apr. 2019
Barrinha (SP)	15	21°26’20.1”S	48°02’06.8”W	CTC 4	1.50	2	Dec. 2018	Mar. 2019	Apr. 2019
Populina (SP)	16	19°53’39.40”S	50°28’35.93”W	RB96-6928	1.50	1	Jan. 2019	Apr. 2019	Jun. 2019
Igaraçú do Tiête (SP)	17	22°33’39.3” S	48°28’39.5” W	RB96-6928	0.90 x 1.50	2	Feb. 2019	May. 2019	Jun. 2019
Luís Antônio (SP)	18	21°33'03.2"S	47°49'48.4"W	RB85-5156	1.50	4	Dec. 2018	Mar. 2019	Apr. 2019

*****First application was performed at vegetative stage, second application at maturation stage.

Table 2. Soil classification and chemical characteristics of experiments at each site.

Site	Experiment	Soil classification	Depth	pH	S.O.M.	$P_{(resin)}$	SO_4^{-2}	Al^{+3}	$H+Al^{+3}$	K	Ca	Mg	BS	CEC	V%
				$CaCl_2$	g dm^{-3}	mg dm^{-3}			----------	mmol$_c$ dm^{-3}			----------		%
Site 1 Pontal (SP)	1	Rhodic Hapludox	0.00-0.25	5.7	31	23	17	0.0	19	14	48	23	85	104	82
			0.25-0.50	5.7	20	3.0	10	0.0	19	7.9	22	10	40	59	68
Site 2 Indiaporã (SP)	2, 8 and 9	Rhodic Eutrudox	0.00-0.25	5.3	35	31	15	0.0	23	2.6	40	17	60	79	76
			0.25-0.50	5.2	16	54	21	0.0	19	0.9	21	9.0	31	50	62
Site 3 Iturama (MG)	3	Rhodic Eutrudox	0.00-0.25	5.6	21	36	18	0.0	17	8.8	34	9.0	52	71	73
			0.25-0.50	5.2	21	58	25	0.0	18	6.0	18	8.0	32	51	63
Site 4 Santa Maria da Serra (SP)	4	Typic Quartzipsamment	0.00-0.25	5.4	24	18	17	0.0	24	3.6	51	12	67	91	74
			0.25-0.50	5.2	13	6.0	34	1.0	19	2.3	28	6.0	36	55	66
Site 5 Luis Antônio (SP)	5, 12, 13 and 18	Typic Quartzipsamment	0.00-0.25	5.4	20	30	10	0.0	14	1.2	29	15	45	59	76
			0.25-0.50	5.5	10	8.0	13	0.0	12	0.5	13	6.0	20	32	62
Site 6 Pontal (SP)	6 and 7	Rhodic Hapludox	0.00-0.25	5.7	13	11	16	0.0	23	1.6	31	17	50	73	68
			0.25-0.50	5.5	12	5.0	36	0.0	19	0.6	18	11	30	49	61
Site 7 Igaraçú do Tiête (SP)	10, 11 and 17	Typic Quartzipsamment	0.00-0.25	5.2	10	12	6.0	0.9	11	0.6	13	8.0	22	33	66
			0.25-0.50	5.0	9.0	8.0	9.0	1.1	14	0.6	15	6.0	22	36	61
Site 8 Barrinha (SP)	14	Rhodic Hapludox	0.00-0.25	5.1	2.1	28	8.0	0.0	24	0.4	37	17	54	73	74
			0.25-0.50	5.4	1.3	17	19	0.4	27	0.1	16	8.0	24	43	56
Site 9 Populina (SP)	15	Typic Eutrudox	0.00-0.25	5.6	35	31	12	0.0	23	2.6	40	17	60	79	76
			0.25-0.50	5.5	16	54	23	0.0	19	0.9	21	9.0	31	50	62
Site 10 Guará (SP)	16	Rhodic Eutrudox	0.00-0.25	6.7	25	un.**	un.	0.1	14	11.5	59	33	104	123	84
			0.25-0.50	6.5	23	un.	un.	0.1	14	10.5	51	27	88	107	82

***** SOM: Soil Organic Matter; BS: Base Saturation; CEC: Cation Exchange Capacity.

**un. Uninformed.

Figure 1. Rainfall (mm) and mean maximum and minimum temperatures in all experiments: in 2017: exp 1 (Pontal, SP), exp 2 (Indiaporã, SP), exp 3 (Iturama, MG), exp 4 (Santa Maria da Serra, SP), and exp 5 (Luís Antônio-SP); in 2018: exp 6 and 7 (Pontal, SP), exp 8 and 9 (Indiaporã, SP), exp 10 and 11 (Igaraçu do Tiête, SP), and exp 12 and 13 (Luís Antônio, SP); in 2019: exp 14 (Barrinha, SP), exp 15 (Populina, SP), exp 16 (Guará, SP), exp 17 (Igaraçú do Tiête, SP) and exp 18 (Luís Antônio, SP).

1.2.2 Experimental design

The experimental design was completely randomized blocks with 8 principal treatments (foliar and ripener applications) and 18 secondary treatments (sites) in a split-plot design with four replicates. The experiments were installed in sugarcane of different ages, that is, ratoons and spacings, and all cultivars used in the experiments had similar characteristics (Daros et al., 2010; UDOP, 2018) (Table 1). Each experiment consisted of eight rows with a length of 10 m and an inter-row spacing of 0.9 m × 1.6 m for experiments 1, 6, and 7; 0.9 m × 1.5 m for experiments 4, 10, and 11; and 1.5 m for all other experiments (Table 1).

The principal treatments were isolated applications of foliar fertilizer (at the vegetative and maturation stages) and ripener (at the maturation stage) and all possible combinations of the two foliar application timings with ripener. Therefore, the treatments were as follows: (*1*) control with no application of multinutrient foliar fertilizer and ripener (control), (*2*) multinutrient foliar fertilizer application at the sugarcane vegetative stage (V), (*3*) multinutrient foliar fertilizer application at the sugarcane maturation stage (M), (*4*) ripener application at the maturation stage (R), (*5*) multinutrient foliar fertilizer application at both the vegetative and maturation stages (VM), (*6*) multinutrient foliar fertilizer application at the vegetative stage plus ripener application at the maturation stage (VR), (*7*) multinutrient foliar fertilizer application at the maturation stage plus ripener application at the maturation stage (MR), and (*8*) multinutrient foliar fertilizer application at both the vegetative and maturation stages plus ripener application at the maturation stage (VMR).

The composition of the multinutrient fertilizer is 200 g N ha^{-1}, 400 g K ha^{-1}, 400 g Mg ha^{-1}, 200 g S ha^{-1}, 100 g B ha^{-1}, 150 g Mn ha^{-1}, 150 g Zn ha^{-1} and 30 g Mo ha^{-1} in 7% of chelating agent EDTA. Foliar fertilizer application in the vegetative and maturation stages was performed by spraying 2 L ha^{-1}, all of them were carried out with use of an adjuvant to improve fertilizer application and enhance its efficiency, and occurred an average of 120 and 35 days before harvest (DBH), respectively (Figure 2). An acetolactate synthase (ALS)-inhibiting growth regulator [sulfometuron-methyl (Corteva, Brazil) and bispyribac-sodium (Ihara, Brazil)] was used as the ripener and was applied at the recommended doses, which is 20 g ha^{-1} and 0.075 L ha^{-1}, respectively, at maturation (35 DBH).

The multinutrient foliar fertilizer and ripener were applied using CO_2-pressurized backpack sprayer equipment with a single tip (type 1/4KLC-9 brass fieldjet) coupled to a 2.6-m-long rod. The working pressure was 4 kgf cm^2 or 58.0 PSI equivalent for an average flow of 100 L ha^{-1}. All applications were performed early in the morning, between 7 and 9 a.m., with

milder temperatures and high relative humidity, respecting the appropriate climatic conditions for foliar applications. In treatments with both foliar fertilizer and ripener applications, foliar fertilization was performed separately before ripener application.

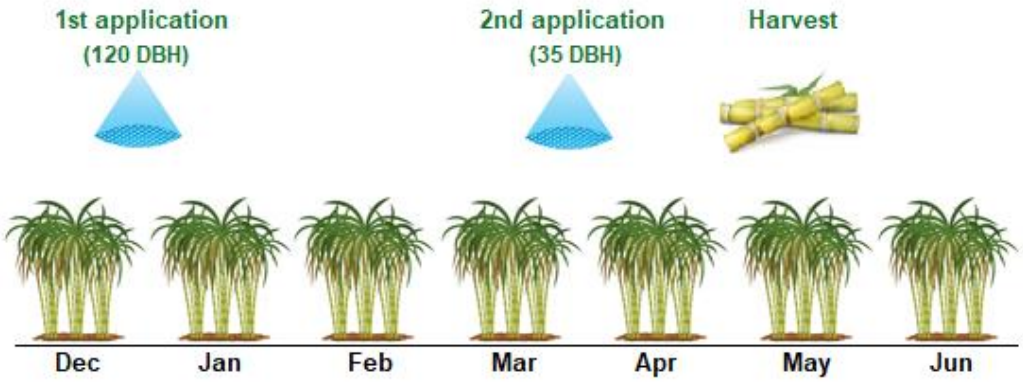

Figure 2. Timing of applications and harvest in the early harvest season. The first application was performed 120 days before harvest (DBH) and the second application was performed 35 DBH.

1.2.3 Sugarcane measurements

Biometric parameters, including plant height (PH), stalk diameter (D), and stalk yield (StY), were analyzed to evaluate sugarcane growth. PH was measured from the root–shoot junction to the auricular region of leaf +3, according to the numbering suggested by Kujiper (VAN DILLEWIJN, 1952), with a measuring tape. D was measured at the third internode above the soil surface, using a digital caliper. PH and D were measured using 20 stalks randomly collected from each plot at the ripening stage prior to harvest. StY was defined by collecting all sugarcane stalks in 2 m of the two central rows of each plot. Uniform, flawless stalks were weighed, and the result was extrapolated to yield in tons ha^{-1}.

To measure the technological parameters [sucrose concentration (%), purity (PUR; %), fiber (FIB; %), reducing sugars (RS; kg sugar Mg^{-1} of stalk), and theoretical recoverable sugar (TRS; kg sugar Mg^{-1} of stalk)] and evaluate the quality of the sugarcane raw material, 10 adjacent stalks were collected from the central row of each plot in each experiment, topped at the apical bud height, defoliated, and transported to the sugarcane technology laboratory of each sugar mill to be processed according to the methodology of Fernandes (2011). Sugar yield (SY) was calculated by multiplying StY by TRS and dividing by 1000. FIB and StY were used to calculate bagasse yield at 50% moisture. Straw production (Mg ha^{-1}) was calculated from StY, considering 140 kg of straw per Mg of stalks. Energy production was calculated by assuming

that 1 Mg of straw has 4.96 MWh of primary energy and 1 Mg of bagasse has 4.94 MWh of primary energy (Waldheim et al., 2001).

The activities of the enzymes sucrose synthase (SuSy), sucrose phosphate synthase (SPS), neutral invertase (NI), and soluble acid invertase (SAI) were assayed in experiment 4 (Santa Maria da Serra-SP) on 19 April 2017 (27 DBH) and 12 May 2017 (4 DBH) and correlated with the biometric and technological data from this experiment. Enzyme activity was evaluated only in this experiment due to the great distances between the other experimental sites and the availability of a biochemical analysis laboratory. Samples from three internodes in the middle third of the stalk were chopped into small pieces, frozen in 50-ml Falcon tubes with liquid nitrogen, and transported to the laboratory on dry ice in a thermal box. All samples were collected at approximately 9 a.m., and the procedure was performed quickly in the field to minimize diurnal variations in enzyme activities and sugar levels. The material was stored at -80 °C until use in enzyme assays.

To measure enzyme activity, extracts of the stalk samples were obtained using the method of Grof et al. (2007). The total protein concentration in the extract was determined according to Bradford (1976). Enzyme activities were determined using the method of Zhu et al. (1997). SPS, SAI, and NI were assayed at 37°C, and SuSy was assayed at 30°C. At 0 (t0), 30 (t30), 60 (t60), and 90 (t90) min, an aliquot of the assay was transferred to a screw-threaded test tube, which was immediately placed in a water bath at 100°C for 3 min to stop the enzymatic reaction. The concentrations of sucrose produced by SPS and SuSy were determined using the anthrone test (van Handel, 1968). Enzyme activity, expressed in µmol of sucrose gMF^{-1} min^{-1}, was calculated considering the greatest difference in sucrose formation at intervals of 30 min. Absorbance was read in a spectrophotometer at 620 nm, and the sucrose concentration was calculated from a standard sucrose curve (0.5–4 mM). The concentration of RS formed by the breakdown of sucrose by SAI and NI was determined using the Somogyi-Nelson method (Nelson, 1944; Somogyi, 1945, 1952). Enzyme activity, expressed in µmol of glucose gMF^{-1} min^{-1}, was calculated considering the greatest difference in sucrose formation at intervals of 30 min. Absorbance was read in a spectrophotometer at 560 nm, and the concentration of RS was calculated from a standard glucose curve (0.5–4 mM).

1.2.4 Statistical analysis

The experimental design was arranged in randomized blocks with split-split plots. The homogeneity of variances and normality of all data were assessed with the F-Snedecor and Shapiro–Wilk tests, respectively. The data were then submitted to analysis of variance (ANOVA), and means were compared between foliar fertilizer and ripening treatments and between sites using the Scott–Knott test (LSD) ($p < 0.1$) in SAS software. As there was no significant difference between sites, results are average of sites in each year. Year was not tested due to sugarcane age being different between years.

1.3 RESULTS

1.3.1 Enzymatic activity

SuSy activity was highest in M at 27 DBH [2.75 µmol fresh weight (FW) min^{-1}] and in M and VR at 4 DBH (1.00 and 0.98 µmol FW min^{-1}, respectively) (Figure 3A). SuSy activity was lowest in the control, R and VM at 27 DBH (1.03, 1.05 and 1.01 µmol FW min^{-1}, respectively) and in VMR at 4 DBH (0.20 µmol FW min^{-1}). Although the differences between 27 DBH and 4 DBH were not analyzed for significance, SuSy activity decreased by 1.75 µmol FW min^{-1} in M and 1.48 µmol FW min^{-1} in V, whereas SuSy activity in the control was almost unchanged (Figure 3A).

At 27 DBH, SPS activity was highest in the control and MR (1.83 and 1.48 µmol FW min^{-1}, respectively), which differed significantly from the other treatments, and was lowest in V and VMR (0.39 and 0.30 µmol FW min^{-1}, respectively) (Figure 3B). At 4 DBH, SPS activity was significantly higher in VR (0.90 µmol FW min^{-1}) than in the other treatments and was lowest in V and VM (0.28 and 0.25 µmol FW min^{-1}) (Figure 3B). Between 27 and 4 DBH, SPS activity increased by 0.32 µmol FW min^{-1} in VMR but decreased by 1.15 µmol FW min^{-1} in the control.

Neutral invertase activity did not differ significantly between the treatments at 27 DBH but was highest in M (0.81 µmol FW min^{-1}) and lowest in VM (0.47 µmol FW min^{-1}) (Figure 3C). At 4 DBH, NI was significantly higher in VMR (2.49 µmol FW min^{-1}) than in the other treatments. Between 27 and 4 DBH, NI increased by 1.79 µmol FW min^{-1} in VMR and decreased by 0.61 µmol FW min^{-1} in the control (Figure 3C).

Compared to the other treatments, SAI activity was highest in VR at 27 and 4 DBH (0.91 and 0.77 µmol FW min^{-1}, respectively) and lowest in V (0.23 and 0.20 µmol FW min^{-1},

respectively) (Figure 3D). Compared to 27 DBH, SAI activity at 4 DBH was lower in V, R, and VR and higher in the control, M, MR and VMR (Figure 3D).

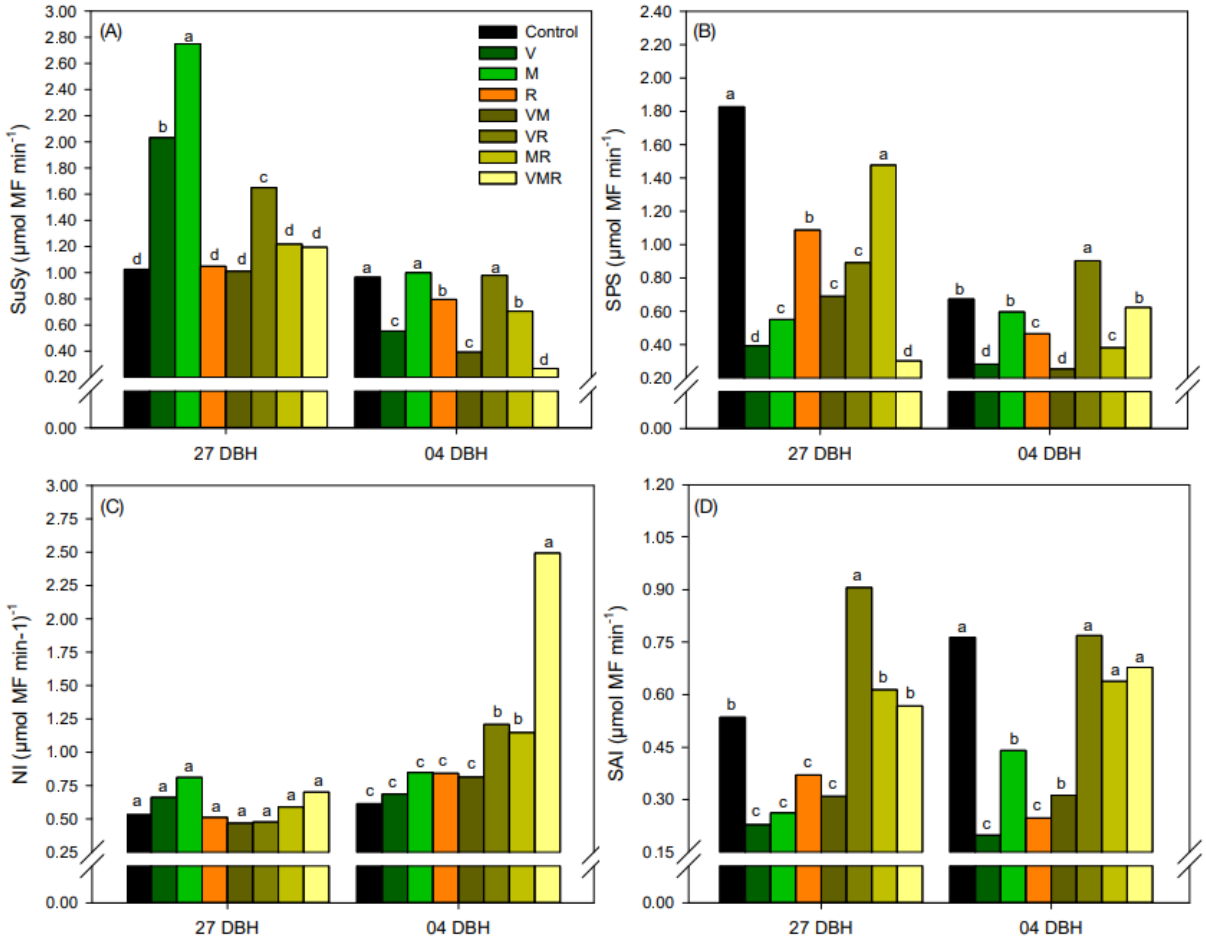

Figure 3. SuSy (A), SPS (B), NI (C) and SAI (D) as a function of multi-nutrient foliar fertilization and ripener applications. The treatments were as follows: (*1*) control, no applications of multinutrient foliar fertilizer or ripener; (*2*) V, application of multinutrient foliar fertilizer at the sugarcane vegetative stage; (*3*) M, application of multinutrient foliar fertilizer at the sugarcane maturation stage; (*4*) R, application of ripener at the maturation stage; (*5*) VM, application of multinutrient foliar fertilizer at both the vegetative and maturation stages; (*6*) VR, application of multinutrient foliar fertilizer at the vegetative stage plus ripener at the maturation stage; (*7*) MR, application of multinutrient foliar fertilizer at the maturation stage plus ripener at the maturation stage; (*8*) VMR, application of multinutrient foliar fertilizer at both the vegetative and maturation stages plus ripener at the maturation stage. Means followed by the same letter do not differ by the Scott–Knott test at 10% probability.

1.3.2 Quality parameters

Purity (PUR) was significantly higher in all treatments with ripener application (R, VR, MR and VMR) in 2017 and 2019 and in R, VR and VMR in 2018, indicating a positive effect of ripener application on sugarcane quality. In all treatments, PUR reached the level

recommended by Ripoli & Ripoli (2004) as an indicator of raw material quality for industry, i.e., >85% (Figure 4A).

Compared with PUR, fiber, sucrose, and TRS, the treatments had opposing effects on reducing sugar (RS). RS tended to be highest in the treatments without ripener application. Compared with the other treatments, RS was significantly higher in the control, V and M in 2017; the control and M in 2018; and the control, V, M and VM in 2019 (Figure 4B). The apparent effect of ripener application on reducing RS content, which means higher sucrose synthesis, suggests that lower glucose and fructose synthesis increases RS levels in sugarcane.

In all three years, the treatments with ripener (R, VR, MR and VMR) significantly increased sucrose and TRS content compared to the treatments without ripener (control, V, M and VM) (Figure 4D and 4E). On average, R, VR, MR and VMR increased TRS by 6.5, 7.9, 6.1 and 8.0 Mg ha^{-1}, respectively, compared to the control, corresponding to gains of 4.9%, 6.0%, 4.6%, and 6.0% (Figure 4E). Thus, regardless of foliar multinutrient fertilizer application, ripener application improved harvest planning, anticipation of maturation, and industrial technological quality. When averaged across the three years, the effects of ripener on TRS did not differ significantly between treatments with or without foliar fertilizer, although TRS was highest in the treatments with foliar multinutrient fertilizer application (Figure 4E).

In 2017 and 2018, sucrose content was significantly higher in VR and VMR than in the other treatments, indicating a positive influence of the combination of foliar fertilization in the vegetative stage and ripener application (Figure 4D). Compared with the control, sucrose content was 7.0% higher in both treatments in 2017 and 6.0% and 6.2% higher in VR and VMR, respectively, in 2018. A similar pattern was observed for TRS content, which was an average of 8.4 Mg ha^{-1} and 8.1 Mg ha^{-1} higher in VR and VMR in 2017 and 2018, respectively (Figure 4E). In addition, sucrose and TRS contents were lowest in the control treatment, M, V and VM in all years, indicating lower industrial quality of the raw material. These results highlight the importance of ripener application for achieving higher sucrose concentrations in the stalks.

Sugar yield (SY) differed significantly between the treatments and was highest in VMR. SY was 2.58, 1.97 and 1.83 Mg ha^{-1} higher in VMR than in the control in 2017, 2018 and 2019, respectively, an average increase of 15.4% (Figure 4F). On average, foliar multinutrient fertilizer application in the maturation stage (M, R and MR) increased SY by 0.7, 0.5 and 1.0 Mg ha^{-1} compared to the control and was inferior to fertilization in the vegetative stage. V, VR, VM and VMR increased SY by 1.2, 1.6, 1.7 and 2.1 Mg ha^{-1}, respectively, compared to the control, although SY was higher in MR than in V in 2019 (Figure 4F).

Foliar multinutrient fertilization in association with ripener application, i.e., VR, MR and VMR, increased SY by averages of 12.0%, 7.0% and 15.4%, respectively, compared to the control (13.8 Mg ha^{-1}) over the three years. Ripener application alone (R) increased SY by only 3.5% compared to the control (Figure 4F). Among the isolated applications, i.e, V, M and R, the increase in SY compared to the control was significantly higher in V (8.8%) than in M (4.8%) and R (3.5%), especially in 2017.

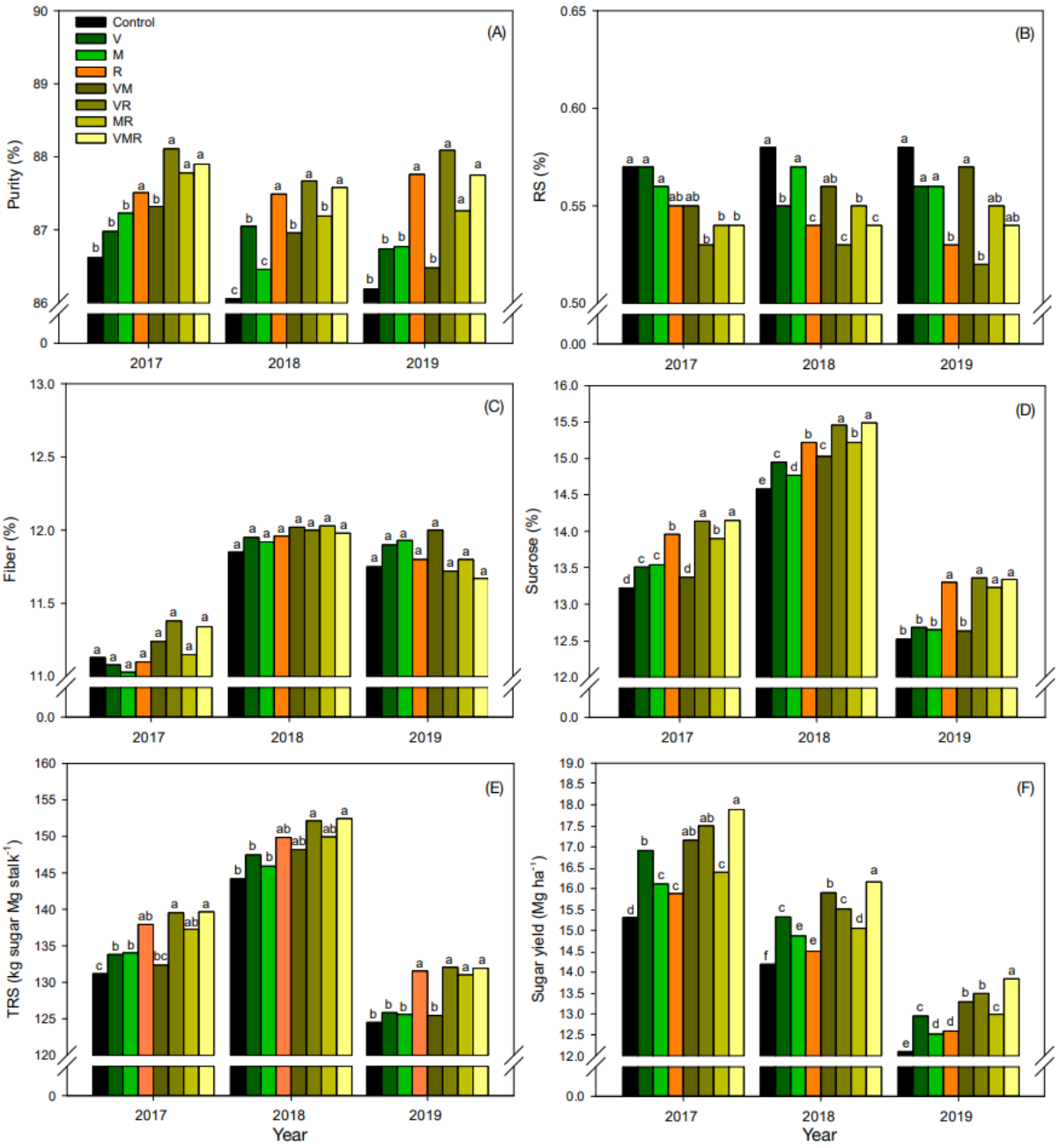

Figure 4. Purity (A), RS (B), fiber (C), sucrose (D), TRS (E) and sugar yield (F) as a function of multi-nutrient foliar fertilization and ripener applications. The treatments were as follows: (*1*) control, no applications of multinutrient foliar fertilizer or ripener; (*2*) V, application of multinutrient foliar fertilizer at the sugarcane vegetative stage; (*3*) M, application of multinutrient foliar fertilizer at the sugarcane

maturation stage; (*4*) R, application of ripener at the maturation stage; (*5*) VM, application of multinutrient foliar fertilizer at both the vegetative and maturation stages; (*6*) VR, application of multinutrient foliar fertilizer at the vegetative stage plus ripener at the maturation stage; (*7*) MR, application of multinutrient foliar fertilizer at the maturation stage plus ripener at the maturation stage; (*8*) VMR, application of multinutrient foliar fertilizer at both the vegetative and maturation stages plus ripener at the maturation stage. Means followed by the same letter do not differ by the Scott–Knott test at 10% probability.

1.3.3 Biometric parameters

Plant height (PH) was significantly higher in VM and VMR, which received two multinutrient fertilizer applications, than in the other treatments in all three years of the study (Figure 5A). Averaged across the three years, PH was 4.7% and 3.9% higher in VM and VMR, respectively, than in the control. By contrast, PH was lowest in R (ripener application only) and was 1.9% lower in this treatment on average than in the control.

The treatments had similar effects on D. In 2017, D was significantly higher in VM, VMR, VR and V than in the other treatments, whereas in 2018 and 2019, D was significantly higher in VM and VMR (Figure 5B). When averaged across the three years of the study, D was 2.5% and 2.3% higher in VM and VMR, respectively, than in the control (Figure 5B). Thus, foliar application of the multinutrient fertilizer improved both PH and D (Figure 5A and 5B).

Since PH and D were highest in VM and VMR, StY was also significantly higher in these treatments than in the other treatments in all years (Figure 5C). Compared with the control, VM and VMR increased StY by 12.7 and 11.3 Mg ha^{-1} in 2017; 9.1 and 7.7 Mg ha^{-1} in 2018; and 9.6 and 8.5 Mg ha^{-1} in 2019. On average, VM and VMR increased StY by 10% and 8.8% (Figure 5C). StY was lowest in R in all years because application of ripener alone retarded growth.

Compared with R, StY was an average of 7.1%, 3.7%, and 10.3% higher in VR, MR, and VMR, respectively, which combined ripener application with foliar multinutrient fertilization (Figure 5C). Compared with the control, combining ripener application with fertilization in the vegetative stage (VR and VMR) increased the average StY by 5.9 and 9.2 Mg ha^{-1}, respectively. These increases were larger than those obtained by combining ripener application with fertilization in the maturation stage (R and MR: -1.4 and 2.4 Mg ha^{-1}, respectively).

Among the foliar multinutrient fertilization treatments with a single application, whether in the vegetative or maturation stage or in association or not with ripener (V, VR, M, and MR), StY was highest in V, with increases of 9.4, 5.7, and 6.5 Mg ha^{-1} in 2017, 2018 and 2019,

respectively, compared with the control. Regardless of whether other applications were performed, PH, D, and StY were highest in the treatments that received foliar multinutrient fertilization in the vegetative stage (V, VM, VR and VMR) (Figure 5A, 5B and 5C). It is important to note that the greatest results for PH, D and StY, considering all years, were obtained in 2017, which had higher mean maximum temperatures and rainfall data.

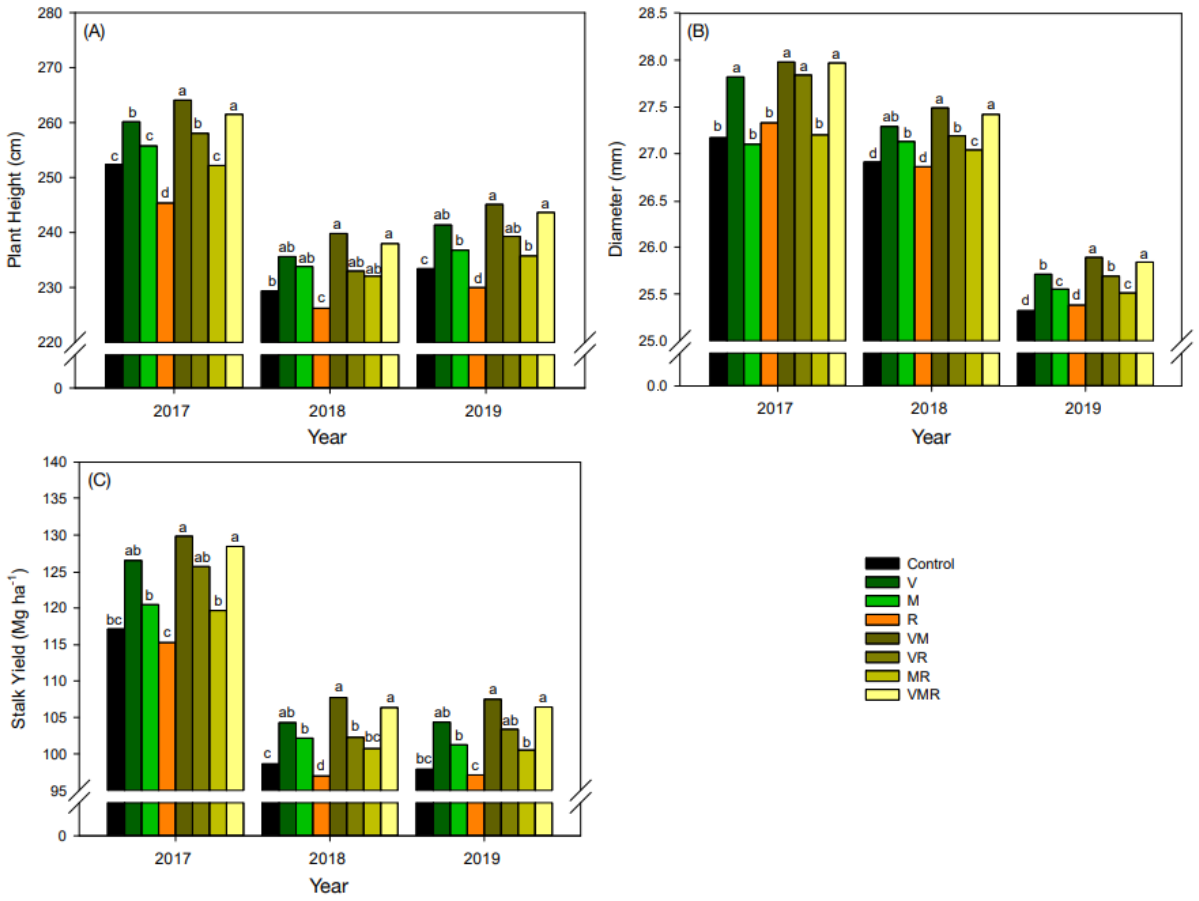

Figure 5. Sugarcane plant height (A), stalk diameter (B) and stalk yield (C) as a function of multi-nutrient foliar fertilization and ripener applications. The treatments were as follows: (*1*) control, no applications of multinutrient foliar fertilizer or ripener; (*2*) V, application of multinutrient foliar fertilizer at the sugarcane vegetative stage; (*3*) M, application of multinutrient foliar fertilizer at the sugarcane maturation stage; (*4*) R, application of ripener at the maturation stage; (*5*) VM, application of multinutrient foliar fertilizer at both the vegetative and maturation stages; (*6*) VR, application of multinutrient foliar fertilizer at the vegetative stage plus ripener at the maturation stage; (*7*) MR, application of multinutrient foliar fertilizer at the maturation stage plus ripener at the maturation stage; (*8*) VMR, application of multinutrient foliar fertilizer at both the vegetative and maturation stages plus ripener at the maturation stage. Means followed by the same letter do not differ by the Scott–Knott test at 10% probability.

1.3.4 Energy parameters

Foliar multinutrient fertilizer application significantly increased bagasse production, and this increase was greatest when two foliar applications were performed (Figure 6A). Compared with the control, VM and VMR increased bagasse production by 11.96% and 11.81% in 2017 and 10.79% and 8.90% in 2018. In 2019, VM increased bagasse production by 11.73% (Figure 6A). Because bagasse is calculated from StY and FIB, it was lowest in the control and R in all three years. Bagasse production was highest when the multinutrient fertilizer was applied in the vegetative stage (Figure 6A).

Straw production followed the same trend as bagasse production and was highest in the treatments with foliar multinutrient fertilization (Figure 6B). Compared with the control, straw production was 10.3% and 9.3% higher in VM and VMR, respectively, in 2017 and 2019 and 9.2% higher in VM in 2018 (Figure 6B).

Estimate energy production was highest in VM and VMR in 2017, with values of 90.2 and 89.2 kWh, respectively, 10.9% and 9.7% more than in the control treatment (Figure 6C). In 2019, these treatments produced 74.6 and 73.9 kWh, corresponding to gains of 9.8% and 8.7%, respectively. In 2018, energy production was highest only in VM, with an increase of 9.2% compared to the control (Figure 6C). Following the patterns of bagasse and straw production, energy production was lowest in R in 2017 and 2018 and in R and the control in 2019 (Figure 6C). On average over the three years, energy production was 71.6 and 72.6 kWt in R and the control, respectively. Thus, foliar application of fertilizer in the vegetative stage (V, VM, VR and VMR) was more beneficial for energy content than application only in the maturation stage (M, R and MR), with increases of 4.1–7.3 kWh (Figure 6C).

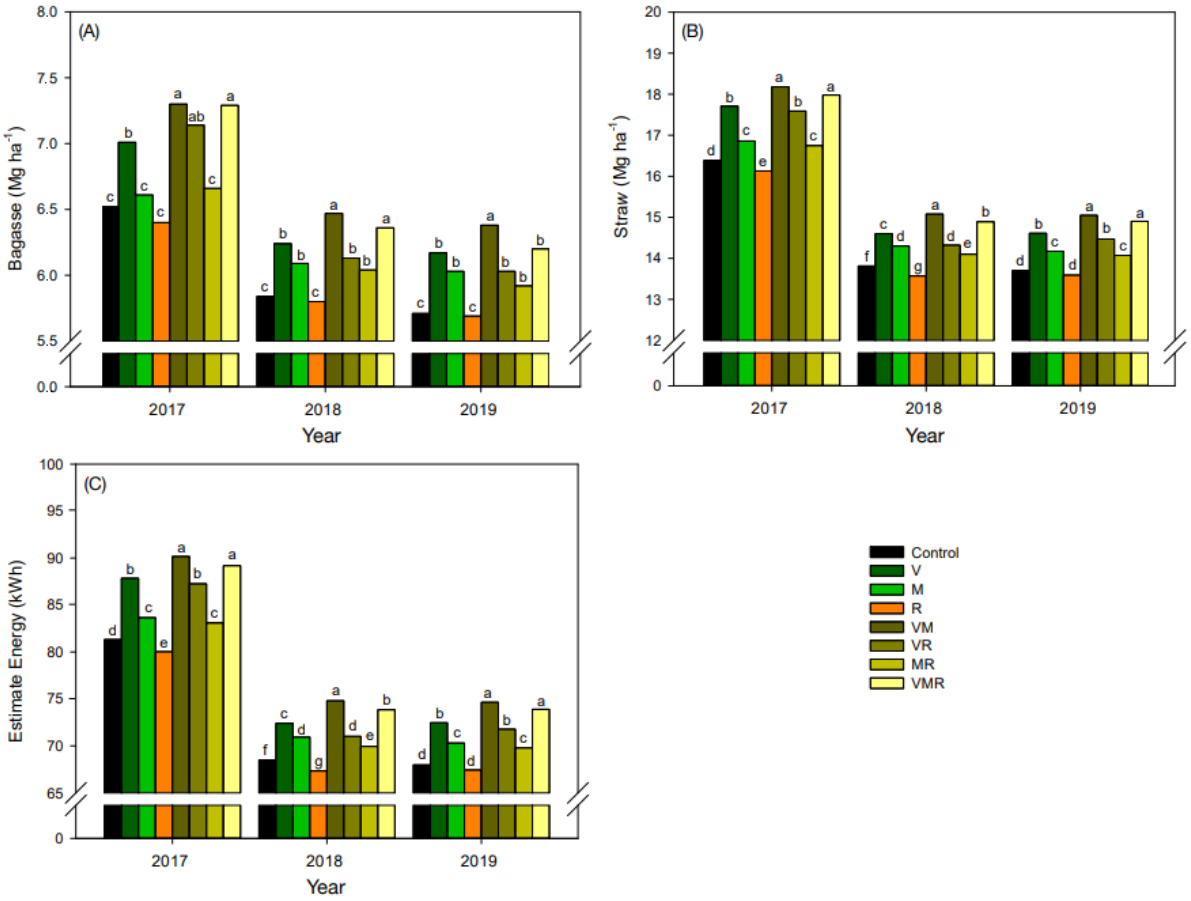

Figure 6. Bagasse (A), straw (B) and estimate energy (C) as a function of multi-nutrient foliar fertilization and ripener applications. The treatments were as follows: (*1*) control, no applications of multinutrient foliar fertilizer or ripener; (*2*) V, application of multinutrient foliar fertilizer at the sugarcane vegetative stage; (*3*) M, application of multinutrient foliar fertilizer at the sugarcane maturation stage; (*4*) R, application of ripener at the maturation stage; (*5*) VM, application of multinutrient foliar fertilizer at both the vegetative and maturation stages; (*6*) VR, application of multinutrient foliar fertilizer at the vegetative stage plus ripener at the maturation stage; (*7*) MR, application of multinutrient foliar fertilizer at the maturation stage plus ripener at the maturation stage; (*8*) VMR, application of multinutrient foliar fertilizer at both the vegetative and maturation stages plus ripener at the maturation stage. Means followed by the same letter do not differ by the Scott–Knott test at 10% probability.

1.4 DISCUSSION

The smart management of foliar fertilizers and ripeners to enhance stalk and sugar yields in sugarcane production is highly desirable. In the present study, applying a multinutrient fertilizer at both the vegetative and maturation stages had positive effects on sugarcane biometric parameters (PH, D and StY) compared to isolated application at the vegetative or maturation stage (Fig. 5). These increases in PH and StY occurred because supplying multinutrients at the stages of greatest demand stimulates the photosynthetic rate, which increases productivity and activates the plant's defense system, including nutrient-dependent antioxidant enzymes that mitigate reactive oxygen species and reduce plant stress (Montañez et al., 2009; Rengel et al., 2011a, 2011b; Bedin et al., 2020; Tavanti et al., 2021).

A similar pattern of increases in PH, D and StY was observed when the two foliar multinutrient fertilizer applications were combined with ripener application (VMR treatment) (Niklas and Enquist, 2001). We can infer that applying the multinutrient fertilizer at the vegetative stage promoted greater development through the growth of new leaves, which have surface features (e.g., more permeable trichrome or stomata) that favor the absorption and translocation of both fertilizer and ripener applied at the maturation stage (Wójcik, 2004; Koch and Ensikat, 2008; Marschner, 2012; Férnandez et al., 2013; Mattiello et al., 2015; Fernandez et al., 2020). Supplying multinutrients to older leaves extends their period of metabolic activation and consequently their productivity (Benbella and Paulsen, 1998; Rossi et al., 2015; Lira et al., 2017).

StY was highest in the treatments with two applications of foliar multinutrient fertilizer (VM and VMR). On average (considering all years), VM and VMR increased StY by 10.0% and 8.8%, respectively, compared to the control by stimulating photosynthesis and the production of photosynthates, which were used for growth in the vegetative stage and to mitigate premature leaf senescence in the maturation stage (Papadakis et al., 2007; Blandino and Reyneri, 2009; El-Mogy et al., 2019). The sequential application of nutrients, such as at the vegetative and maturation stages in the same field year, supports the maintenance and nutritional balance of newly expanded leaves, which otherwise may be vulnerable to low internal translocation of certain nutrients. In addition, sequential application stimulates photosynthesis and secondary plant metabolism (Alston, 1979; Papadakis et al., 2007).

Foliar fertilization supplies several precursor nutrients for photosynthesis and respiration reactions that occur in plants at the vegetative and maturation stages, as a result of nutrient function in plant metabolism. Constituent of all amino acids, amides, proteins,

nucleotides and polyamides, nitrogen participates in the photochemical process, acting in the "antenna" complex, which captures light energy for chlorophyll oxidation, producing the necessary energy for photosynthesis (Buchanan et al., 2015).

Among the macronutrients, potassium is the most required one by sugarcane, activating a higher number of enzymes involved in sucrose and starch synthesis, playing important roles in plant growth and sugar yield (Otto et al., 2010; Moore et al., 2014). Other nutrients are directly involved in metabolic process related to photosynthesis. Sulfur acts in photosynthetic and respiratory electron transport through Fe-S grouping, also participates in the structure and regulation of proteins, and under S imbalance plants have a low concentration of chlorophyll, resulting in lower photosynthetic rate and consequently less sucrose synthesis (Epstein and Bloom, 2005; Marschner, 2012).

The growth of roots and stalks and the development of new leaves and flowers depend on sucrose import by photosynthetically active leaves (source), which therefore function as sinks. During the first two developmental stages (early and fast-growing stages), the stalks and leaves are the main sinks; at the maturation stage, the stalks are the main sinks due to sucrose accumulation (Ribeiro et al., 2020). Magnesium is an essential component in the photoassimilate partioning between source and sink organs and it is linked to the phloem loading of sucrose, thus, its deficiency affects the proton-motive force generated by H^+/ATPase, which requires Mg-ATP for its operation (Hermans et al., 2005), the translocation of photoassimilates to the sink organs and may affect the amount of sucrose in the stalks (Führs and Gerendás, 2013).

Thus, adequate nutrient management of sugarcane at maturation (pre-harvest) can provide favorable results, especially in areas where aerial chemical application is restricted or in sugarcane fields with inadequate purity for ripener use. Combining foliar fertilizers and ripeners has positive effects on leaf nutrition compared to applying ripener alone and leads to superior stalk and sugar yields (Jain et al., 2013). In summary, the PH and StY results in this study suggest that combining foliar multinutrient fertilization at the vegetative and maturation stages with ripener application can promote plant growth and enhance production per unit area, mainly when climate conditions are favorable for natural sugarcane development (higher temperature and rainfall), as well as occurred in 2017 season.

Combining foliar multinutrient fertilization with ripener application also increased TRS content. Regardless of stage, applying only multinutrient fertilizer (M, VM and V) reduced TRS compared to applying ripener. However, applying the multinutrient fertilizer at the vegetative and maturation stages, especially in association with ripener, provided a sufficient supply of

nutrients to enhance SY, with an increase of 15.4% in VMR (Alexander, 1986). Fertilizer and ripener application increased the concentration of sucrose, which is involved in sugarcane growth, plant development, storage, signal transduction and acclimatization to environmental stresses (Hartt et al., 1963; Salerno and Curatti, 2003).

Sugarcane is an efficient, renewable source of ethanol, energy, animal feed, and fertilizer. Foliar multinutrient fertilization increased energy production, bagasse production and FIB when performed at the vegetative stage (Figure 6). Bagasse and energy production are energy parameters that are directly related to biomass production (Coelho et al., 2019) and are important components of energetic diversification. Bagasse can reduce greenhouse gas emissions when used as a biofuel and increase energy generation from biomass (Rípoli et al., 2000; Waldheim et al., 2001). Foliar fertilization supplies essential nutrients, such as Mg, B, and K, and promotes biomass accumulation at the time of greatest sugarcane growth, increasing the return in productivity per unit of fertilizer applied.

The metabolic response of sugarcane to foliar fertilizer and ripener application was explored by measuring the enzymatic activities of SuSy, SPS, SAI, and NI, which play important roles in the maintenance of sucrose synthesis, sucrose concentration, respiration, non-photosynthetic cells, and sugar transport in the plant (Kingston-Smith et al., 1999; Chandra et al., 2012; Lemoine et al., 2013). In general, foliar multinutrient fertilization at the maturation stage and ripener application strongly increased SuSy activity at 27 DBH; however, at 4 DBH, only foliar fertilization at the maturation stage and foliar fertilizer application at the vegetative stage combined with ripener increased SuSy activity (Fig. 3). In addition, there was a positive effect of foliar multinutrient fertilization on SuSy activity at 27 DBH, but SuSy activity declined at 4 BDH in all treatments that received foliar fertilizer and ripener. The increases in stalk yield and TRS were probably a consequence of this intense sucrose synthesis observed at 27 DBH; the sharp decline in sucrose synthesis at 4 DBH culminated in sucrose degradation into RS, increased growth and reduced sucrose accumulation (Zhu et al., 1997; Botha and Black, 2000). However, SuSy activity was not correlated with StY or TRS in any of the treatments. This lack of correlation probably reflects the opposing functions of SuSy in sucrose synthesis and hydrolysis; these reactions are reversible, and the function and direction of the reaction depend on the sugarcane phenological stage, tissue sugar levels and energy demand from plant metabolism (Huber and Akazawa, 1986).

SPS activity was highest in the control at 27 DBH and in the treatments in which fertilizer was applied at the vegetative stage and combined with ripener at 4 DBH (Fig. 3). This pattern of activity did not follow the effects of the treatments on biometric and quality

parameters. We expected SPS activity to be highest in the treatments with superior sugarcane growth and yields, such as VM and VMR, because SPS activity is critical for sucrose synthesis and is often directly correlated with sucrose accumulation in the stalks (Hatch and Glasziou, 1963; Wendler et al., 1991; Lingle, 1999; Botha and Black, 2000; Grof et al., 2007). However, Zhu et al. (1997) did not find differences in SPS activity between varieties with high and low stalk contents of sucrose. It is possible that the sugarcane that did not receive foliar fertilizer or ripener had greater SPS activity because sucrose synthesis had just begun and was not yet reflected in the biometric and quality parameters and yields. The lack of correlation of SPS activity with sucrose accumulation or plant yields may also be attributable to variations in sugarcane genotypes, phenological stages, and time and day of sample collection.

The maturation process begins with an increase in sucrose content and a reduction in hexose content (Grof et al., 2007). The increase in the sucrose content in the stalks is partially associated with increased SPS activity (Verma et al., 2011). Verma et al. (2011) compared varieties with high and low sucrose contents in the stalks and found increases in SPS transcription and activity in the basipetal direction of the stalks. Although several studies have shown a positive correlation between SPS activity and sucrose levels in sugarcane stalks (Gutiérrez-Miceli et al., 2002), other studies suggest that sucrose accumulation in stalks is not directly controlled by SPS (Ebrahim et al., 1998; Lingle and Tew, 2008), consistent with our findings.

Sucrose synthesis in the leaves and stalks usually occurs through the activities of SuSy and SPS (Zhu et al., 1997; Grof et al., 2007), but the predominant enzyme activity varies depending on the plant growth stage (Botha and Black, 2000; Gutiérrez-Miceli et al., 2002). Sucrose synthesis varies temporally and spatially in stalks and is higher in the older or basal internodes at the early stages of sugarcane growth. Thus, the relationship between sucrose levels and sugarcane development remains unclear; sucrose levels in the stalks do not increase linearly during development but increase rapidly with internode maturation (Botha and Black, 2000).

With respect to invertases, SAI activity was high in all treatments except V, M, R, and VM at 27 DBH and was highest in VR. During the maturation stage, the stalk growth rate decreases, and sucrose accumulation in the stalks increases, which may be related to NI activity (Jain et al., 2013). NI activity did not differ significantly between the treatments but was highest in M at 27 DBH and VMR at 4 DBH. These results followed the patterns of StY, SY and TRS. Previous studies have also reported that increases in NI activity are usually related to superior TRS, StY and SY (Lingle, 1999; Crusciol et al., 2017). In addition, the differences in sucrose

accumulation in the stalks between the treatments with fertilizer or ripener, which could not be explained by low SAI activity, may be attributable to the increase in NI activity.

The active accumulation of sucrose is governed by tissue maturity; that is, sucrose accumulation differs between mature and immature tissues due to differences in the levels of invertases and the need for growth (Alexander, 1973). SAI levels decline in mature stalk tissues, where growth processes are at the end stage, and NI becomes the predominant enzyme in the cytoplasm, leading to sucrose accumulation in the vacuole. In mature tissues, sucrose is stored in intercellular spaces and reaches a content of approximately 15%–20% (Lingle, 1999). This process occurs in sugarcane treated with ripeners because ripeners are plant regulators that affect plant morphology and physiology and modify qualitative and quantitative production parameters. In addition, ripener application decreases plant growth and increases sugar translocation and invertase activity to accumulate sucrose in the stalks (Martins and Castro, 1999; Castro et al., 2002; Crusciol et al., 2017).

Physiologically, the sucrose content of sugarcane stalks increases when the activities of enzymes that hydrolyze sucrose are surpassed by the activities of enzymes that synthesize sucrose. Under adequate growth conditions, this phase occurs in response to not only reduced carbon demand for stalk growth when the plant reaches maturity but also environmental factors such as water restriction and reduced air temperature (Ebrahim et al., 1998; Grof et al., 2007; Cardozo and Sentelhas, 2013). In general, sucrose accumulation occurs through the action of enzymes (Botha and Black, 2000; Grof et al., 2007), and discrepancies in sucrose accumulation reported in the literature appear to be attributable to differences in variety (Gutiérrez-Miceli et al., 2002; Grof et al., 2007; Lingle and Tew, 2008; Verma et al., 2011), phenological stage (Botha and Black, 2000; Verma et al., 2011), and enzyme reaction rates (Botha and Black, 2000).

In summary, management with foliar multinutrient fertilization is a tool to boost the nutrient supply in sugarcane production during the period of highest growth and improve vegetative development. Foliar multinutrient fertilizers can be applied with other chemical products, such as ripeners, and such combined applications are already being implemented in sugarcane management. Foliar multinutrient fertilization elicits a fast response from sugarcane and enhances nutrient use efficiency. In addition, ripener application induces sugarcane maturation and, when associated with foliar fertilization, allows the plant to better utilize nutrients stored in leaves (source) and stalks (sink) to reach the sucrose and sugar production potential of sugarcane.

1.5 CONCLUSIONS

Applying foliar multinutrient fertilizers and ripeners in sugarcane field production efficiently increases plant growth, yields, and byproduct production, especially when the fertilizer is applied at the vegetative and maturation stages and combined with ripener application. These practices enhance the activity of metabolic enzymes in sugarcane and are very feasible for implementation by mills in sugarcane production. The magnitude of the effect of multinutrient fertilization depends on the timing of application and its combination with ripener application.

The evaluation of biometric, quality, and energy parameters and enzyme activities in this study revealed differences in nutrient supply between sugarcane development stages that would be missed by typical sugarcane studies. Supplying nutrients by foliar fertilization enhances sugarcane growth in the early development (vegetative) stage and provides the nutrients required for enzymatic activity in the late (maturation) stage. The application of a plant regulator as a ripener reduces growth and increases sucrose accumulation. When combined, these practices improve stalk and sugar yields, total recoverable sugars, and neutral invertase activity. These findings provide a foundation for selecting appropriate management practices to enhance sucrose, energy and ethanol production by sugarcane mills. Further investigation is needed to determine which plant enzymes are the main drivers of sucrose synthesis and sucrose accumulation in sugarcane stalks.

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CHAPTER 2

TANDEM NUTRIENT AND RIPENER APPLICATION CAN IMPROVE THE YIELD AND QUALITY OF MIDDLE AND LATE HARVEST SUGARCANE

ABSTRACT

The global demand for sugar and renewable fuels is growing. Sugarcane will play an important role in meeting the demand for sugar and ethanol because it is a highly productive, economically viable, and ecologically well-managed crop. Applying multinutrient foliar fertilizers to sugarcane at the end of the vegetative stage and beginning of the maturation stage increases productivity by stimulating the plant's defense system, which increases antioxidant enzyme activity, mitigates stress, reduces premature leaf senescence, and promotes photosynthesis. Applying ripeners accelerates sugarcane maturation and sugar accumulation in stalks, increasing the quality of the raw material. In this study, we evaluated the effects of multiple combinations of multinutrient foliar fertilizer and ripener applications on the development, yields, productivity and sucrose synthesis of middle (winter) and late (spring) harvest sugarcane. An multinutrient composed by N, K, Mg, S, B, Mn, Zn and Mo, and its foliar fertilization at the vegetative stage was performed 150 days before harvest (DBH) in winter or 60 DBH in late harvest, and fertilization at the maturation stage was performed at 30 DBH. Multinutrient fertilization at the vegetative and maturation stages increased plant height, stalk diameter and stalk yields. Sucrose content (%) and theoretical recoverable sugar (TRS) were highest in sugarcane treated with ripener alone or in combination with multinutrient foliar fertilizer. Sugar yield was highest when two applications of multinutrient foliar fertilizer were combined with ripener application (VMR treatment), with increases of 10.0% and 14.0% in the middle and late harvest, respectively, compared to the control. Applying multinutrient foliar fertilizer at the vegetative and maturation stages increased stalk and sugar yields and, in turn, bagasse and energy production. Like stalk yield, the accumulation of reducing sugars and sucrose in leaves was highest when multinutrient foliar fertilizer was applied at both the vegetative and maturation stages. Thus, applying multinutrient foliar fertilizer at the vegetative and maturation stages stimulates photosynthetic activity, resulting in greater sugar production and productivity. Applying multinutrient foliar fertilizer in association with ripener enhances sucrose accumulation and increases both productivity and raw material quality.

Keywords: *Saccharum* spp., foliar fertilizer, maturation regulator, starch, metabolites.

2.1 INTRODUCTION

The global need for green fuel has increased demand for ethanol as a replacement for fossil fuels. Sugarcane is a major raw material in ethanol production, and Brazil is the leading producer of sugarcane. In the 2022/2023 season, Brazil contributed 21% of global sugar production and more than 90% of global sugarcane-derived ethanol (CHAGAS et al., 2016; OECD, 2023). The most common sugarcane harvest period is the early harvest between March and April, when production is high. In Brazil, sugarcane harvest times have expanded to include middle and late harvests (from June to December), but these harvests typically have low stalk levels of theoretical recoverable sugar (TRS) and low yields because the longer photoperiod, high temperatures and high rainfall stimulate vegetative growth (CAPUTO et al., 2007; CARDOZO; SENTELHAS, 2013; KEATING et al., 1999).

Sucrose accumulation determines the economic viability of sugarcane production. The effects of environmental variations, such as low temperatures and water deficit, on carbohydrate metabolism depend on the growth stage. These conditions can impair plant growth and stalk yields in the vegetative stage but increase sucrose accumulation in stalks in the maturation stage. Adverse climatic conditions also influence sucrose content in the stalks by stimulating sugar synthesis and storage or breakdown into glucose and fructose (CARDOZO et al., 2020). Glucose and fructose are reducing sugars that are used for new cell production, i.e., vegetative growth (CARDOZO; SENTELHAS, 2013; SCARPARI; BEAUCLAIR, 2004). Thus, sugarcane cultivation and harvesting must be carefully managed to ensure an appropriate timing of crushing. Producers also aim to improve supply by using time management to ensure high-quality canes throughout the harvest (CRUSCIOL et al., 2017).

By increasing stalk yield and TRS, the application of multinutrient foliar fertilizers and ripeners can help maintain and improve sugar yield and the timing of harvest for product delivery to the mill (JAIN; CHANDRA; SOLOMON, 2013). Multinutrient foliar fertilizers increase the activity of enzymatic and non-enzymatic antioxidants to stimulate plant defense systems, increase plant resilience to biotic and abiotic stresses, minimize premature leaf senescence, stimulate sucrose metabolism enzymes, promote photosynthesis and ultimately increase stalk yield (BOLOURI-MOGHADDAM et al., 2010; TAVANTI et al., 2021). Ripeners accelerate the sugarcane maturation process by directing photosynthates to accumulate in the stalks, which increases raw material quality and advances the harvest period (VIANA et al., 2017). The benefits of ripeners for increasing TRS by mobilizing sucrose for storage in the stalks are well recognized, and nutrient application can minimize delays in stalk

development (JAIN; CHANDRA; SOLOMON, 2013; LEITE; CRUSCIOL, 2008). The use of these growth-regulating compounds can aid crop management planning and improve the quality of the sugarcane harvest, increasing profitability (MESCHEDE; VELINI; CARBONARI, 2010).

Maturation is the most economically important stage of sugarcane development, as this stage is marked by the greatest accumulation of photosynthates and production of sucrose. Maturation is induced by periods of low rainfall and temperature or by natural or synthetic ripeners (SILVA; CAPUTO, 2012). The final product of photosynthesis is sucrose, a non-reducing sugar that is the main route by which carbon assimilated in leaves is transported to drains (BLAKELEY; DENNIS, 1993). Sugars produced by photosynthesis are stored in leaves in the form of sucrose and transient starch (NOZUE; MALOOF, 2006; SMITH; STITT, 2007; SULPICE et al., 2014; WALTER; SCHURR, 2005). Sucrose is involved in growth, development, signal transduction and acclimatization to environmental stresses (SALERNO; CURATTI, 2003). Increases in photosynthetic processes and sucrose metabolism due to variations in metabolite levels during sugarcane development stages can greatly enhance biomass accumulation (DU et al., 2000a, 2000b; USUDA et al., 1987). Changes in the photosynthetic rate can influence the dynamics of the production of metabolites, especially non-structural carbohydrates (NSCs) that maintain plant metabolism and growth (SMITH; STITT, 2007). The progressive accumulation of sucrose in sugarcane stalks directly interferes with the source–sink relationship by increasing the photosynthetic rate (MCCORMICK; CRAMER; WATT, 2008). When the sink is active due to growth and/or storage stimulation, NSCs are degraded, and their energy is directed to the collecting organs. When the sink is inactive, NSCs accumulate in the leaves, mainly as starch, signaling a reduction in photosynthetic processes through repression (STITT, 1991). In 12-month-old sugarcane, sucrose accumulation is usually greatest in the organs, whereas starch is mainly present in the leaves (DE SOUZA et al., 2018).

We hypothesize that the use of multinutrient foliar fertilizers and ripeners improves the agronomic and metabolic parameters of sugarcane, sugar yields, and raw quality. To test this hypothesis, we evaluated the efficiency of applying a multinutrient complex via the leaves in association with a ripener at the vegetative and maturation stages in increasing sugarcane productivity, sugar yield and plant metabolites involved in the biosynthesis processes of sugarcane in the middle and late harvest seasons.

2.2 MATERIALS AND METHODS

2.2.1 Characteristics of the study sites

Twenty-one experiments were conducted over two years (2017 and 2019) in the middle harvest (winter, June-August) and in 2017 late harvest (spring, September-November) of sugarcane in commercial areas in the states of São Paulo (SP) and Minas Gerais (MG) in south-central Brazil (Figure 1). In 2018 middle and late harvest, as well as 2019 late harvest season, the weather conditions were very unfavorable to nutritional applications in the vegetative stage, due to extreme drought conditions, low humidity in the soil, and absence of green leaves in adequate conditions to absorb nutrients, therefore the study were not carried out in these respective periods.

According to Köppen's classification, the local climate is Cfa (humid, hot summers) at Igaraçú do Tiête-SP (Sites 4 and 10) and Aw (dry winters) at all other sites (Figure 1). Climate data were obtained from meteorological stations located near the experimental sites (Figure 2). The average temperature ranges were 21.0–23.5 °C and 21.9–23.2 °C in middle harvest (winter) in 2017/2018 and 2019/2020, respectively, and 21.0–22.8 °C in late harvest (spring) in 2017/2018.

The soil at the experimental sites was chemically characterized at depths of 0.00-0.25 m and 0.25-0.50 m according to van Raij et al. (2001). The soil classifications according to Soil Survey Staff (2014) and soil chemical characteristics are shown in Table 1. The soil at the experimental sites was ameliorated and fertilized as recommended by van Raij et al. (1997). Phytosanitary management was carried out within the regular standards for sugarcane production, and cultivars were chosen according to the characteristics of the winter (middle harvest) and spring (late harvest) periods at each site (DAROS et al., 2010; UDOP, 2018). The sugarcane stage (age) differed among the experimental sites; consequently, the row spacings and the dates of the harvests and treatments varied (Table 2).

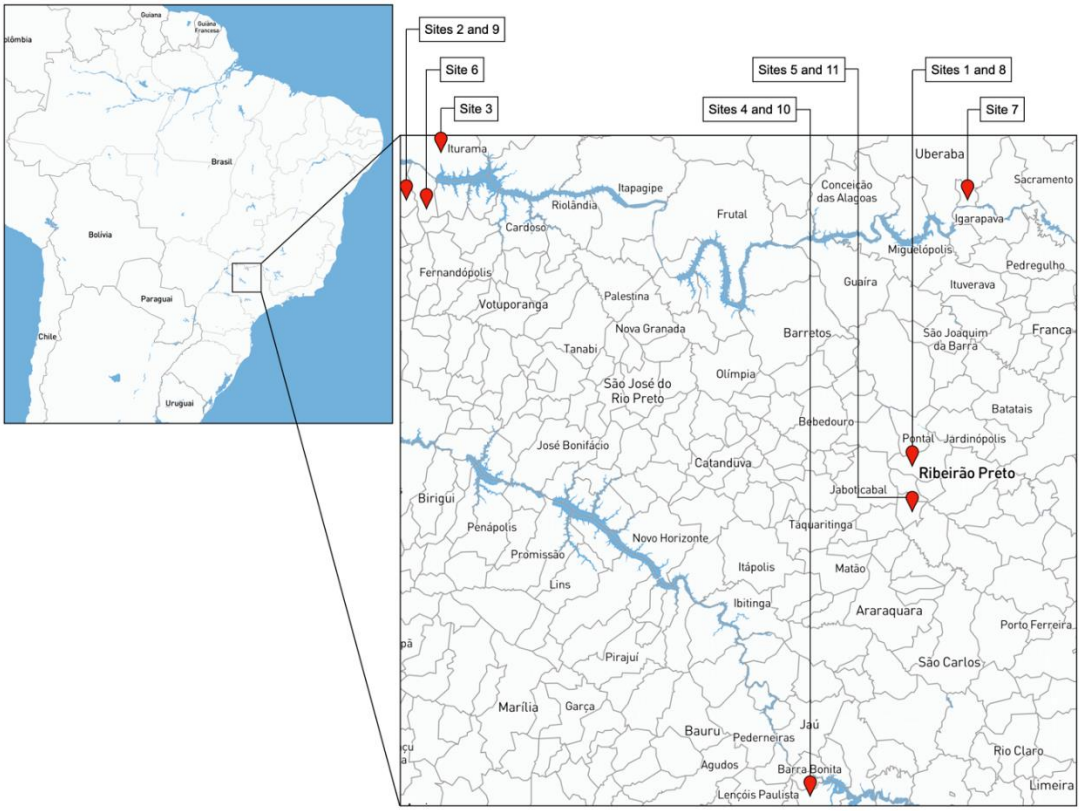

Figure 1: Experimental sites. The 2017 middle harvest (winter) trials were located at Sertãozinho-SP (Site 1, exp 1– 21°08'43.95"S 48°04'23.74"W, exp 2 – 21°08'29.82"S 48°06'46.94"W), Populina-SP (Site 2, exp 3 – 19°53'10.93"S 50°31'44.49"W, exp 4 – 19°52'23.71"S 50°31'12.15"W), Iturama-MG (Site 3, exp 5 – 19°42'29.59"S 50°18'55.05"W, exp 6 – 19°50'29.15"S 50°17'44.46"W), Igaraçú do Tiête-SP (Site 4, exp 7 – 22°30'06.93"S 48°28'23.74"W, exp – 8 – 22°30'13.77"S 47°27'56.56"W), and Pradópolis-SP (Site 5, exp 9 – 21°26'20.10"S 48°02'06.80"W, exp 10 – 21°26'46.23"S 48°02'30.67"W). The 2019 middle harvest (winter) trials were located at Ouroeste-SP (Site 6, exp 11 – 19°56'10.02"S 50°30'08.67"W) and Delta-MG (Site 7, exp 12 – 19°51'09.69"S 47°46'44.3"W, exp 13 – 19°51'35.97"S 47°46'32.72"W). The late harvest (spring) 2017 trials were located at Sertãozinho-SP (Site 8, exp 14 – 21°05'57.19"S 48°05'18.50"W, exp 15 – 21°06'16.27"S 48°05'21.24"W), Populina-SP (Site 9, exp 16 – 19°53'39.40"S 50°28'35.93"W, exp 17 – 19°53'18.49"S 50°28'51.47"W), Igaraçú do Tiête-SP (Site 10: exp 18 – 22°33'44.06"S 48°33'11.58"W, exp 19 – 22°33'40.26"S 48°33'25.12"W) and Pradópolis-SP (Site 11, exp 20 – 21°21'59.01"S 47°59'25.53"W, exp 21 – 21°21'15.54"S 47°59'53.22"W).

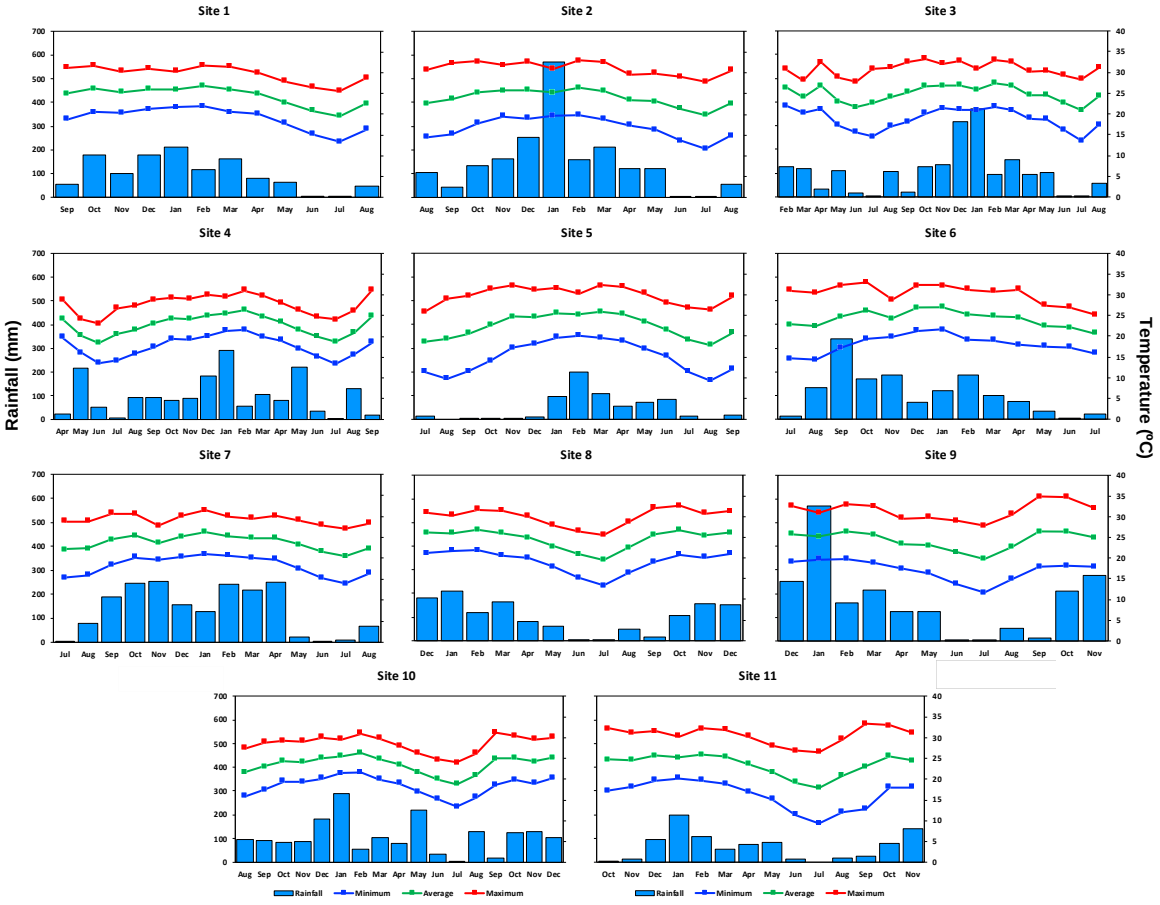

Figure 2: Rainfall (mm) and mean maximum and minimum temperatures during the experimental period. Middle harvest 2017: Sertãozinho-SP (Site 1: exp 1–2), Populina-SP (Site 2: exp 3–4), Iturama-MG (Site 3: exp 5–6), Igaraçú do Tiête-SP (Site 4: exp 7–8), and Pradópolis-SP (Site 5: exp 9–10). Middle harvest 2019: Ouroeste-SP (Site 6: exp 11) and Delta-MG (Site 7: exp 12–13). Late harvest 2017: Sertãozinho-SP (Site 8: exp 14–15), Populina-SP (Site 9: exp 16–17), Igaraçú do Tiête-SP (Site 10: exp 18–19) and Pradópolis-SP (Site 11: exp 20-21).

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

Harvest	Site	Soil Classification	Depth (m)	pH $CaCl_2$	S.O.M. g dm^{-3}	$P_{(resin)}$ mg dm^{-3}	SO_4^{-2}	Al^{+3}	$H+Al^{+3}$	K	Ca	Mg	BS	CEC	V%
									---------- $mmol_c$ dm^{-3} ----------						
Middle harvest (winter)	1	Rhodic Eutrudox	0.00-0.25	5.5	58	14	8.0	0.0	44	12	82	22	116	160	73
			0.25-0.50	5.5	41	4.0	10	0.0	37	8.0	53	17	78	115	68
	2	Rhodic Hapludox	0.00-0.25	6.0	45	42	22	0.0	31	8.0	45	22	75	106	71
			0.25-0.50	5.0	23	57	36	0.0	28	6.0	32	20	58	86	67
	3	Rhodic Hapludox	0.00-0.25	5.0	17	7.0	3.0	2.0	29	2.0	20	9.0	31	60	51
			0.25-0.50	4.6	13	5.0	6.0	7.0	30	1.0	14	8.0	23	53	44
	4	Rhodic Eutrudox	0.00-0.25	5.3	19	10	10	0.2	19	1.0	38	21	60	79	76
			0.25-0.50	5.2	20	9.0	15	0.3	20	1.0	46	17	64	84	76
	5	Rhodic Eutrudox	0.00-0.25	5.8	55	54	2.0	0.0	27	10	37	22	69	96	72
			0.25-0.50	5.5	42	45	8.0	0.0	21	7.0	23	12	42	63	67
	6	Rhodic Hapludox	0.00-0.25	5.8	27	40	36	0.0	21	9.0	30	12	51	72	71
			0.25-0.50	5.5	22	48	50	0.0	20	9.0	20	14	43	63	68
	7	Rhodic Eutrudox	0.00-0.25	5.7	55	54	5.0	0.0	25	12	40	19	71	96	74
			0.25-0.50	5.4	42	45	10	0.0	20	6.0	25	12	43	63	68
Late harvest (spring)	8	Rhodic Eutrudox	0.00-0.25	5.6	1.8	80	25	0.0	7.0	2.0	33	9	44	51	86
			0.25-0.50	5.6	1.6	20	65	0.0	14	2.0	21	7	30	44	68
	9	Rhodic Hapludox	0.00-0.25	5.0	42	36	30	0.0	27	3.0	42	20	65	92	71
			0.25-0.50	5.0	25	48	44	0.0	18	1.0	26	12	39	57	68
	10	Rhodic Eutrudox	0.00-0.25	5.5	10	22	4.0	0.0	15	3.0	19	11	33	48	70
			0.25-0.50	5.2	7.1	9.7	12	0.3	13	3.0	17	5.0	25	38	66
	11	Rhodic Eutrudox	0.00-0.25	6.2	49	38	34	0.0	32	9.0	33	25	67	99	68
			0.25-0.50	6.0	39	35	22	0.0	29	8.0	18	16	42	71	59

*SOM: Soil Organic Matter; BS: Base Saturation; CEC: Cation Exchange Capacity.

Table 2. Sugarcane cultivar, ratoon stage, row spacing and date of establishment and first and second applications and harvest in each season.

Harvest Period	Site	Cultivar	Ratoon	Row spacing	1st Aplication*	2nd Aplication	Harvest
Middle harvest (winter)	1	CTC4	3	0.9 x 1.60	Mar. 2017	Jul. 2017	Aug. 2017
	2	IAC95 5000	3	1.5	Mar. 2017	Jul. 2017	Aug. 2017
	3	RB85-5536	2	1.5	Mar. 2017	Jul. 2017	Aug. 2017
	4	SP80 3280	2	0.9 x 1.50	Mar. 2017	Jul. 2017	Aug. 2017
	5	CTC4	3	1.5	Mar. 2017	Jul. 2017	Aug. 2017
	6	IAC91-1099	3	1.5	Feb. 2019	Jun. 2019	Jul. 2019
	7	SP80-1816	5	1.5	Mar. 2019	Jul. 2019	Aug. 2019
Late harvet (spring)	8	SP80-3280	2	0.9 x 1.60	Sep. 2017	Oct. 2017	Nov. 2017
	9	CTC15	1	1.5	Sep. 2017	Oct. 2017	Nov. 2017
	10	SP80-3280	1	0.9 x 1.50	Oct. 2017	Nov. 2017	Dec. 2017
	11	SP80-3280	2	1.5	Sep. 2017	Oct. 2017	Nov. 2017

*First application was performed at vegetative stage, second application at maturation stage.

2.2.2 Experimental design

The experimental design comprised 8 treatments in completely randomized blocks with four replications in the middle harvest (winter) and late harvest (spring periods). For both periods, the treatments were as follows: (*i*) control, no application of multinutrient foliar fertilizer or ripener; (*ii*) V, application of multinutrient foliar fertilizer at the sugarcane vegetative stage; (*iii*) M, application of multinutrient foliar fertilizer at the maturation stage; (*iv*) R, application of ripener at the maturation stage; (*v*) VM, application of multinutrient foliar fertilizer at both the vegetative and maturation stages; (*vi*) VR, application of multinutrient foliar fertilizer at the vegetative stage and ripener at the maturation stage; (*vii*) MR, application of both multinutrient foliar fertilizer and ripener at the maturation stage; and (*viii*) VMR, application of multinutrient foliar fertilizer at both the vegetative and maturation stages and ripener at the maturation stage.

The composition of the multinutrient fertilizer is 200 g N ha^{-1}, 400 g K ha^{-1}, 400 g Mg ha^{-1}, 200 g S ha^{-1}, 100 g B ha^{-1}, 150 g Mn ha^{-1}, 150 g Zn ha^{-1} and 30 g Mo ha^{-1} in 7% of chelating agent EDTA. The same multinutrient foliar fertilizer was used in both the vegetative and maturation stages and was applied at 2 L ha^{-1}, all of them were carried out with use of an adjuvant to improve fertilizer application and enhance its efficiency. The first application of multinutrient foliar fertilizer was performed 150 and 60 days before harvest (DBH) in the middle harvest (winter) and late harvest (spring) periods, respectively. The second application of foliar fertilizer was performed at 30 DBH in the middle harvest (winter) and late harvest (spring) periods (Figure 3). Growth regulators that inhibit acetolactate synthase (ALS) [sulfometuron-methyl (Corteva, Brazil) and bispyribac-sodium (Ihara, Brazil)] were used as ripeners at the recommended doses, which is 20 g ha^{-1} and 0.075 L ha^{-1}, respectively, in the field. All ripener applications were performed at 30 DBH, at the maturation stage of sugarcane.

The multinutrient foliar fertilizer and ripener were applied using CO_2-pressurized backpack sprayer equipment with a single tip (type 1/4KLC-9 brass fieldjet) coupled to a 2.6-m-long rod. The working pressure was 4 kgf cm^2 or 58.0 PSI equivalent, for an average flow of 100 L ha^{-1}. In treatments with both foliar fertilizer and ripener applications, the products were sprayed separately, following the sequence of foliar fertilization before ripener application. All applications were performed early in the morning, between 7 and 9 a.m., with milder temperatures and high relative humidity, respecting the appropriate climatic conditions for foliar applications.

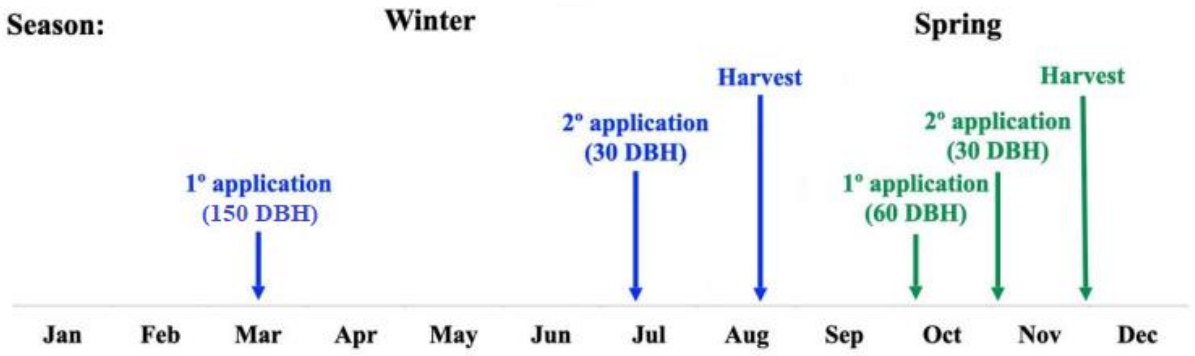

Figure 3. Timing of applications and harvest in the winter (middle harvest) and spring (late harvest) seasons. In middle harvest, the first application was performed 150 days before harvest (DBH); in late harvest, the first application was performed 60 DBH. The second application was 30 DBH in both periods.

2.2.3 Sugarcane measurements

Sugarcane growth was evaluated by measuring the following biometric parameters: plant height, stalk diameter, and stalk yield. Plant height was measured with a measuring tape from the root–shoot junction to the auricular region of leaf +3, according to the numbering suggested by Kujiper (VAN DILLEWIJN, 1952). The stalk diameter was measured at the third internode with the aid of a digital caliper. Plant height and stalk diameter were measured in 20 stalks randomly collected from each plot at the ripening phenological stage prior to harvest. Stalk yield was defined by collecting sugarcane stalks sequentially in a 2-m length established at random in the two central rows of each plot. Uniform, flawless stalks were collected and weighed, and the results were extrapolated to obtain the yield in tons per hectare.

Sugarcane raw material quality was evaluated by determining the following technological parameters: sucrose concentration (%), purity (%), fiber content (%), RS (kg Mg^{-1} of stalk) and theoretical recoverable sugar (TRS) (kg Mg^{-1} of stalk). Ten stalks were collected in sequence in the central row of all plots at each site, topped at apical bud height, defoliated, and transported to the sugarcane technology laboratory of each sugar mill to be processed according to the previously developed methodology of Fernandes (2011) for TRS. Sugar yield was calculated by multiplying the stalk yield by TRS and dividing by 1000. The results for fiber content and stalk yield were used to calculate bagasse yield at 50% moisture. The stalk yield was used to calculate straw production assuming 140 kg of straw per Mg of stalks. To estimate energy production, 1 Mg of straw was assumed to have 4.96 MWh of primary energy, and 1 Mg of bagasse was assumed to have 4.94 MWh of primary energy (Waldheim et al., 2001).

Metabolite analyses were performed at sites 1, 3 and 7 to encompass all locations and sugarcane cultivars. Fully expanded (leaf + 1) or TVD leaves were sampled and immediately frozen in liquid N for storage at -80 °C. The samples were ground to a fine powder in a mortar and pestle and lyophilized. To extract alcohol-soluble sugars, 20 mg of lyophilized powder was extracted with 1 mL of 80% (v/v) ethanol at 80 °C for 20 min. This process was repeated six times for complete extraction of alcohol-soluble sugars. The supernatants were dried under vacuum, and the pellets were resuspended in 1 mL of deionized water (DE SOUZA et al., 2014). Pigments were extracted in 0.5 mL of 99% chloroform and analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD). The alcohol-soluble sugars were separated on a CarboPac PA1 column by isocratic elution in 150 mM NaOH (1 mL min^{-1}). Starch quantification was performed using the remaining pellets after drying in an oven at 40 °C for 12 h. Leaf reducing sugar (mg g^{-1} dry weight), sucrose (mg g^{-1} dry weight), starch (mg g^{-1} dry weight) and total sugar (mg g^{-1} dry weight) were quantified by reference to calibration curves created using standard solutions of each sugar. Enzymatic activities were quantified according to the method of DE SOUZA et al. (2014).

2.2.4 Statistical analysis

The experimental design was arranged in randomized blocks with split–split plots. The homogeneity of variances and normality of the data were assessed with the F-Snedecor and Shapiro–Wilk tests, respectively. The data were then submitted to analysis of variance (ANOVA), and means were compared between foliar fertilizer and ripening treatments and between sites using the Scott–Knott test (LSD) ($p < 0.1$) in SAS software. As there was no significant difference between sites, results are average of sites in each year. Year was not tested due to sugarcane age being different between years.

2.3 RESULTS

2.3.1 Leaf metabolite levels

Leaf metabolite content differed significantly between treatments (Figures 4A, 4B, 4C and 4D). Total sugar content was 22.9, 12.2 and 15.9 mg g^{-1} dry weight higher in VM, MR, and VMR, respectively, than in the control (Figure 4D). Reducing sugar content (mg g^{-1} dry weight) was highest in R, with an increase of 15.8% compared to the control, and lowest in VM (Figure 4A). Starch content was highest in R and VR, with increases of 6.6 and 4.5 mg g^{-1} dry weight,

respectively, compared to the control. Similar to reducing sugar content, starch content was lowest in VM, with a decrease of 7.1 mg g^{-1} dry weight compared to the control (Figure 4C). Multinutrient foliar fertilization significantly ($p \leq 0.1$) increased sucrose content. Sucrose content was highest in VM (56.47 mg g^{-1} dry weight), with an increase of 81.3% compared with the control (31.15 mg g^{-1} dry weight) (Figure 4B), and lowest in the control and R (31.1 and 30.0 mg g^{-1} dry weight, respectively).

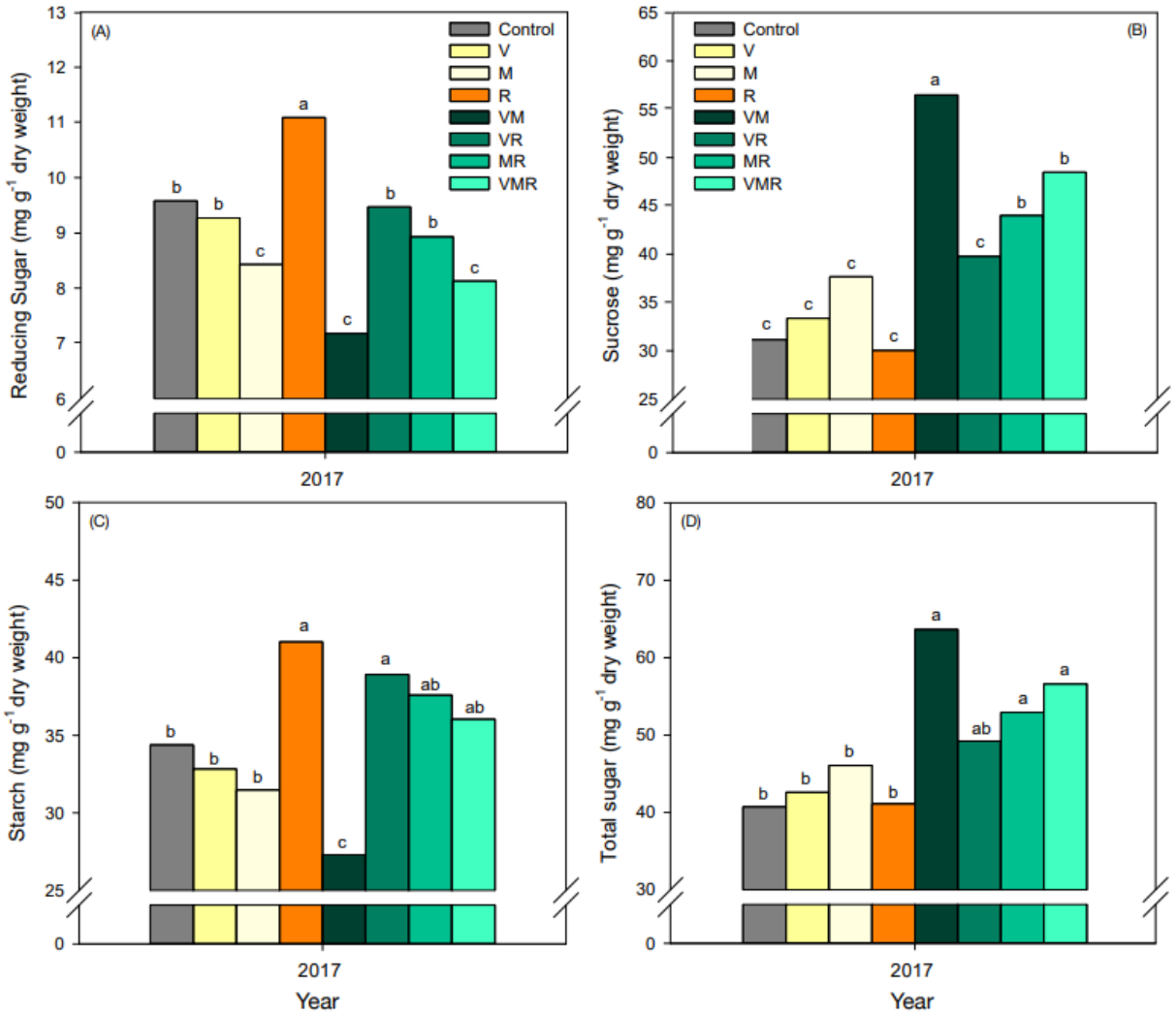

Figure 4. Reducing sugar (A), sucrose (B), starch (C) and total sugar (D) as a function of multinutrient foliar fertilization and ripener applications in the winter (middle harvest) season. The treatments were as follows: (*1*) control, no applications of multinutrient foliar fertilizer or ripener; (*2*) V, application of multinutrient foliar fertilizer at the sugarcane vegetative stage; (*3*) M, application of multinutrient foliar fertilizer at the sugarcane maturation stage; (*4*) R, application of ripener at the maturation stage; (*5*) VM, application of multinutrient foliar fertilizer at both the vegetative and maturation stages; (*6*) VR, application of multinutrient foliar fertilizer at the vegetative stage plus ripener at the maturation stage; (*7*) MR, application of multinutrient foliar fertilizer at the maturation stage plus ripener at the maturation stage; (*8*) VMR, application of multinutrient foliar fertilizer at both the vegetative and maturation stages plus ripener at the maturation stage. Means followed by the same letter do not differ by the Scott–Knott test at 10% probability.

2.3.2 Quality parameters

The sugarcane quality parameters reducing sugar content (%), fiber content (%), and purity (%) did not differ significantly between the treatments in either middle harvest. However, in late harvest 2017, purity was higher in all treatments with ripener application (R, VR, MR, and VMR) than in the control, M and VM, with an average increase of 1.65 percentage points (Figure 5B). The treatments had opposing effects on reducing sugar content compared with fiber content and purity. Accordingly, reducing sugar content was lowest in R, VR, MR, and VMR due to the greater synthesis of sucrose from glycose and fructose (Figure 5D).

In middle harvest 2017, sucrose content (%) and TRS were significantly higher in VR and VMR than in the other treatments. Compared with the control, VR and VMR increased sucrose content by 3.2 and 3.4 percentage points and TRS by 4.4 and 4.8 kg sugar Mg stalk^{-1}, respectively (Figures 6A and 6C). In middle harvest 2019, sucrose content was significantly higher in R, VR, MR, and VMR than in the other treatments. Compared with the control, R, VR, MR, and VMR increased sucrose content by 2.9%, 3.2%, 2.3%, and 2.9% and TRS by 4.4, 4.8, 3.5, and 4.3 kg sugar Mg stalk^{-1}, respectively (Figures 6A and 6C). A similar pattern was observed in late harvest 2017; TRS was significantly higher in R, VR, MR, and VMR than in the other treatments, and these four treatments increased TRS by 7.3, 7.8, 6.0, and 8.2 kg sugar Mg stalk^{-1}, respectively, compared with the control (Figure 6D). Regardless of season or year, sucrose and TRS were lowest in the control (Figures 6A, 6B, 6C and 6D).

In middle harvest 2017, sugar yield (Mg ha^{-1}) was highest in VM and VMR, with increases of 1.35 and 1.58 Mg ha^{-1}, respectively, compared with the control. In middle harvest 2019, SY was highest in V, VM, VR and VMR, with gains of 0.94, 1,12, 1.18 and 1.28 Mg ha^{-1}, respectively, compared with the control (Figure 6E). These results showed that, in middle harvest season, with unfavorable weather conditions for sugarcane development (low temperatures and rainfall) and favorable to natural ripening, as we observed in climate data from 2017 and 2019, the V and VM treatments had a great performance, proving to be an interesting management alternative, without the need to ripener application, once they were statistically similar to VMR (Figure 6E).

Finally, in late harvest 2017, sugar yield was significantly higher in VMR than in the other treatments, with an increase of 1.56 Mg ha^{-1} compared with the control (Figure 6F). Similar to TRS, sugar yield was lowest in the control in all years and seasons.

Sugar yield was highest in V, VM, VR and VMR in middle harvest and VMR in late harvest, and considering only VMR treatment, it presented an average increase, compared to

the control, of 10.0% in middle harvest and 14.0% in late harvest. These results demonstrate that combining two applications of multinutrient foliar fertilizer at the vegetative and maturation stages with ripener application improves sugarcane yield and quality (Figures 6E and 6F).

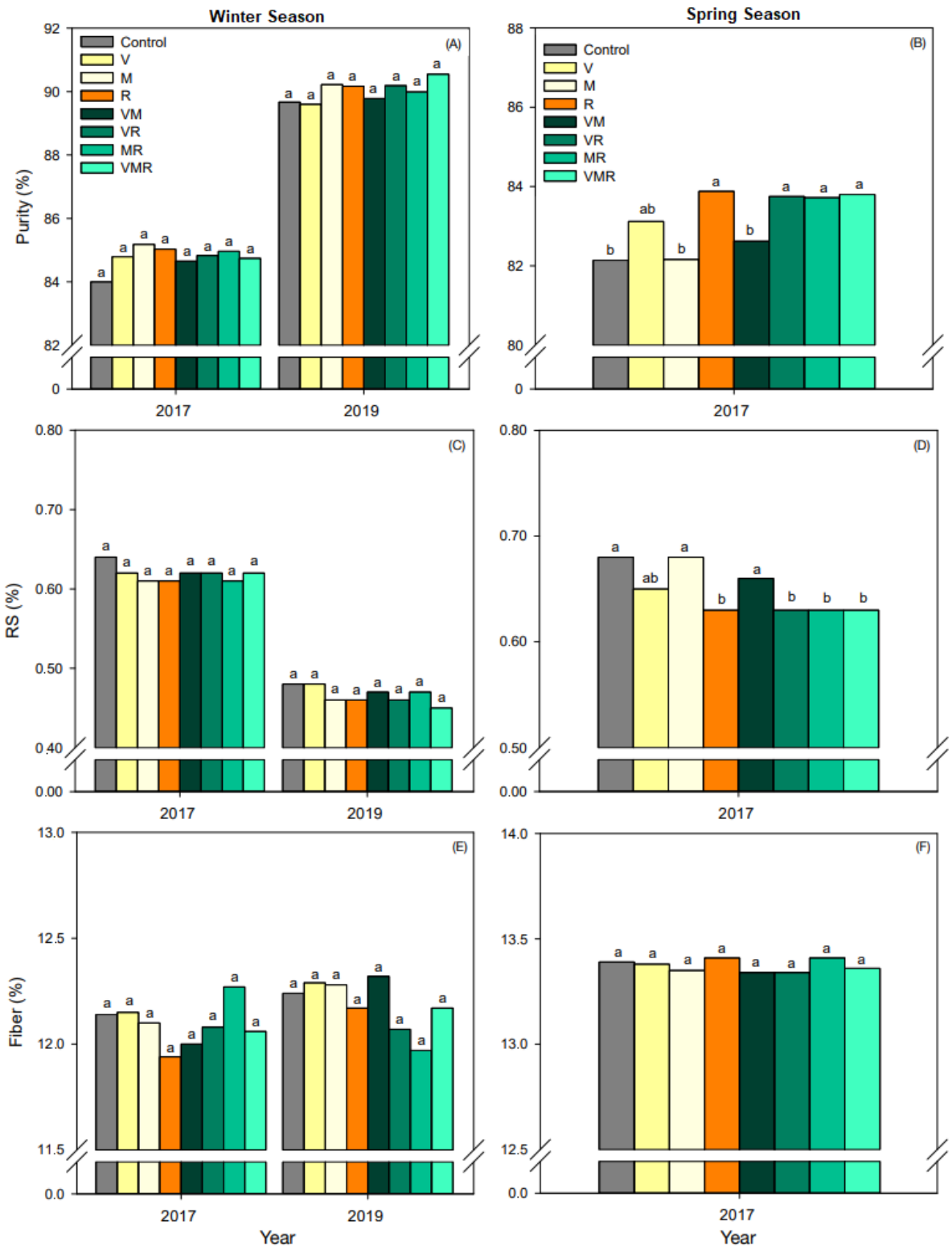

Figure 5. Purity (A and B), reducing sugar (RS) (C and D), fiber (E and F) as a function of multinutrient foliar fertilization and ripener applications in the winter (middle harvest) and spring (late harvest) seasons. The treatments were as follows: (*1*) control, no applications of multinutrient foliar fertilizer or ripener; (*2*) V, application of multinutrient foliar fertilizer at the sugarcane vegetative stage; (*3*) M, application of multinutrient foliar fertilizer at the sugarcane maturation stage; (*4*) R, application of ripener at the maturation stage; (*5*) VM, application of multinutrient foliar fertilizer at both the vegetative and maturation stages; (*6*) VR, application of multinutrient foliar fertilizer at the vegetative stage plus ripener at the maturation stage; (*7*) MR, application of multinutrient foliar fertilizer at the maturation stage plus ripener at the maturation stage; (*8*) VMR, application of multinutrient foliar fertilizer at both the vegetative and maturation stages plus ripener at the maturation stage. Means followed by the same letter do not differ by the Scott–Knott test at 10% probability.

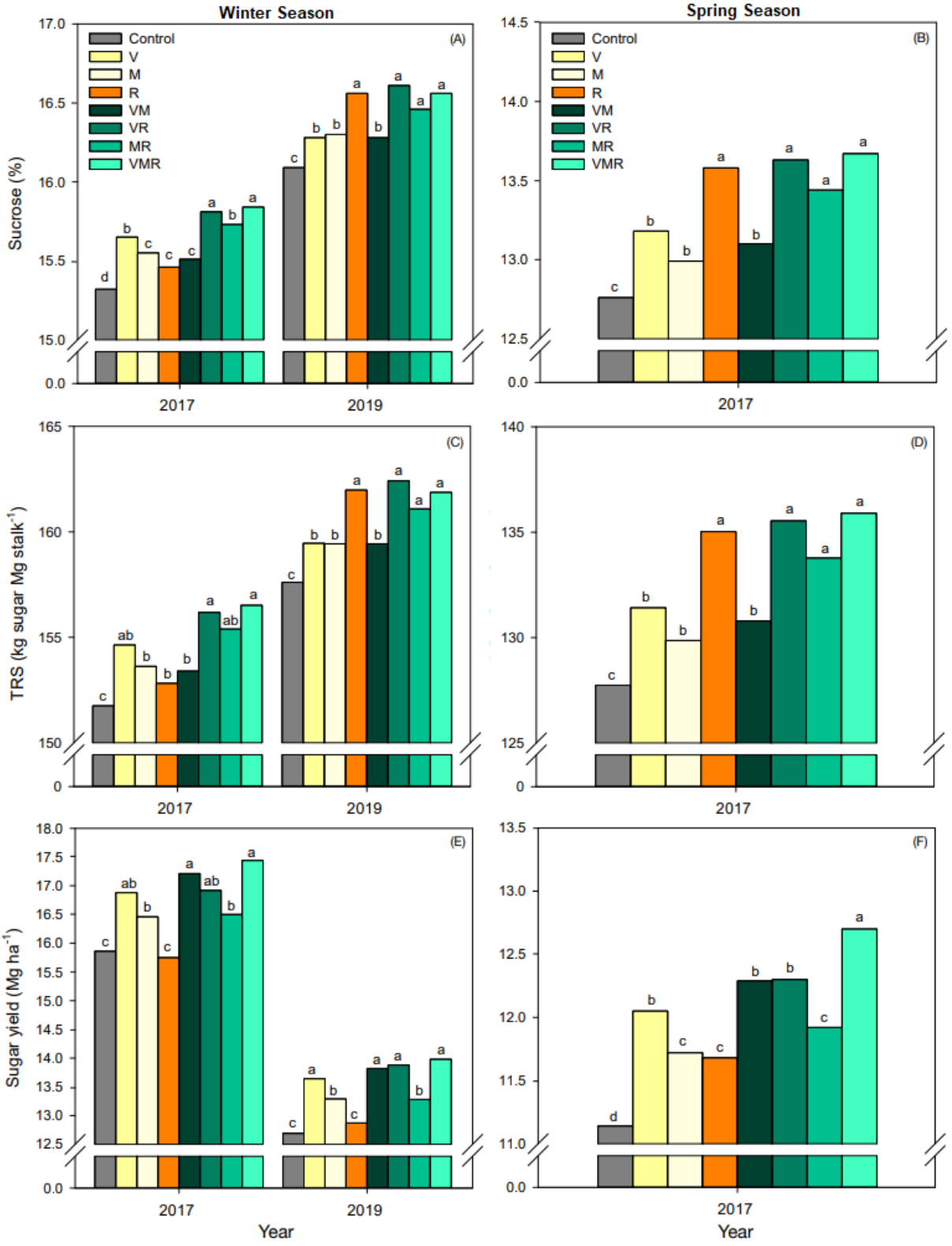

Figure 6. Sucrose (A and B), TRS (C and D) and sugar yield (E and F) as a function of multinutrient foliar fertilization and ripener applications in the winter (middle harvest) and spring (late harvest) seasons. The treatments were as follows: (*1*) control, no applications of multinutrient foliar fertilizer or ripener; (*2*) V, application of multinutrient foliar fertilizer at the sugarcane vegetative stage; (*3*) M, application of multinutrient foliar fertilizer at the sugarcane maturation stage; (*4*) R, application of

ripener at the maturation stage; (*5*) VM, application of multinutrient foliar fertilizer at both the vegetative and maturation stages; (*6*) VR, application of multinutrient foliar fertilizer at the vegetative stage plus ripener at the maturation stage; (*7*) MR, application of multinutrient foliar fertilizer at the maturation stage plus ripener at the maturation stage; (*8*) VMR, application of multinutrient foliar fertilizer at both the vegetative and maturation stages plus ripener at the maturation stage. Means followed by the same letter do not differ by the Scott–Knott test at 10% probability.

2.3.3 Biometric parameters

In middle harvest 2019, VM and VMR significantly ($p \leq 0.1$) increased plant height by 15.8 and 14.5 cm, respectively, compared with the control (Figure 7A). Similar effects of these treatments were observed in middle and late harvest 2017, although the increases were not significant. These results suggest that two applications of multinutrient foliar fertilizer provide a greater response of growth parameters than a single application. Plant height was lowest in the control and R regardless of season, although the differences were only significant in 2019. The average plant height in the control treatment and R in 2019 was 280 cm, 5.4% lower than in VM and VMR (Figures 7A and 7B). ANOVA indicated that there were no significant differences in stalk diameter between the treatments in any season or year, although like plant height, stalk diameter was highest in VM and VMR (Figures 7C and 7D).

Multinutrient foliar fertilization also increased stalk yield in all seasons and years. The response to fertilization was greatest in VM and VMR. Compared to the control, VM and VMR increased stalk yield by 6.6 Mg ha^{-1} (7.1%) and 6.0 Mg ha^{-1} (6.4%), respectively, when averaged over the two middle harvest seasons and by 7.0 Mg ha^{-1} (8.1%) and 6.3 Mg ha^{-1} (7.3%), respectively, in the late harvest season (Figures 7E and 7F). Furthermore, in middle harvest 2019, stalk yield was significantly higher in V and VR than in the control, with an average gain of 4.6 Mg ha^{-1} (5.6%). These results indicate a positive influence of applying multinutrient foliar fertilizer at the sugarcane vegetative stage (V, VM, VR and VMR), i.e., within 150 DBH in the middle harvest and 60 DBH in the late harvest season. Stalk yield was lowest in the control and R in both seasons and years. Moreover, compared to the control, R decreased stalk yield by 1.5 Mg ha^{-1} when averaged over the two middle harvest and by 0.8 Mg ha^{-1} in late harvest 2017, highlighting the growth-inhibiting effects of ripeners on sugarcane (Figures 7E and 7F).

In summary, the application of multinutrient foliar fertilizer ameliorated the effects of ripener use on stalk yield (VR, MR and VMR) compared to R in all seasons and years, indicating that this combination could be beneficial for sugarcane yield and quality.

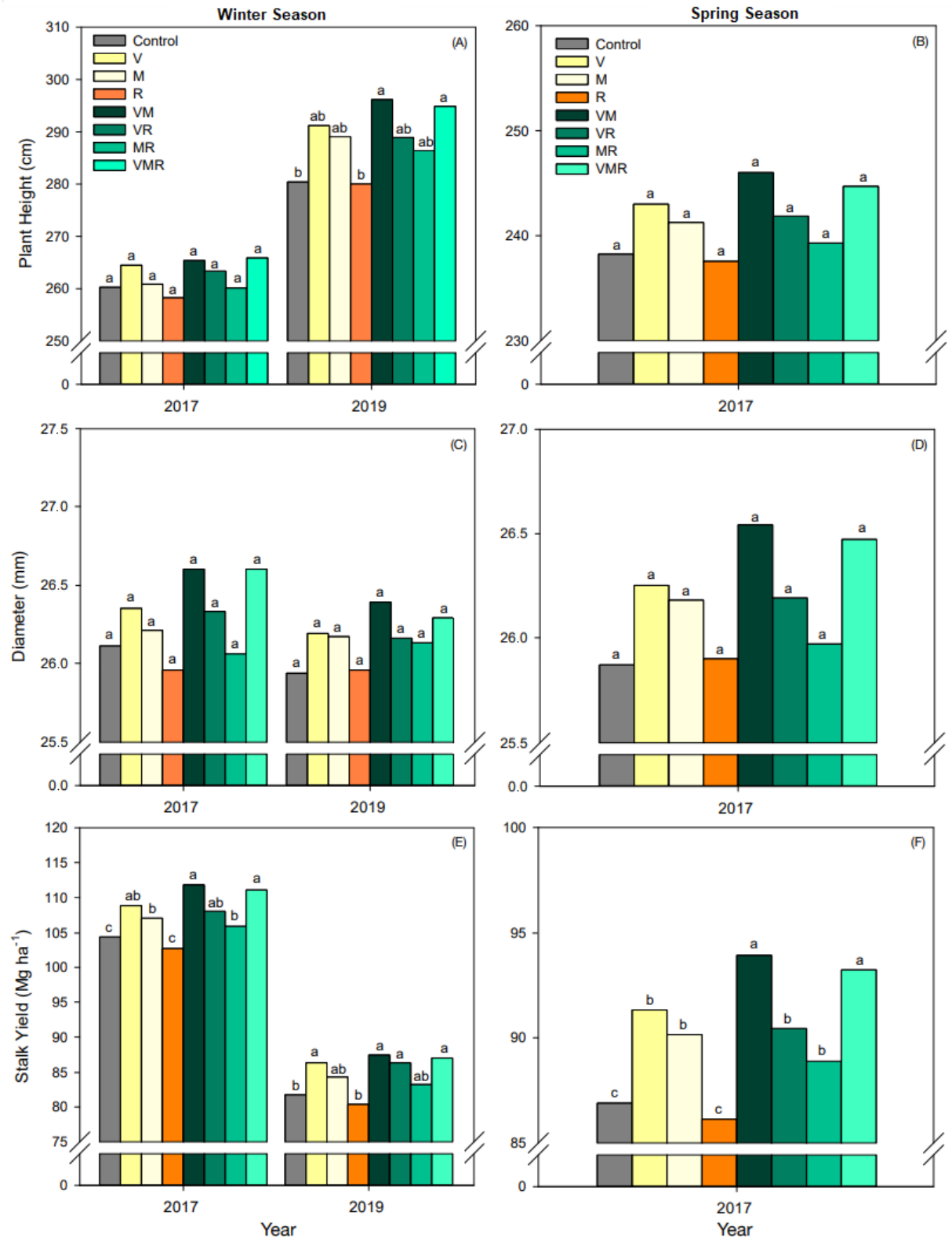
Winter Season
Spring Season
Control
V
M
R
VM
VR
MR
VMR
(A)
(B)
(C)
(D)
(E)
(F)
Plant Height (cm)
Diameter (mm)
Stalk Yield (Mg ha-1)
2017
2019
Year

Figure 7. Sugarcane plant height (A and B), stalk diameter (C and D) and stalk yield (E and F) as a function of multinutrient foliar fertilization and ripener applications in the winter (middle harvest) and spring (late harvest) seasons. The treatments were as follows: (*1*) control, no applications of multinutrient foliar fertilizer or ripener; (*2*) V, application of multinutrient foliar fertilizer at the sugarcane vegetative stage; (*3*) M, application of multinutrient foliar fertilizer at the sugarcane maturation stage; (*4*) R, application of ripener at the maturation stage; (*5*) VM, application of multinutrient foliar fertilizer at both the vegetative and maturation stages; (*6*) VR, application of multinutrient foliar fertilizer at the vegetative stage plus ripener at the maturation stage; (*7*) MR, application of multinutrient foliar fertilizer at the maturation stage plus ripener at the maturation stage; (*8*) VMR, application of multinutrient foliar fertilizer at both the vegetative and maturation stages plus ripener at the maturation stage. Means followed by the same letter do not differ by the Scott–Knott test at 10% probability.

2.3.4 Energy parameters

Multinutrient foliar fertilization and ripener application significantly ($p \leq 0.1$) increased bagasse content (Mg ha^{-1}) and the estimated energy yield (kWh) (Figures 8C and 8D). In middle harvest 2017, bagasse content was highest in V, VM, and VMR, with increases of 4.43%, 6.17%, and 5.70%, respectively, compared with the control. In middle harvest 2019, bagasse content was significantly higher in V, M, VM, VR, and VMR than in the other treatments, with gains of 6.01%, 4.01%, 7.41%, 4.61%, and 6.01%, respectively, compared with the control (Figure 8C). In late harvest 2017, bagasse content was highest in V, VM and VMR, with increases of 5.00%, 8.28%, and 7.59%, compared with the control, or an average increase of 6.95% (Figure 8D).

Similar to bagasse content, straw yield was highest in the treatments with multinutrient foliar fertilization (Figures 8A and 8B). In middle harvest 2017, straw yield was significantly higher in VM and VMR than in the control, with gains of 7.1% and 6.4%, respectively. In 2019, straw yield was highest in V, VM, VR, and VMR, with increases of 5.7%, 7.0%, 5.6%, and 6.5%, respectively, compared with the control (Figure 8A). In late harvest 2017, straw yield was 8.1% and 7.3% higher in VM and VMR, respectively, compared with the control (Figure 8B).

In middle harvest 2017, the estimated energy yield was 77.6 kWh (7.2%) and 77.1 kWh (6.5%) higher in VM and VMR, respectively, than in the control. The estimated energy yield in middle harvest 2019 was significantly higher in V, VM, VR, and VMR than in the other treatments, with increases of 3.18, 3.96, 3.18, and 3.65 kWh, respectively, compared with the control (Figure 8E). In late harvest 2017, the estimated energy yield was highest in VM and VMR, with increases of 4.88 kWh (8.1%) and 4.40 kWh (7.3%), respectively, compared with

the control (Figure 8F). Bagasse content and the estimated energy yield were lowest in the control and R regardless of season or year (Figures 8C, 8D, 8E and 8F).

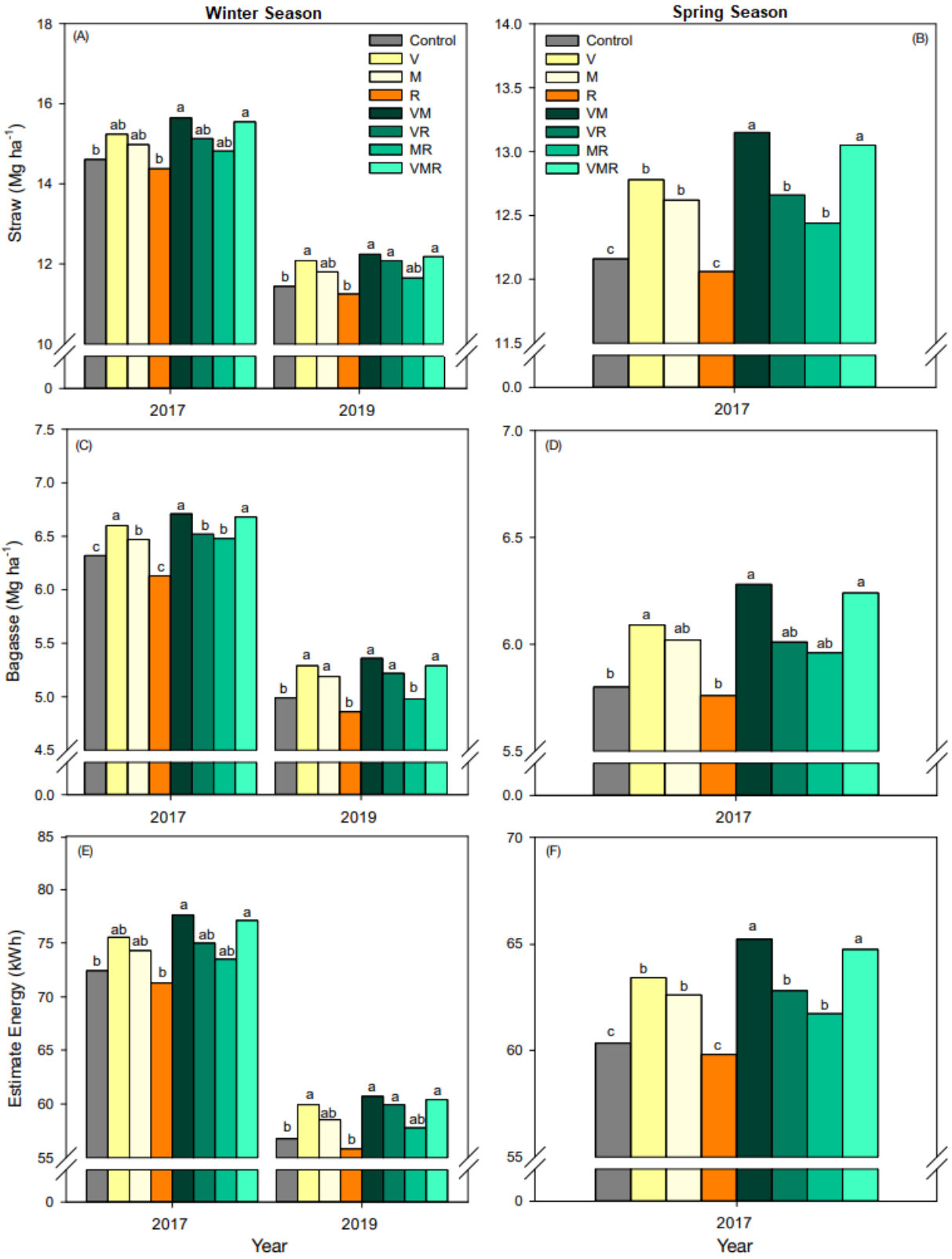

Figure 8. Straw (A and B), bagasse (C and D) and estimate energy (E and F) as a function of multinutrient foliar fertilization and ripener applications in the winter (middle harvest) and spring (late

harvest) seasons. The treatments were as follows: (*1*) control, no applications of multinutrient foliar fertilizer or ripener; (*2*) V, application of multinutrient foliar fertilizer at the sugarcane vegetative stage; (*3*) M, application of multinutrient foliar fertilizer at the sugarcane maturation stage; (*4*) R, application of ripener at the maturation stage; (*5*) VM, application of multinutrient foliar fertilizer at both the vegetative and maturation stages; (*6*) VR, application of multinutrient foliar fertilizer at the vegetative stage plus ripener at the maturation stage; (*7*) MR, application of multinutrient foliar fertilizer at the maturation stage plus ripener at the maturation stage; (*8*) VMR, application of multinutrient foliar fertilizer at both the vegetative and maturation stages plus ripener at the maturation stage. Means followed by the same letter do not differ by the Scott–Knott test at 10% probability.

2.4 DISCUSSION

In this study, multinutrient foliar fertilizer application improved sugarcane plant growth parameters. In all years and seasons, sugarcane stalk growth and yield were highest when fertilizer was applied at the vegetative stage, i.e., an average of 150 DBH in the middle harvest season or 60 DBH in the late harvest season (Figure 7). These results are consistent with previous reports that applying foliar fertilizers at the vegetative and maturation stages increases sugarcane yields (KARMOLLACHAAB et al., 2016; NAGA MADHURI et al., 2013).

Foliar fertilization at the vegetative stage coincides with a period of high nutritional demand and stimulates the emergence of new leaves, which are more metabolically active, have thinner cuticles and waxy layers, and thus are more permeable to substance entry (FÉRNANDEZ; BROWN, 2013; FÉRNANDEZ; SOTIROPOULOS; BROWN, 2013; ROSSI; BERMUDEZ; CARRARI, 2015). Consequently, foliar fertilization in the vegetative stage typically improves the absorption of subsequent applications of fertilizer and ripener. This makes dual application (at the vegetative and maturation stages) a promising strategy to meet plant nutritional demands and increase nutrient use efficiency. Additionally, these applications can increase yield by improving the nutrient supply in leaves, mitigating low nutrient translocation in plants (PAPADAKIS; SOTIROPOULOS; THERIOS, 2007).

Plant biomass is approximately 90% carbohydrates and the sugar metabolism enzymes involved in sucrose and starch synthesis are extremely important for regulating carbohydrate partitioning, plant development, productivity, and crop quality, especially under stress (LECLERE; SCHMELZ; CHOUREY, 2010; RUAN et al., 2010). These enzymes are mainly stimulated by nutrients (Isla et al., 1988). Accordingly, in this study, plant height and stalk yield were lower when no multinutrient foliar fertilizer was applied at the vegetative and/or maturation stages, i.e., the control and R treatments. These results corroborate those of previous studies (BANASIHAN; MACALAGUIN; MENDOZA, 2007; VIATOR et al., 2008).

Ripener application in 2017 late harvest season increased technological parameters, such as sucrose content and TRS, especially when ripener application was associated with foliar fertilization at the vegetative and/or maturation stages. In these treatments, sucrose synthesis and accumulation in the stalks increased (Figure 6), consistent with previous findings (INOUE et al., 2015; MESCHEDE; VELINI; CARBONARI, 2010). In middle harvest season, both 2017 and 2019, ripener application also increased TRS and sucrose content, however, the V and VM treatments (without ripener) had a positive effect on sugar yield, which consider stalk yield and TRS, due to the higher results in biometric parameters. These results were obtained because of the climate conditions observed in 2017 and 2019, that stimulates the natural sugarcane maturation, with lower temperatures, rainfall, and humidity, reducing the ripener effect.

TRS represents all sugarcane sugars in the stalk, i.e., both reducing sugars (glucose and fructose) and total sugars (sucrose). Thus, the present results confirm that ripener application is an efficient tool to promote sucrose accumulation and sugar yield. TRS is an essential indicator of the quality of processed sugarcane, especially when it is destined for ethanol production (FERNANDES, 2011), and of economic productivity.

The application of the multinutrient foliar fertilizer composed by N, K, Mg, S, B, Mn, Zn and Mo, stimulated sucrose synthesis, probably by increasing photosynthetic activity, through the functions of these nutrients. Nitrogen is present in the structure of enzymes, such as RuBP carboxylase (Rubisco) and phosphoenolpyruvate carboxylase (PEPcase), increasing the photosynthetic rate due to its adequate supply on leaves (GASTAL; LEMAIRE, 2002; FAGAN et al., 2016). Potassium acts in protein synthesis as a cofactor in the activation of amino acids and indirectly in the supply of ATP, and the K concentration in the plant regulates the stomatal opening and closing and under its imbalance there is an influx of water from the guard cells, causing the closure of the stomata and avoiding CO_2 entry, consequently lower photosynthetic rate and less sucrose synthesis, accumulation and transport, due to lower water content (TAIZ; ZEIGER, 2004; FRANZÉ, 2010).

Depending on climatic conditions, photosynthates are directed to growth or storage in stalks. Ripener application promoted the targeting of synthesized sucrose to parenchymal storage cells in the stalks. In the initial phase of oxidative stress, stored sucrose functions as a primary antioxidant (STOYANOVA et al., 2011; YUANYUAN et al., 2009) against reactive oxygen species (ROS). ROS have deleterious effects on cells through lipid peroxidation, which results in extravasation of cellular compounds. ROS are continuously produced in chloroplasts during photosynthesis, in mitochondria during cellular respiration, in peroxisomes and in the plasma membrane (NADPH oxidases). When photosynthetic activity and sucrose production

are high, it is thought that sugars neutralize (directly or indirectly) lipid peroxidation, that is, stress, facilitating cellular ROS homeostasis (GILL; TUTEJA, 2010; SHEN; JENSEN; BOHNERT, 1997). Sucrose is also involved in the biosynthesis of enzymatic and non-enzymatic antioxidants (BOLOURI-MOGHADDAM et al., 2010; MUNDREE et al., 2002; PARVANOVA et al., 2004).

Although the treatments in which only multinutrient foliar fertilizer was applied (V, M and VM) did not increase TRS and sucrose content as much as the treatments with ripener applications (R, VR, MR and VMR), the former treatments were still superior to the control. Thus, multinutrient foliar fertilization can provide a competitive advantage against ROS-induced stress by increasing sucrose levels (BOLOURI-MOGHADDAM et al., 2010; COUÉE et al., 2006; SMEEKENS et al., 2010; XIANG et al., 2011) and also by function of the nutrients as constituents of enzymes or in the activation of these, i.e., zinc, which is required in superoxide dismutase (SOD) (FRAÚSTO DA SILVA; WILLIAMS, 1991). Zinc foliar application probably promotes an increase in photosynthesis by activating carbonic anhydrase, which is an important enzyme in fixing CO_2, stimulating the carbohydrate metabolism (FAGAN et al., 2016)

The positive effects of the combined fertilizer and ripener treatment (VMR) on technological and growth parameters (Figures 5 to 7) can be explained by several factors. First, VMR increased photosynthetic activity and consequently the synthesis of sucrose, which is necessary for cell growth and elongation (AMAO; TAKAI; OHASHI, 2010) and accumulates in the vacuole in parenchymal storage cells (JAIN; CHANDRA; SOLOMON, 2013). Second, nutrients stimulate the activity of enzymes responsible for synthesizing and degrading sucrose (ISLA et al., 1988). Third, VMR probably stimulated specialized plant metabolic pathways involved in defense, including enzymatic and non-enzymatic antioxidants that eliminate ROS (MESQUITA et al., 2019; SARKER; OBA; DARAMY, 2020). Finally, VMR reduced premature leaf senescence (BLANDINO; REYNERI, 2009; RIOU-KHAMLICHI et al., 2000).

In addition to mitigating leaf senescence, multinutrient foliar fertilization can increase leaf lifetime, which is critical for achieving a higher stalk growth rate and raw material quality. A greater biomass of green and photosynthetically active leaves increases the synthesis of sucrose and starch during the day and subsequent degradation at night. As the plant ages at the end of the crop cycle, the growth rate decreases, resulting in greater accumulation of sucrose in stalks, leaves and roots and starch in leaves (DE SOUZA et al., 2018). ROS generation is a major limitation for reaching sugarcane yield potential. Although ROS do not directly affect leaf senescence, they induce gene expression processes that stimulate programmed cell death,

promoting a balance between ROS production and elimination or homeostasis (AHMAD; SARWAT; SHARMA, 2008; GILL; TUTEJA, 2010). During the final stage of crop development, the concentrations of antioxidant enzymes are low, whereas the concentrations of ROS are high (KOVTUN et al., 2000; NEILL; DESIKAN; HANCOCK, 2002; SLESAK et al., 2007). Antioxidants that can mitigate the effects of ROS damage include sugars such as soluble carbohydrates (glucose, fructose and sucrose) and oligosaccharides of the raffinose family (RFOs) (Stoyanova et al., 2011), which have excellent capacity to eliminate ROS (VAN DEN ENDE; VALLURU, 2009). In summary, increasing sucrose synthesis also contributes to ROS elimination.

In addition to increasing sugar and stalk yields (Figures 6 and 7), multinutrient foliar fertilization increased bagasse production and, consequently, estimated energy yields (Figure 8), similar to results reported by Amorim et al. (2011) and Soccol et al. (2010). Straw and bagasse biomass (Figure 8) are used to generate energy by burning or as feedstocks in the production of second-generation ethanol (DE SOUZA et al., 2013). Multinutrient foliar fertilization of sugarcane at the vegetative and maturation stages was associated with the highest straw and bagasse production in all years and seasons. Sugarcane bagasse is the main byproduct of the sugarcane industry and is mainly lignocellulosic biomass. Large quantities of bagasse are used as substrates for second-generation ethanol and energy cogeneration, enhancing sugarcane profitability. The remaining lignocellulosic biomass can increase bioethanol production by 40%, even if part of the bagasse from sugar production is used for electricity co-generation and 50% of the leaf straw is left in the field to promote soil coverage and sustainability (BUCKERIDGE; DE SOUZA; DOS SANTOS, 2010). Thus, applying multinutrient foliar fertilizer at the vegetative and maturation stages of sugarcane in all seasons could have profound economic benefits.

The evaluation of leaf metabolites (Figure 4) showed that leaf sucrose content increased only when multinutrient foliar fertilizer was applied at both stages and followed by ripener application in the maturation stage. A similar result was obtained for leaf total sugar content. These improvements can be explained by the daily metabolic variations that occur during the plant vegetative cycle. The accumulation of sucrose and starch in leaves induces an increase in stalk sucrose concentrations and a reduction in the photosynthetic rate at the end of the vegetative stage and beginning of the maturation stage. As a result, daily metabolic variations decrease and become undetectable (DE SOUZA et al., 2018). Increases in leaf metabolite content reduce carbon availability, which reduces plant growth by affecting cell division, cell expansion and morphogenesis (COOKSON; VAN LIJSEBETTENS; GRANIER, 2005;

FLEMING, 2006; TSUKAYA; BEEMSTER, 2006). When carbon availability is low, the transcription of genes related to protein biosynthesis (activation of amino acids, ribosomal proteins, rRNA), cell division and cycle, and DNA synthesis and repair decrease (SMITH; STITT, 2007). This relationship between carbon availability and plant vegetative growth is apparent in the G1-S phase transition in the cell cycle and is also related to sugars. Cells deficient in sugars stop at the G1 phase of the cell cycle, a stage where nutritional, hormonal and developmental signals are integrated to determine whether DNA replication will occur and whether possible further division is compromised (MENGES et al., 2006; MENGES; MURRAY, 2002). The resulting accumulation of sucrose in stalks alters the plant's source–sink relationship and the behavioral pattern of NSCs through changes in photosynthetic rates (DE SOUZA et al., 2018).

In summary, stimulating photosynthetic activity through the application of multinutrient foliar fertilizer (once or twice) increases sugar production and consequently productivity. When the multinutrient foliar fertilizer is applied in association with ripener, sucrose accumulation increases, enhancing both productivity and the industrial quality of the raw material.

2.5 CONCLUSIONS

Combining the application of multinutrient foliar fertilizer at the vegetative and maturation stages with ripener application is a great option for increasing sucrose synthesis, plant development and sugarcane stalk and sugar yields.

In middle harvest season, under unfavorable conditions for sugarcane vegetative development, within lower temperatures, humidity and less rainfall, the natural ripening process of the plant are stimulated, which turns the ripener application not effectively in sugar yield. Thus, only the multinutrient foliar application, in both vegetative and maturation stages (V or VM), showed to be the best alternative in this harvest season.

In late harvest season, with higher temperatures and rainfall, the sugarcane plants are stimulated to growth, then ripener application is very efficient, and performed even better when associated with multinutrient foliar fertilizer (VMR), as we observed in this study.

Optimization of these applications can increase sugarcane growth under adverse conditions by promoting sugarcane tillering and stimulating sucrose accumulation. In addition, this strategy can facilitate harvest management for more sustainable bioenergy production and higher raw quality for industry.

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GENERAL CONSIDERATIONS

Foliar multinutrient fertilizer composed by N, K, Mg, S, B, Mn, Zn and Mo, and ripener applications in sugarcane field production efficiently raise plant growth, sugar yields, energy production and favoured enzymes activity in sucrose metabolism, especially when fertilizer applied at vegetative and maturation stages combined with ripener application. This management proved to be a great alternative to increase sucrose synthesis, contributing to higher plant development as well as increasing quality for industry.

Considering the results found in this study, it should be noted that use of ripener is an essential tool to enhance theoretical recoverable sugar and sucrose accumulation in sugarcane, mainly in early harvest season, when the climate conditions are not favorable to natural maturation, and the association with multi-nutrient foliar contributes to achieving greater results in tons of sugar per hectare (sugar yield).

It has been clear the positive effect of multinutrient fertilization mainly in the vegetative stage, on average of 120 DBH at early harvest season, 150 and 60 days at middle and late harvest, respectively, which enhanced sugarcane growth through a longer period. The combined effects of nutrient supply by fertilizer at early development stage (vegetative) and at late stage (maturation) to complement the necessary nutrients for reactions, were reflected in improvements in productivity, mainly stalk and sugar yields, emphasizing the importance of an adequate supply of nutrients to crops. Furthermore, due to the capacity of the leaves to absorb nutrients, foliar applications represent an excellent fertilization strategy to improve the internal concentration of required nutrient at the period of greatest demand in plants.

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