Sugarcane growth, sucrose accumulation and invertase activities under trinexapac-ethyl treatment[1]

Eduardo Rossini Guimarães[2], Miguel Angelo Mutton[3], João Martins Pizauro Junior[2], Márcia Justino Rossini Mutton[2,4]

[1] Part of first author's master dissertation.

[2] Departamento de Tecnologia, Faculdade de Ciências Agrárias e Veterinárias de Jaboticabal (FCAV), Unesp. Via de Acesso Prof. Paulo Donato Castellane, s/n. CEP 14884-900, Jaboticabal (SP), Brasil.

[3] Departamento de Produção Vegetal, Unesp-FCAV.

[4] Corresponding author: mjrmut@fcav.unesp.br

Abstract

Since the 1980's Brazilian sugarcane growers and mills have used chemical ripeners to reach higher industrial yield, increase profits and broaden the harvesting season of sugarcane. Trinexapac-ethyl (TE), one of the main ripeners used in Brazil, suppresses vegetative growth and favors sucrose accumulation in sugarcane stems by inhibiting the biosynthesis of GA_1 (Gibberelic acid isoform 1) from GA_{20}. So far, little research has been done on the physiological effects of this ripener. The present research was undertaken to determine the effects of the chemical ripener trinexapac-ethyl upon sugarcane growth, sucrose accumulation and soluble and wall bound invertase activities. The experiment was installed in a second ratoon area of the sugarcane variety SP81-3250 in Pradópolis, state of São Paulo, Brazil, and arranged in randomized blocks (split-plots). Trinexapac-ethyl was applied at a rate of 0.2 L of active ingredient per hectare. The results suggest that this ripener can be used to increase sugar yield and its repressive effect on vegetative growth does not affect stalk productivity. The activity of soluble acid invertase in young stem tissues can be used as a reliable biochemical parameter to determine the response of sugarcane to the trinexapac-ethyl.

Additional keywords: *Saccharum*; gibberellin inhibition; sugar metabolism; sucrose yield; chemical ripener.

Resumo

GUIMARÃES, E. R.; MUTTON, M. A.; PIZAURO JUNIOR, J. M.; MUTTON, M. J. R. Crescimento, acúmulo de sacarose e atividade de invertases em cana-de-açúcar submetida ao tratamento com etil-trinexapac. **Científica**, Jaboticabal, v.33, n.1, p.20-26, 2005.

Desde os anos 80, fornecedores de cana e usinas têm utilizado maturadores químicos, na busca de maior valorização da cana, rendimento industrial e antecipação da safra. Apesar disso, poucas pesquisas têm sido desenvolvidas sobre os efeitos fisiológicos desses produtos. A presente pesquisa foi realizada com o intuito de determinar os efeitos do maturador químico etil-trinexapac sobre o crescimento, a atividade de invertases e a qualidade tecnológica na cana-de-açúcar durante a maturação. O experimento foi instalado em área de segundo corte, localizada no município de Pradópolis (SP), e a variedade empregada foi a SP81-3250, de maturação média. O delineamento experimental foi o de blocos casualizados, com parcelas subdivididas e quatro repetições. Os resultados indicam que o maturador permitiu um acréscimo em Açúcar Total Recuperável (ATR), e seu efeito repressivo sobre o crescimento não foi suficiente para reduzir a produtividade de colmos. O produto inibiu a atividade das invertases ácidas solúveis e ligadas à parede celular em tecidos jovens do colmo. A atividade da isoforma ácida solúvel pode ser usada como um parâmetro bioquímico confiável na determinação da resposta da cana-de-açúcar ao ethyl-trinexapac.

Palavras-chave adicionais: *Saccharum*; matéria seca; produtividade de açúcar; maturador químico.

Introdução

Brazilian sugarcane growing regions provide ideal conditions to the natural ripening of sugarcane. During the dry season (May to September), the growth rate is slowed up while the sucrose content in storage parenchyma tissues rises. The economic success of sugarcane crops is determined by the accumulation of sucrose (BATTA et al. 2002).

Since the 1980's growth regulators known as "chemical ripeners" have been used in Brazil to reach higher industrial yield, increase profits, and to broaden the harvesting season of sugarcane.

Trinexapac-ethyl (TE) is one of the main ripeners used in Brazil. According to RESENDE et al. (2000), it reduces endogenous levels of an active form of gibberellic acid, GA_1, by repressing its biosynthesis from GA_{20}. Gibberellic acids play an important regulatory role in the activities of invertases in different plant species (TYMOWSKA-LALANNE & KREIS, 1998). Therefore, the

expected physiological response of sugarcane to TE treatment is stem elongation inhibition and possible changes in the photoassimilate partitioning, favoring sucrose accumulation.

Several authors emphasize the need of further studies on the regulation of the sucrose accumulation in the sugarcane stem (MOORE, 1995; WHITTAKER & BOTHA, 1997; ZHU et al., 1997; BATTA et al. 2002).

A previous work (RESENDE et al., 2000) showed that TE significantly increases sugar yield when applied at a rate of 200 g of active ingredient per hectare, 45 to 60 days before harvest in many Brazilian commercial varieties, and provides better tillering, root growth and distribution in the following harvests. On the other hand, some cane growers and mills report that this ripener may reduce vegetative growth even at lower doses, resulting in consistent stalk productivity loss.

The present research was undertaken to determine the effects of TE on sugarcane growth, technological quality and invertase activities, and to verify if its growth repression is likely to bring sugar yield loss.

Material and methods

The experiment was conducted in a second ratoon area of 'Usina São Martinho' in Pradópolis, state of São Paulo, Brazil (latitude 21° 21' 34'' S, longitude 48° 03' 56'' W). We used the sugarcane mid-season variety SP81-3250, known as very productive (COPERSUCAR, 2003). The experiment was arranged in randomized blocks (split plots comprising five rows of 15 m, 1.5 m between rows) with four replications. The main treatments (plots) were the trinexapac-ethyl (TE) levels 0 (control) and 0.2 L ha^{-1}, and the split plots were the five sampling dates, which were not the same in all parameters and therefore are detailed in their description. Data were analyzed using analysis of variance (Table 1) and Tukey test (P = 0.05).

Table 1 – Analysis of variance scheme.

Source of variation	Degrees of freedom
Blocks	3
Trinexapac-ethyl (T)	1
Residue a	3
Plots	(7)
Sampling dates (S)	4
Interaction (T x S)	4
Residue b	24
Total	39

Trinexapac-ethyl treatment

Trinexapac-ethyl was broadcast applied at a rate of 0.2 L of active ingredient per hectare with a CO_2 pressurized spray boom, as described by RESENDE et al. (2000) on April 15, 2003 (air temperature 25° C, relative humidity 70%, wind northwest at 15-20 km h^{-1} and clear sky).

Invertase extraction and assay

For invertase assays, one representative stem was chosen per plot. Stem tissues (0.3 g samples, excluding the rind) were collected two days before TE treatment and at 15, 64, 81 and 104 days after TE treatment. Samples were taken from mature, young and maturing internodes (third oldest, +1 Kuijper system (DILLEWJIN, 1952), and intermediate, respectively) always around 10:00 am, and immediately frozen in liquid nitrogen.

Both soluble and wall bound invertase assays were performed as described by ALBERTSON et al. (2001) with the following exceptions: glucose determinations were made in 80 µL aliquots of the stop reaction mix by reaction with Nelson's arsenomolybdate reagent (NELSON, 1944); protein concentrations were determined in 200 µL aliquots of the desalted enzyme extracts using Bradford's colorimetric method (BRADFORD, 1976); the pellet-wash procedure for cell wall bound invertases was adapted to eight washes with 2 mL of the extraction buffer lacking polyvinylpolypyrrolidone (PVPP) minimizing the contamination of soluble invertases to levels below 10%.

Sugar yield

Ten stalks were collected sequentially in each plot, submitted to topping at the apex bud line and taken to the Laboratory of Cane Analyses of 'Usina São Martinho' at the day of TE treatment and at 35, 60, 89 and 106 days after. After stalk grinding and homogenization, a 500 g sample was crushed as described by TANIMOTO (1964). The resulting juice was used to the following determinations: pol (polarimeter), Brix (refratometer at 20° C), reducing sugars (NELSON, 1944), and fiber % cane (TANIMOTO, 1964). Purity was obtained by dividing pol per Brix. The percentage of soluble solids (Brix) was also determined in top (+1 Kuijper system (DILLEWJIN, 1952) mid and bottom internodes of one representative stalk per plot in the same day of growth analyses. Total recoverable sugar (TRS, kilograms of sugar per ton of stalks) was calculated as recommended by CONSECANA (2003).

Growth analyses and stalk productivity

Ten stalks were collected sequentially in each plot two days before and at 19, 31, 78, and 100 days after TE treatment. The following measurements were made as described.

Stalk length: from the oldest internode to the last visible auricular region of +1 leaf, Kuijper system (DILLEWJIN, 1952).

Number and length of nodes: nodes were counted (from +1 kuijper system to oldest) and their average length estimated by dividing the number of nodes by the stalk height.

Stalk dry matter weight: five stalks per plot were cut in three equal parts (top, mid and bottom) weighed, each part divided in four longitudinal slices and dried up to constant weight.

Stalk productivity (tons per hectare) was estimated through the fresh weight of milleable stalks, considering 12 plants per meter (average) and 1.5 m between rows.

Results and discussion

Invertase activities

Trinexapac-ethyl significantly reduced the activity of soluble acid invertase (SAI) in the youngest internodes (top), but no effect was found in older tissues (Table 2). Since TE represses GA_1 biosynthesis, it is expected that acid invertase activities decrease, especially in growing tissues, as seen in this experiment. In their experiment on the physiological functions of invertases, GAYLER & GLASZIOU (1972) report high correlation (r = 0.92) between acid invertase activities and plant growth. These authors showed that the pre-treatment with gibberellic acid increased invertase activities and internode elongation.

Table 2 – Analysis of variance for the activity of soluble acid invertase in µmol of glucose min^{-1} g^{-1} protein.

Source of variation	Internode		
	Top	Mid	Bottom
Trinexapac-ethyl (T)			
1. Control	437.31A	137.41A	80.68A
2. 0.2 L ha^{-1}	417.50B	136.71A	80.09A
F test (T)	37.73**	0.03^{ns}	5.48^{ns}
LSD Tukey 5%	10.26	12.01	0.80
Sampling dates (S)			
1- 2 DBT(1)	495.87A	155.52A	73.44B
2- 15 DAT(2)	429.15B	148.72A	93.03A
3- 64 DAT	414.59B	133.54B	81.30B
4- 81 DAT	399.94B	128.74B	80.21B
5- 104 DAT	397.46B	118.79B	73.94B
F test (S)	8.72**	17.39**	10.35**
LSD Tukey 5%	56.95	14.94	10.25
F test (T x S)	0.42 ns	0.39 ns	0.37 ns
CV % (T)	2.39	8.71	0.99
CV % (S)	9.04	7.39	8.65

Capital letters compare means vertically within treatments; *: significant at 5% of probability by Tukey test; **: significant at 1% of probability by Tukey test; ns: non-significant by Tukey test; (1): days before treatment; (2): days after treatment.

A more recent article by TYMOWSKA-LALANNE & KREIS (1998) reports that GAs can regulate the activities of invertases in different plant species, since this class of plant growth regulators is involved mainly with stem elongation. As it can be seen in Table 2, soluble acid invertase activities decrease with tissue age and maturation. These results corroborate previous studies (GAYLER & GLASZIOU, 1972; BATTA & SINGH, 1986; ZHU et al., 1997; ALBERTSON et al., 2001; BATTA et al., 2002). In immature (i.e., stem top) storage tissues, where cell elongation is predominant, the sucrose stored in the vacuole is rapidly hydrolyzed by soluble acid invertases and the resulting hexoses return to the metabolic compartment by diffusion movement in the direction of the prevailing gradient (GAYLER & GLASZIOU, 1972; ALEXANDER, 1973; LINGLE, 1997).

Several studies have associated soluble neutral invertases (SNI) with the regulation of sucrose turnover in mature stem tissues (HATCH & GLASZIOU, 1963; GAYLER & GLASZIOU, 1972; BATTA & SINGH, 1986; VORSTER & BOTHA, 1998; ROSE & BOTHA, 2000; BATTA et al., 2002). However, in this experiment the activity of SNI was low if compared to SAI in any of the stem portions, and it was not influenced by the treatment with TE, except in bottom internode tissues (Tables 3 and 5), where the application of ripener decreased SNI activity. This result is in accordance with ALBERTSON et al. (2001), who found small changes in the activity of SNI in tissues of varying ages. These authors suggested that SNI may play a 'house-keeping' role, since it maintains hexose concentrations in the cytosol (VORSTER & BOTHA, 1998). Varying tendencies were found to SNI activities in mid and bottom portions of the sugarcane stem along sampling dates (Tables 4 and 5).

Table 3 – Analysis of variance for the activity of SNI in µmols of glucose min^{-1} g^{-1} protein.

Source of variation	Internode		
	Top	Mid	Bottom
Trinexapac-ethyl (T)			
1. Control	96.54A	47.78A	75.27A
2. 0.2 L ha^{-1}	98.29A	47.38A	64.89B
F test (T)	2.17 ns	0.01 ns	154.36**
LSD Tukey 5%	3.77	3.66	2.66
Sampling dates (S)			
1- 2 DBT(1)	74.53D	90.20A	60.07D
2- 15 DAT(2)	118.39A	40.81B	80.75A
3- 64 DAT	102.02B	34.74C	73.33B
4- 81 DAT	101.07B	29.15D	70.49B
5- 104 DAT	91.07C	42.25B	65.74C
F test (S)	53.86**	1086.28**	54.59**
LSD Tukey 5%	9.16	3.10	4.41
F test (T x S)	0.56 ns	19.97**	9.39**
CV % (T)	3.84	7.66	3.77
CV % (S)	6.37	4.43	4.26

Capital letters compare means vertically within treatments; *: significant at 5% of probability by Tukey test; **: significant at 1% of probability by Tukey test; ns: not significant by Tukey test; (1): days before treatment; (2): days after treatment.

Table 4 – Tukey and F tests of the interaction between main treatments (plots) and sampling dates (split plots) for soluble neutral invertase activity in mid internodes.

Treatments	2 DBT[(1)]	15 DAT[(2)]	64 DAT	81 DAT	104 DAT	F test
Control	90.20Aa	38.04Bb	40.45Ab	27.65Ac	41.04Ab	543.83**
TE[(3)] 0.2 L ha^{-1}	90.20Aa	43.59Ab	29.02Bc	30.65Ac	43.63Ab	562.42**
F test	0.00ns	9.97**	42.31**	2.91ns	1.91ns	

Capital letters compare means vertically and lower case letters compare horizontally within treatments; *: significant at 5% of probability by Tukey test; **: significant at 1% of probability by Tukey test; ns: non-significant by Tukey test; [(1)]: days before treatment; [(2)]: days after treatment; [(3)]: Trinexapac-ethyl.

Table 5 – Tukey and F tests of the interaction between main treatments (plots) and sampling dates (split plots) for soluble neutral invertase activity in bottom internodes.

Treatments	2 DBT[(1)]	15 DAT[(2)]	64 DAT	81 DAT	104 DAT	F test
Control	60.07Ad	86.97Aa	82.11Aab	76.66Abc	70.55Ac	49.16**
TE[(3)] 0.2 L ha^{-1}	60.07Aa	74.53Ba	64.56Bb	64.34Bb	60.93Bb	14.82**
F test	0.00ns	36.25**	72.308**	35.54**	21.67**	

Capital letters compare means vertically and lower case letters compare horizontally within treatments; *: significant at 5% of probability by Tukey test; **: significant at 1% of probability by Tukey test; ns: non-significant by Tukey test; [(1)]: days before treatment; [(2)]: days after treatment; [(3)]: Trinexapac-ethyl.

TE increased the activity of neutral invertases 15 days after treatment (DAT) (Table 3). A previous research (VIEIRA, 1988) reported higher SNI activities in high-sucrose and low-reducing sugar stems. Together with our result, this is an indication of the 'house-keeping' role suggested by ALBERTSON et al. (2001). The activity of SNI decreased systematically from the second to the last sampling date, except in mid internodes, which is a ripening indication.

The activity of cell wall bound invertase (CWI) sistematically decreased along sampling dates in all stem portions, except from the first to the second date in bottom tissues, when a significant increase took place. This may be due to the need of replacing the sucrose concentrations lost by these tissues with the increase of CWI activity in top and mid tissues after TE application (Table 6). This enzyme is believed to be necessary to the uptake of sucrose by stem parenchyma cells (ALEXANDER, 1973; MOORE, 1995). Therefore, the response to TE treatment only in growing tissues in the last sampling can be explained by the fact that even physiologically mature tissues depend on CWI to facilitate the uptake of sucrose as glucose and fructose. As observed in SAI, the activities of CWI were lower in mature tissues.

Table 6 – Analysis of variance for the activity of cell wall bound invertase in ηmol of glucose min^{-1} g^{-1} fresh weight.

Source of variation	Internode		
	Top	Mid	Bottom
Trinexapac-ethyl (T)			
1. Control	9.37A	2.19A	1.91A
2. 0.2 L ha^{-1}	9.16A	2.24A	1.87A
F test (T)	8.53 ns	0.76 ns	1.78 ns
LSD Tukey 5%	0.24	0.18	0.11
Sampling dates (S)			
1- 2 DBT[(1)]	10.85A	2.52A	1.65C
2- 15 DAT[(2)]	9.89B	2.56A	2.25A
3- 64 DAT	8.87C	2.19B	2.04B
4- 81 DAT	8.63CD	2.11B	1.93B
5- 104 DAT	8.09D	1.72C	1.60C
F test (S)	72.87**	50.50**	54.47**
LSD Tukey 5%	0.54	0.20	0.15
F test (T x S)	1.32 ns	1.51 ns	1.01 ns
CV % (T)	2.52	8.01	5.65
CV % (S)	3.94	6.15	5.55

Capital letters compare means vertically within treatments; *: significant at 5% of probability by Tukey test; **: significant at 1% of probability by Tukey test; ns: non-significant by Tukey test; [(1)]: days before treatment; [(2)]: days after treatment.

Technological analyses

The concentrations of pol, Brix, reducing sugars (RS), fiber and purity were not affected by the application of trinexapac-ethyl (Table 7). Over the sampling dates an expected increase of pol, Brix, purity and fiber and decrease of RS took place, due to the maturity behavior of sugarcane during fall and winter (Table 7). The measurement of Brix in internodes of different ages showed that trinexapac-ethyl could accelerate ripening. As it can be seen in Tables 9, 10, and 11, its effect is more expressive on younger, immature tissues. This result is expected since the main effect of TE is repressing the biosynthesis of an active form of gibberellic acid, a growth hormone present mainly in developing tissues. Other authors (RESENDE et al., 2000) found significant increase in the concentration of pol, Brix and purity (pol/Brix)

Table 7 – Analysis of variance for the technological analyses.

Source of variation	Brix	pol	RS(1)	Purity	Fiber	TRS(2) (kg t-1)
Trinexapac-ethyl (T)						
1. Control	18.71A	14.30A	1.02A	85.09A	8.68A	169.72A
2. 0.2 L ha-1	18.86A	14.48A	1.00A	85.23A	8.51A	170.88A
F test (T)	0.22 ns	0.56 ns	0.18 ns	0.08 ns	0.34 ns	0.32 ns
LSD Tukey 5%	0.45	0.31	0.17	1.58	0.90	6.49
Sampling dates (S)						
1- 0 DAT(3)	16.15D	11.71D	1.51A	79.99D	8.04B	136.06D
2- 35 DAT	18.06C	13.59C	1.15B	83.75C	8.53AB	175.31BC
3- 60 DAT	18.82B	14.80B	0.76C	87.56AB	8.55AB	190.46A
4- 89 DAT	20.15A	15.44B	0.94BC	85.74BC	8.84A	170.54C
5- 106 DAT	20.75A	16.42A	0.68C	88.76A	9.01A	179.15B
F test (S)	127.37**	112.59**	24.73**	48.91**	6.87**	166.08**
LSD Tukey 5%	0.71	0.73	0.28	2.06	0.59	6.64
F test (T x S)	0.74 ns	0.10 ns	0.58 ns	0.83 ns	0.69 ns	14.87**
CV % (T)	5.61	5.3	17.21	1.85	10.41	3.79
CV % (S)	2.42	3.37	18.87	1.64	4.63	2.64

Capital letters compare means vertically within treatments; *: significant at 5% of probability by Tukey test; **: significant at 1% of probability by Tukey test; ns: non-significant by Tukey test; (1): reducing sugars; (2) : total recoverable sugar; (3): days after treatment.

Table 8 – Tukey and F tests of the interaction between main treatments (plots) and sampling dates (split plots) for total recoverable sugar.

Treatments	0 DAT(1)	35 DAT	60 DAT	89 DAT	106 DAT	F test
Control	135.15Ad	164.30Bc	193.05Aa	174.97Ab	181.14Ab	95.14**
TE(2) 0.2 L ha-1	136.98Ad	186.31Aab	187.87Aa	166.11Bc	177.16Ab	85.81**
F test	0.27ns	39.50**	2.19ns	6.41*	1.30 ns	

Capital letters compare means vertically and lower case letters compare horizontally within treatments; *: significant at 5% of probability by Tukey test; **: significant at 1% of probability by Tukey test; ns: non-significant by Tukey test; (1):days after treatment; (2) : Trinexapac-ethyl.

Table 9 – Analysis of variance for Brix in different stem portions.

Source of variation	Internode		
	Top	Mid	Bottom
Trinexapac-ethyl (T)			
1. Control	8.96B	18.10A	19.42B
2. 0.2 L ha-1	12.85A	18.94A	20.71A
F test (T)	243.15 **	1.94ns	28.32*
LSD Tukey 5%	0.79	1.92	0.77
Sampling dates (S)			
1- 2 DBT(1)	5.78D	14.62C	17.10C
2- 15 DAT(2)	9.18C	17.88B	19.40B
3- 64 DAT	10.90B	19.02AB	20.05B
4- 81 DAT	14.05A	20.50A	21.68A
5- 104 DAT	14.62A	20.58A	22.10A
F test (S)	85.86 **	42.08**	44.90**
LSD Tukey 5%	1.64	1.57	1.24
F test (T x S)	17.17**	3.72*	0.77ns
CV % (T)	7.23	10.29	3.82
CV % (S)	10.19	5.77	4.20

Capital letters compare means vertically within treatments; *: significant at 5% of probability by Tukey test; **: significant at 1% of probability by Tukey test; ns: non-significant by Tukey test; (1): days before treatment; (2): days after treatment.

concomitant with the reduction in the levels of reducing sugars and percentage of fiber after TE treatment in several sugarcane varieties, but no effect on sugar yield was shown. Regarding the reduction in the activities of soluble acid invertase induced by TE (Table 2), our results may sound curious. However, the level of Total Recoverable Sugar (TRS) was higher at 35 DAT and lower at 89 DAT (Table 8). This means the ripener can be economically effective by increasing sugar yield, but crops treated with TE should be monitored for sugar concentrations in order to avoid yield loss. By slightly suppressing vegetative growth, TE seems to favor sucrose accumulation and thus sugar yield.

Sugarcane growth and stalk yield

This is the first report on the effect of TE upon cane growth during maturation. None of the parameters (stalk length, number and length of internodes, stalk dry matter) were affected by TE treatment (Table 12). Together with the results for sugar yield and invertase activities, this means that the doses employed in this experiment seem to be adequate for promoting sugar accumulation increase, without significant growth inhibition.

Table 10 – Tukey and F tests of the interaction between main treatments (plots) and sampling dates (split plots) for Brix in top stem portions.

Treatments	2 DBT[1]	15 DAT[2]	64 DAT	81 DAT	104 DAT	F test
Control	6.00Ab	7.05Bb	10.00Ba	10.30Ba	11.45Ba	17.39**
TE[3] 0.2 L ha^{-1}	5.55Ac	11.30Ab	11.80Ab	17.80Aa	17.80Aa	85.63**
F test	0.36ns	32.46**	5.82*	101.09**	72.47**	

Capital letters compare means vertically and lower case letters compare horizontally within treatments; *: significant at 5% of probability by Tukey test; **: significant at 1% of probability by Tukey test; ns: non-significant by Tukey test; [1]: days before treatment; [2]: days after treatment; [3]: Trinexapac-ethyl.

Table 11 – Tukey and F tests of the interaction between main treatments (plots) and sampling dates (split plots) for Brix in mid stem portions.

Treatments	2 DBT[1]	15 DAT[2]	64 DAT	81 DAT	104 DAT	F test
Control	14.95Ab	16.80Bb	19.40Aa	19.40Ba	19.95Aa	16.15**
TE[3] 0.2 L ha^{-1}	14.30Ac	18.95Ab	18.65Ab	21.60Aa	21.20Aa	29.65**
F test	0.52ns	5.64*	0.69ns	5.91*	1.91ns	

Capital letters compare means vertically and lower case letters compare horizontally within treatments; *: significant at 5% of probability by Tukey test; **: significant at 1% of probability by Tukey test; ns: non-significant by Tukey test; [1]: days before treatment; [2]: days after treatment; [3]: Trinexapac-ethyl.

Table 12 – Analysis of variance for growth parameters and stalk productivity.

Source of variation	IL[1] (cm)	SL[2] (cm)	SP[3] (t ha^{-1})	NI[4]	DM[5](%)
Trinexapac-ethyl (T)					
1. Control	12.72A	253.63A	113.75A	20.14A	26.62A
2. 0.2 L ha^{-1}	12.73A	253.10A	112.89A	20.08A	27.06A
F test (T)	0.00ns	0.10ns	0.04ns	0.03ns	0.89ns
LSD Tukey 5%	0.54	14.19	14.18	1.19	1.49
Sampling dates (S)					
1- 2 DBT[6]	13.79A	244.10BC	114.16AB	17.72C	22.19C
2- 19 DAT[7]	13.96A	239.56C	97.39B	17.20C	26.08B
3- 31 DAT	12.97B	262.87A	120.67A	20.20B	27.82AB
4- 78 DAT	11.68C	261.12A	116.12AB	22.37A	28.63A
5- 100 DAT	11.23C	259.17AB	118.26A	23.07A	29.47A
F test (S)	49.31**	7.52**	4.04*	42.69**	43.35**
LSD Tukey 5%	0.73	16.32	19.15	1.69	1.83
F test (T x S)	2.29ns	1.26ns	0.72ns	2.19ns	0.39ns
CV % (T)	4.19	5.57	12.44	5.89	5.53
CV % (S)	3.9	4.37	11.46	5.70	4.62

Capital letters compare means vertically within treatments; *: significant at 5% of probability by Tukey test; **: significant at 1% of probability by Tukey test; ns: non-significant by Tukey test; [1]: internode length; [2]: stalk length; [3]: stalk productivity; [4]: number of internodes; [5]: weight of dry matter; [6]: days before treatment; [7]: days after treatment.

Since previous studies have not determined stalk productivity, it was difficult to say if the growth inhibition promoted by TE was severe enough to bring loss in sugar per area. As seen in Table 12, TE had no significant effect on stalk productivity. Thus, the increase in sugar yield reported here could reflect in more sugar per area.

Conclusions

The results suggest that trinexapac-ethyl allows an increase on sugar yield and its repressive effect on vegetative growth does not affect stalk productivity. In addition to that, the activity of soluble acid invertase in young stem tissues seems to be useful as a biochemical parameter to determine the response of sugarcane to the trinexapac-ethyl.

Acknowledgements

The authors are thankful to Usina São Martinho S/A for their help with the carrying out of the sugarcane crop. We also thank Daniel Bordonal Kalaki, Gisele

Cristina Ravaneli and Rafael José Navas da Silva for their help with laboratory analyses. Eduardo Rossini Guimarães was supported by a scholarship from CAPES (Brazil).

References

ALBERTSON, P. L.; PETERS, K. F.; GROF, C. P. L. An improved method for the measurement of the cell wall invertase activity in sugarcane tissue. **Australian Journal of Plant Physiology**, Melbourne, v.28, p.323-328, 2001.

ALEXANDER, A. G. **Sugarcane physiology**. Amsterdam: Elsevier, 1973. 752p.

BATTA, S. K.; KAUR, S.; MANN, A. P. S. Sucrose accumulation and maturity behaviour in sugarcane is related to invertase activities under subtropical conditions. **International Sugar Journal**, Glamorgan, v.104, p.10-13, Jan/Feb 2002.

BATTA, S. K.; SINGH, R. Sucrose metabolism in sugarcane grown under varying climatic conditions: synthesis and storage of sucrose in relation to the activities of sucrose synthase, sucrose phosphate synthase and invertase. **Phytochemistry**, New York, v. 25, p.2431-2437, 1986.

BRADFORD, M. M. A rapid sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, New York, v.72, p.248-254, 1976.

CONSECANA. **Normas de avaliação da qualidade da cana-de-açúcar**. Available at: <http://www.unica.com.br/files/consecana/normaseprecos>. Access on: 19 nov. 2003.

COPERSUCAR. Boletim Técnico da nona geração de variedades de cana-de-açúcar Piracicaba: Copersucar, 2003. 16p. Edição Especial.

DILLEWIJN, C. van. **Botany of sugar cane**. Waltham: Chronica Botanica, 1952. 371p.

GAYLER, K. R.; GLASZIOU, K. T. Physiological functions of acid and neutral invertases in growth and sugar storage in sugar cane. **Physiologia Plantarum**, Copenhagen, v. 27, p.25-31, 1972.

HATCH, M. D.; GLASZIOU, K. T. Sugar accumulation cycle in sugar cane. II. Relationship of invertase activity to sugar content and growth rate in storage tissue of plants grown in controlled environments. **Plant Physiology**, Minneapolis, v.38, p.344-348, 1963.

LINGLE, S. E. Seasonal internode development and sugar metabolism in sugarcane. **Crop Science**, Madison, v. 37, p.1222-1227, 1997.

MOORE, P. H. Temporal and spatial regulation of sucrose accumulation in the sugarcane stem. **Australian Journal of Plant Physiology**, Melbourne, v.22, p.661-679, 1995.

NELSON, N. A photometric adaptation of the Somogyi method for the determination of glucose. **Journal of Biological Chemistry**, Bethesda, v.153, p.375-380, 1944.

RESENDE, P. A. P.; SOARES, J. E.; HUDETZ, M. Moddus®, a plant growth regulator and management tool for sugarcane production in Brazil. **International Sugar Journal**, Glamorgan, v.102, p.5-9, Apr. 2000.

ROSE, S.; BOTHA, F. C. Distribution patterns of neutral invertase and sugar content in sugarcane internodal tissues. **Plant Physiology and Biochemistry**, Paris, v.38, p.819-824, 2000.

TANIMOTO, T. The press method of cane analysis. **Hawaiian Planter's Record**, Aiea, v.57, p.133-150, 1964.

TYMOWSKA-LALANNE, Z.; KREIS, M. The plant invertases: physiology, biochemistry and molecular biology. **Advances in Botanical Research**, London, v. 28, p.71-117, 1998.

VIEIRA, I. M. S. **Relações entre níveis de açúcares e atividades das invertases em tecidos de quatro cultivares de cana-de-açúcar (*Saccharum* spp.) cultivadas a campo.** 1988. 108 f. Tese (Doutorado em Fitotecnia) – Escola Superior de Agricultura Luiz de Queiroz, Universidade de São Paulo, Piracicaba, 1988.

VORSTER, D. J.; BOTHA, F. C. Partial purification and characterization of sugarcane neutral invertase. **Phytochemistry**, New York, v.49, p.651-655, 1998.

WHITTAKER, A.; BOTHA, F. C. Carbon partitioning during sucrose accumulation in sugarcane internodal tissue. **Plant Physiology**, Minneapolis, v.115, p.1651-1659, 1997.

ZHU, Y. J.; KOMOR, E.; MOORE, P. H. Sucrose accumulation in the sugarcane stem is regulated by the difference between the activities of soluble acid invertase and sucrose phosphate synthase. **Plant Physiology**, Minneapolis, v.115, p.609-616, 1997.

Recebido em 29-3-2004.
Aceito para publicação em 1-3-2005.