Chemical ripening of sugarcane with trinexapac-ethyl (Moddus®) – mode of action and comparative efficacy

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Abstract

Chemical ripeners cause growth suppression thus increasing sucrose accumulation in sugarcane by lowering the growth sink demand for sucrose. Trinexapac-ethyl (Moddus®), the most recent chemical to be introduced as a ripener with a hormonal mechanism, inhibits production of the plant hormone gibberellic acid, which leads to restriction of internode elongation. This study reports novel findings on a dual mode of action, by which Moddus® affects growth processes in both the stalk and leaf canopy above a certain concentration, and how this influences ripening efficacy. In addition the efficacy of Moddus® is compared to Ethephon®, the other ripener with a hormonal mechanism. An irrigated field trial was planted to variety N32 at the South African Sugarcane Research Institute's research station in Pongola, South Africa. The plant and subsequent three ratoons were used as experimental crops and harvested in April of each year. Replicated treatment plots consisted of 6 cane rows, 16 m long and spaced 1.4 m apart. Treatments comprised an unsprayed control, Ethephon® applied according to standard practice, and Moddus® applied at three dosages. Products were applied with CO_2-pressurised equipment and a hand-held overhead spray boom. Measurements on the stalks (length, individual internode elongation and juice quality) and leaves (green and dead leaf mass per stalk) were conducted at intervals until harvest. At harvest the cane and sugar yield was determined for each treatment. A novel finding was that Moddus®, above a certain concentration, ripened the crop

through a dual mode of action involving restriction of both internode elongation and leaf growth. Characterisation of effects on individual internode elongation and mass of the green leaf canopy, coupled to yield data, provided insights to be considered in future when attempting to explain varietal differences in response to Moddus®. The easy-to-measure plant processes identified in this study as sensitive indicators of ripening efficacy might enable initial pot-based screening of large numbers of varieties for responsiveness to Moddus®, before embarking on more time-consuming and expensive field-based testing. The data acquired in this study could also be used in attempts to simulate Moddus®-induced ripening with mechanistic sugarcane crop models.

Keywords: Cane quality; Chemical ripening; Moddus®; Sugarcane; Trinexapac-ethyl

1. Introduction

Natural ripening of sugarcane (*Saccharum* spp. hybrids), defined as an increase in stalk sucrose content on a dry matter basis over time, is driven by environmental factors such as low temperature and limited soil water availability (van Heerden et al., 2013 and citations therein). Under these conditions growth potential is restricted without canopy photosynthesis being appreciably affected up to a certain level of stress intensity. The suppression of new stalk and leaf growth lowers the growth sink demand for sucrose, thus leading to increased rate of sucrose storage within the stalk (van Heerden et al., 2013).

Unfavourable environmental conditions for natural ripening (high temperature and abundant soil moisture) often results in a relatively immature (low stalk sucrose content) crop at harvest. In irrigated sugarcane production, drying-off (termination of irrigation before harvesting) is often employed to induce natural ripening under these conditions (Robertson and Donaldson, 1998). However, for this strategy to be effective and synchronized with on-farm harvesting schedules, it is necessary to have precise control over crop water supply. This is not always possible in all cultivation areas, and is often curtailed by unpredictable rainfall patterns. In wetter than normal years, the benefits from drying-off might not be realized. On the other hand, excessive drying-off during drier than

normal years, could lead to drought stress likely to negatively affect yields of both cane and sucrose (Robertson et al., 1999; Donaldson and Bezuidenhout, 2000).

As a consequence of these limitations there is wide-spread use of chemicals for the purpose of ripening irrigated sugarcane when lack of natural ripening has produced a relatively immature crop (Alexander, 1973; Legendre, 1974; Rostron, 1985; Resende et al., 2000; Rixon et al., 2007; van Heerden et al., 2013). The main advantage of chemical ripeners is that they can suppress stalk and leaf growth much more rapidly and consistently than natural processes such as reduced temperatures or limiting soil moisture (Alexander, 1973; van Heerden et al., 2013). Chemical ripeners are applied to the sugarcane leaf canopy by means of aircraft or ground-operated spray booms. The active compounds are subsequently absorbed through the leaves and causes suppression of shoot and/or leaf growth (Alexander, 1973; van Heerden et al., 2013), either through a hormonal or a herbicidal mechanism, thereby increasing sucrose accumulation by lowering the growth sink demand for sucrose (Clowes, 1978; Solomon et al., 2001; Donaldson, 2002; Morgan et al., 2007; van Heerden et al., 2013).

In order to be effective, a chemical ripener should increase sucrose yields in a rapid and consistent fashion without damaging the sugarcane crop, the following ratoon, neighbouring crops or the environment (Resende et al., 2000). The main ripeners currently used are glyphosate, 2-chloroethyl phosphonic acid (e.g., Ethrel® and Ethephon®), fluazifop-p-butyl (e.g. Fusilade Forte®), and trinexapac-ethyl (Moddus®). Glyphosate and fluazifop-p-butyl have a herbicidal mode of action and induce ripening through the chemical suppression of the stalk apical meristem, which prevents the formation of new stalk tissue (Eastwood and Davis, 1997). In the case of 2-chloroethyl phosphonic acid, which has a hormonal mode of action, the active compound rapidly releases the plant hormone ethylene (Yang, 1969; Jaramillo et al., 1977). The produced ethylene results in a reduction (up to 50%) in lamina size (area) and mass of leaves produced after application, which lowers the growth sink demand for sucrose (Rostron, 1974; Eastwood and Davis, 1997).

Trinexapac-ethyl (Moddus®), the most recent chemical to be introduced as a sugarcane ripener, also has a hormonal mode of action and is used as an anti-lodging agent in cereals and in turf grass management. However, this chemical can be used as a ripener in sugarcane and is registered for

this use in a few countries including USA, Brazil and Australia (Resende et al., 2000; Di Bella et al., 2007; Rixon et al., 2007; Orgeron et al., 2013). Once absorbed through the leaves, the active compound temporarily inhibits the conversion of an inactive precursor (GA_{20}) of the plant hormone gibberellic acid (GA) into one of its main bioactive forms (GA_1). In the process GA_{20} accumulates, while the suppression of GA_1 levels leads to an inhibition of internode elongation (Resende et al., 2000; Rixon et al., 2007), which lowers growth sink demand for sucrose with a concomitant acceleration of sucrose storage (ripening) within the stalk.

Despite its current use as a sugarcane ripener, there are no published reports with detailed (concrete) information (only visual observations, e.g. Di Bella et al., 2007 or datasets lacking evidence of statistical analysis, e.g. Resende et al., 2000) on how Moddus® affects growth processes, not only in the stalk but also the leaf canopy, in terms of time course and dose response. Besides production data from some large-scale commercial trials (Di Bella et al., 2007), there are also no detailed report with statistical evidence on how Moddus® affects yield (cane and sugar) at different application rates. For example, Resende et al., (2000) only reported effects of different application rates on cane quality (sugar content), but not yield. This lack of information makes it difficult to understand why certain sugarcane varieties respond particularly well to Moddus® in terms of ripening efficacy, and why others respond poorly (Resende et al., 2000; Kingston and Rixon, 2007; Rixon et al., 2007).

Findings on the comparative efficacy of Moddus®, relative to other ripener chemicals, was published recently (van Heerden, 2014). However this paper only focused on preliminary yield responses from limited trial work and did not consider the mechanisms underpinning the observed ripening responses. In another recent report (El-Hamd et al., 2013), the efficacy of various sugarcane ripener chemicals were also compared, but the treatments did not include Moddus®.

The present study reports concrete findings on how Moddus® affects growth processes in the stalk and leaf canopy of sugarcane and how this influences ripening efficacy at different dosages. Experiments were conducted over four crop cycles to investigate the possible influence of season on achieved responses. In addition, results are provided on the efficacy of Moddus® compared to Ethephon®, the other ripener with a hormonal mechanism.

2. Materials and methods

2.1. Experimental design and crop growth conditions

A drip-irrigated field trial was planted to sugarcane (*Saccharum* spp. hybrids) variety N32 on 23 April 2010 at the South African Sugarcane Research Institute's (SASRI) research station (27^{o} 25' 14'' S and 31^{o} 35' 39'' E) in Pongola, South Africa. Variety N32 was selected because of its high resistance to lodging, a major constraint in chemical ripener field trials that frequently disrupts application of treatments. In addition, this variety has a proven track-record of responding well to chemical ripeners. Fertiliser application rates were determined from soil samples collected at planting and harvest of the plant, first and second ratoon crops. The plant, first, second and third ratoon crops were harvested on 19 April 2011, 3 April 2012, 5 April 2013 and 16 April 2014 respectively.

In each year the young crop was initially irrigated with overhead sprinklers to bring the water in the soil profile to field capacity and to achieve uniform germination/emergence. Thereafter, water was applied by surface drip irrigation to maintain soil moisture content in the top 60 cm of the soil profile at between 75-95% of field capacity. Soil moisture content was monitored using 10 HS Decagon soil moisture probes (Decagon Devices Inc., Pullman, USA) inserted in pairs into undisturbed soil at 15 cm and 45 cm depths between the cane rows and dripper lines at 5 random locations within the field.

Irrigation was not withheld before harvest in an attempt to increase cane quality any further through the practice known as drying-off. Instead, irrigation was maintained at least until two weeks before harvest, and then terminated to allow drying of the top-soil so that stool damage and soil compaction did not occur during harvest operations.

Trial plots consisted of 6 cane rows, each 16 m long and spaced 1.4 m apart. The experiment was a completely randomised design with five replications per ripener treatment.

2.2. Chemical ripener treatments

Table 1 : Summary of ripener treatments, application rates (l ha^{-1} and g active ingredient ha^{-1}) and planned vs. actual spray-to-harvest intervals (STHIs) in the plant and three ratoon crops of variety N32. Abbreviations: Eth, Ethephon®; Mod, Moddus®; DBH, days before harvest. The three different rates of Moddus® are indicated by the Mod 0.8, Mod 1 and Mod 2 treatments.

Treatment	**Application rate (l ha^{-1})**	**Application rate (g ha^{-1})**	**Planned STHI (DBH)**	**Actual STHI (Plant crop)**	**Actual STHI (1^{st} ratoon)**	**Actual STHI (2^{nd} ratoon)**	**Actual STHI (3^{rd} ratoon)**
Eth	1.5	720	84	82	81	86	85
Mod 0.8	0.8	200	70	70	67	72	71
Mod 1	1.0	250	70	70	67	72	71
Mod 2	2.0	500	70	70	67	72	71

In each of the four crops the experimental design comprised an unsprayed (untreated) control and four ripener treatments. For the ripener treatments, Ethephon® (Eth) was planned for application 84 days before harvest (DBH) at 1.5 l ha^{-1}, while Moddus® (Mod) was planned for application 70 DBH at 0.8, 1 and 2 l ha^{-1}. In Table 1 the various treatments, planned spray-to-harvest intervals (STHIs) and actual STHIs for the four crops are summarised. The slight discrepancies between planned and actual STHIs were caused by weather conditions (wind and rain), which did not always allow product application on the planned dates.

Ripeners were applied to cane rows 2 – 5 in each plot while cane rows 1 and 6 acted as unsprayed guard rows. All spray mixtures were applied early in the morning under calm and dry conditions by CO_2-pressurised spraying equipment with a hand-held overhead boom fitted with two TK-1 flood jet nozzles spaced 1.4 m apart. The spray mixtures were delivered at 175 kPa pressure in a water volume of 57 l ha^{-1}.

2.3. Crop growth measurements

Stalk length was measured at two-weekly intervals between application of the ripener treatments and harvest. For this purpose the length of 20 randomly-selected stalks from rows 3 and 4 in each treatment plot were measured from ground level to the height where the youngest fully expanded leaf attaches to the stalk. At the same time the number of stalks in rows 3 and 4 in each treatment plot were counted for estimation of stalk population (stalks ha^{-1}).

Just before harvest four randomly selected stalks from rows 2 and 5 of each plot were harvested for biomass partitioning measurements. The dead and green leaves were carefully stripped from each stalk and weighed separately to determine green and dead leaf mass per stalk. On each of these defoliated stalks the number of internodes was counted and their individual lengths recorded with a ruler

2.4. Cane quality and yield measurements

A 12-stalk sample was taken randomly from rows 2 and 5 of each plot at harvest to determine a range of milling quality characteristics, including estimated recoverable crystal per cent (ERC%), which is an estimate of the recoverable value of sugarcane delivered to the sugar factory. The ERC% was calculated per stalk fresh weight (FW) as (equation 2):

$$ERC\% = aS - bN - cF \qquad \text{(Eq. 2)}$$

where S = sucrose % per stalk FW; N= non-sucrose % per stalk FW (predominantly hexoses); F = fibre % per stalk FW and a, b, c are industry determined factors (0.978, 0.535, and 0.018 respectively) characterising the efficiency of sucrose extraction at the mill. The three components were estimated with near-infrared spectroscopy (NIR) with a Matrix-F NIR instrument (Bruker Pty. Ltd., South Africa) according to accepted industry protocol.

At harvest, the two centre rows (3 and 4) in each plot were cut and bundled by hand and weighed using a hydraulic grab apparatus equipped with a load cell to determine cane yield (TCH, t ha^{-1}). The ERC yield (TERC, t ha^{-1}) per plot was subsequently calculated as (equation 3):

$$TERC\ (t\ ha^{-1}) = (ERC\% \times TCH)/100 \qquad \text{(Eq. 3)}$$

2.5. Statistical analysis

Within each year all the measured variables were analysed using a one-way ANOVA. Each variable was first tested for normality and homogeneity using the Shapiro-Wilk and Bartlett tests respectively (Genstat v.14). The post hoc test used was the Fischer's LSD (5%) test. Because there was no significant ($F > 0.05$) year x treatment interactions, data measured over the four crop cycles (years) were also combined to allow determination of overall treatment effects. The combined data was analysed using a two-way ANOVA.

3. Results

Table 2 : The effects of ripener treatments on cane quality (ERC%) at harvest over four seasons (plant crop and three ratoons) in variety N32. The overall response (averaged across four crops) is also shown. The change in ERC%, relative to control values, is shown in each case. LSD ($p < 0.05$) values are provided at the bottom of the table. Significant differences between control and treatments within each column are indicated with superscript letters. Means followed by same letters are not significantly ($p = 0.05$) different.

	Plant crop		**First ratoon**		**Second ratoon**		**Third ratoon**		**Overall**	
Treatment	**ERC%**	**Change**	**ERC%**	**Change**	**ERC%**	**Change**	**ERC%**	**Change**	**ERC%**	**Change**
Control	9.6^{a}	-	8.6^{a}	-	9.5^{a}	-	9.8^{a}	-	9.7^{a}	-
Eth	10.7^{ab}	1.1	11.2^{b}	2.5	12.4^{b}	2.9	11.3^{b}	1.5	11.9^{bc}	2.2
Mod 0.8	10.6^{ab}	1	11.7^{bc}	3.1	12.3^{b}	2.8	11.5^{b}	1.6	11.9^{bc}	2.3
Mod 1	11.8^{b}	2.2	11.4^{b}	2.8	12.6^{b}	3.1	12.3^{bc}	2.4	12.4^{bc}	2.8
Mod 2	11.9^{b}	2.3	12.6^{c}	4	13.0^{b}	3.5	12.7^{c}	2.9	12.8^{c}	3.1
$LSD_{0.05}$	*1.45*		*1.01*		*1.8*		*0.997*		*1.04*	

3.1. Effect of chemical ripener treatments on cane quality (ERC%) at harvest

The effects of the various ripener treatments on cane quality (ERC%) at harvest in the plant and three ratoon crops are shown in Table 2. Across the four crops the cane quality in the control treatment ranged between 8.6 - 9.8 %. In the plant crop the ripener treatments increased ERC% by up to 2.3 percentage units in relation to the control treatment, with two of the four ripener treatments achieving significant ($p < 0.05$) increases in cane quality. In the subsequent three ratoon crops all four ripener treatments increased ERC% by between 1.5 – 4 percentage units in relation to the control treatment, with these effects being significant ($p < 0.05$) in all cases.

There were no significant ($F = 0.889$) year x treatment interactions, so the data collected over the four crops cycles (years) were combined to allow assessment of overall comparative treatment responses (Table 2). This revealed that all four ripener treatments resulted in a significant increase in cane quality over the control, which ranged between 2.2 – 3.1 percentage units. The Mod 0.8, Mod 1 and Mod 2 treatments did not increase ERC% significantly more than the current industry standard treatment, Eth. In terms of the three Moddus® application rates, a dose-response trend (not significant) was observed (Mod 2 > Mod 1 > Mod 0.8) with increases in cane quality of 3.1, 2.8 and 2.3 percentage units with descending application rate (Table 2).

3.2. Effect of chemical ripener treatments on cane yield (TCH) at harvest

Across the four crops TCH at harvest showed a gradual decrease in the control treatment from 120 t ha^{-1} in the plant crop to 94.8 t ha^{-1} in the third ratoon (Table 3). In the plant crop TCH in the four ripener treatments did not differ significantly ($p > 0.05$) from the control. In the subsequent three ratoon crops this was also the case (Table 3), except in the third ratoon crop where the Eth treatment resulted in a significant reduction in TCH of 15.8 t ha^{-1} compared to the control.

Table 3 : The effects of ripener treatments on cane yield (TCH, t ha^{-1}) at harvest over four seasons (plant crop and three ratoons) in variety N32. The overall response (averaged across four crops) is also shown. The change in cane yield, relative to control values, is shown in each case. LSD ($p < 0.05$) values are provided at the bottom of the table. Significant differences between control and treatments within each column are indicated with superscript letters. Means followed by same letters are not significantly ($p = 0.05$) different.

	Plant crop		**First ratoon**		**Second ratoon**		**Third ratoon**		**Overall**	
Treatment	**TCH**	**Change**	**TCH**	**Change**	**TCH**	**Change**	**TCH**	**Change**	**TCH**	**Change**
Control	120.0[a]	-	119.5[a]	-	112.3[a]	-	94.8[b]	-	112.1[a]	-
Eth	119.8[a]	-0.2	117.7[a]	-1.8	115.2[a]	2.9	79.0[a]	-15.8	108.5[a]	-3.6
Mod 0.8	117.8[a]	-2.2	110.2[a]	-9.3	113.9[a]	1.6	91.4[ab]	-3.5	107.5[a]	-4.6
Mod 1	114.0[a]	-6	111.4[a]	-8.1	114.4[a]	2.1	91.3[ab]	-3.5	107.3[a]	-4.8
Mod 2	121.1[a]	1.1	113.5[a]	-6	105.9[a]	-6.4	86.6[ab]	-8.2	106.3[a]	-5.8
$LSD_{0.05}$	*13.3*		*12.1*		*12.3*		*11.46*		*9.97*	

There were no significant (F = 0.764) year x treatment interactions and the data collected over the four crops cycles were combined (Table 3). This revealed that none of the ripener treatments resulted in a significant decrease in TCH.

3.3. Effect of chemical ripener treatments on ERC yield (TERC) at harvest

The effect of ripener treatments on ERC yield (TERC) at harvest represents the product of influences of these chemicals on cane quality (ERC%) and cane yield (TCH), whether these influences were significant or not. In Table 4, the effect of the various ripener treatments on TERC in the plant crop and three ratoons are shown.

Across the four crops TERC showed a decrease in the control treatment from 11.3 t ha^{-1} in the plant crop to 9.3 t ha^{-1} in the third ratoon (Table 4). In the plant crop only the Mod 2 treatment resulted in a significant increase in TERC of 3.1 t ha^{-1} in relation to the control treatment. In the first and second ratoon crops all the ripener treatments achieved significant increases in TERC, which ranged between 2.5 – 3.9 t ha^{-1}. In the third ratoon crop only the Mod 1 and Mod 2 treatments increased TERC significantly in relation to the control treatment.

There were no significant (F = 0.234) year x treatment interactions, so the data collected over the four crops cycles (years) were combined (Table 4). This revealed that all four ripener treatments resulted in a significant increase in TERC, which ranged between 1.8 – 2.9 t ha^{-1}. The Mod 0.8 and Mod 1 treatments resulted in similar increases in TERC compared to the current industry standard treatment, Eth. In terms of the three Moddus® application rates, a dose-response trend (not significant) was observed (Mod 2 > Mod 1 > Mod 0.8) with increases in TERC of 2.9, 2.2 and 1.8 t ha^{-1} with descending application rate (Table 4). This resulted in the Mod 2 treatment significantly outperforming the Eth treatment by 1.1 t ERC ha^{-1}.

Table 4 : The effects of ripener treatments on ERC yield (TERC, t ha^{-1}) at harvest over four seasons (plant crop and three ratoons) in variety N32. The overall response (averaged across four crops) is also shown. The change in ERC yield, relative to control values, is shown in each case. LSD ($p < 0.05$) values are provided at the bottom of the table. Significant differences between control and treatments within each column are indicated with superscript letters. Means followed by same letters are not significantly ($p = 0.05$) different.

	Plant crop		**First ratoon**		**Second ratoon**		**Third ratoon**		**Overall**	
Treatment	**TERC**	**Change**	**TERC**	**Change**	**TERC**	**Change**	**TERC**	**Change**	**TERC**	**Change**
Control	11.3^{a}	-	10.3^{a}	-	10.7^{a}	-	9.3^{a}	-	10.2^{a}	-
Eth	12.9^{ab}	1.6	13.2^{b}	2.9	14.3^{b}	3.6	9.0^{a}	-0.3	11.9^{b}	1.8
Mod 0.8	12.4^{ab}	1	12.9^{b}	2.6	14.0^{b}	3.3	10.5^{ab}	1.2	11.9^{bc}	1.8
Mod 1	13.2^{ab}	1.9	12.8^{b}	2.5	14.3^{b}	3.6	11.2^{b}	1.9	12.4^{bc}	2.2
Mod 2	14.4^{b}	3.1	14.2^{b}	3.9	13.8^{b}	3.1	11.0^{b}	1.8	13.1^{c}	2.9
$LSD_{0.05}$	*2.25*		*1.7*		*2.4*		*1.63*		*1.1*	

3.4. Moddus® mode of action – effects on stalk growth

In sections 3.4 and 3.5 below results are presented on the mode of action of Moddus® in terms of effects on stalk and leaf growth processes at the different application rates (Mod 0.8, Mod 1 and Mod 2) in comparison to the effects induced by Ethephon®, the other ripener with a hormonal mechanism. The aim is to demonstrate how effects on these growth processes influences ripening efficacy, using TERC as indicator. This knowledge will also be an important tool in future screening experiments to help understand varietal differences in ripening response to Moddus®.

In Fig. 1 the effects of Ethephon® and Moddus® on stalk length, measured from ground level to youngest fully expanded leaf attachment to stalk, is shown. All three Mod rates resulted in rapid, and near complete, suppression of stalk growth up to approximately 56 days after spraying with no significant dose-response evident. Thereafter stalk growth significantly resumed in the Mod 0.8 and Mod 1 treatments up to harvest, but not in the case of the Mod 2 treatment (Fig. 1). Consistent with its known mode of action, the Eth treatment had no significant effect on stalk growth.

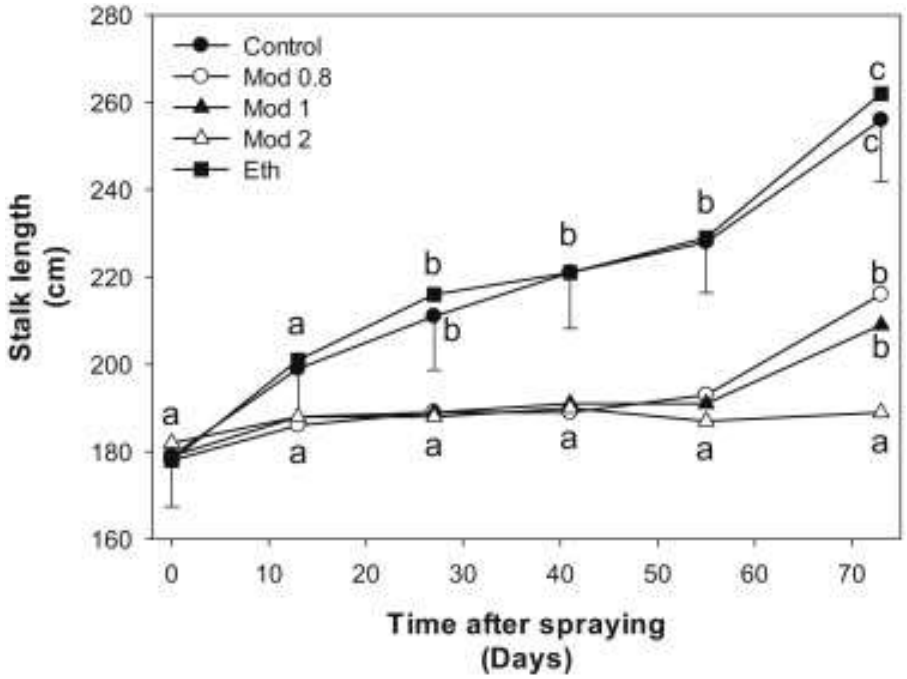

Figure 1 : The effects of Moddus®, applied at 0.8 (Mod 0.8), 1 (Mod 1) and 2 l ha^{-1} (Mod 2), and Ethephon® on standing stalk length (cm) at various time points between application and harvest. Values represent the mean of five replicates and error bars indicate $LSD_{0.05}$. Significant differences ($p < 0.05$) between control and treatment means at each time point are indicated by different letters.

To investigate the effect of Moddus® on stalk elongation at the individual internode level, the length of the internodes situated from internode 8 (counted from ground level upwards) and above were measured at harvest. Internode 8 was chosen as the starting point, because it was determined at the time of Moddus® application, that this was the youngest fully expanded internode. These results are shown in Fig. 2. For clear comparison between the treatments the length of internodes were all expressed as a percentage of the length of internode 8.

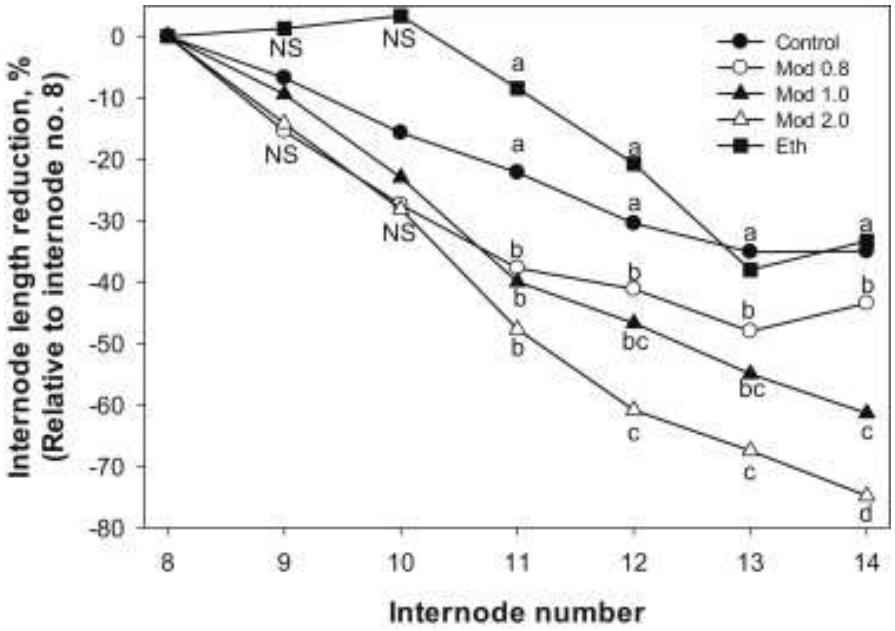

Figure 2 : The effects of three Moddus® application rates (Mod 0.8, Mod 1 and Mod 2) and Ethephon® on the elongation of individual internodes. At the time of Moddus® application the youngest fully expanded internode was number 8. The length of subsequent internodes, elongating after the application of Moddus®, were measured at harvest and expressed as the percentage difference relative to the length of internode no. 8. Values represent the mean of five replicates with significant differences ($p < 0.05$) between control and treatment means at each time point indicated by different letters.

In the unsprayed control a decrease in the length of subsequent internodes was observed, which can be explained by natural growth slow-down, primarily driven by gradually lower air temperatures towards autumn. For example, the length of internode 14 was 34% less than the length of internode 8. The first internode, of which the elongation was significantly reduced by Moddus®, was internode 11 (Fig. 2). The elongation of the subsequent internodes was further reduced in a clear dose-dependent fashion, which was statistically significant ($p < 0.05$). For example the length of internode

14 was 75%, 61% and 43% less than the length of internode 8 in the Mod 2, Mod 1 and Mod 0.8 treatments respectively. Consistent with its known mode of action, the Eth treatment had no significant effect ($p > 0.05$) on individual internode elongation.

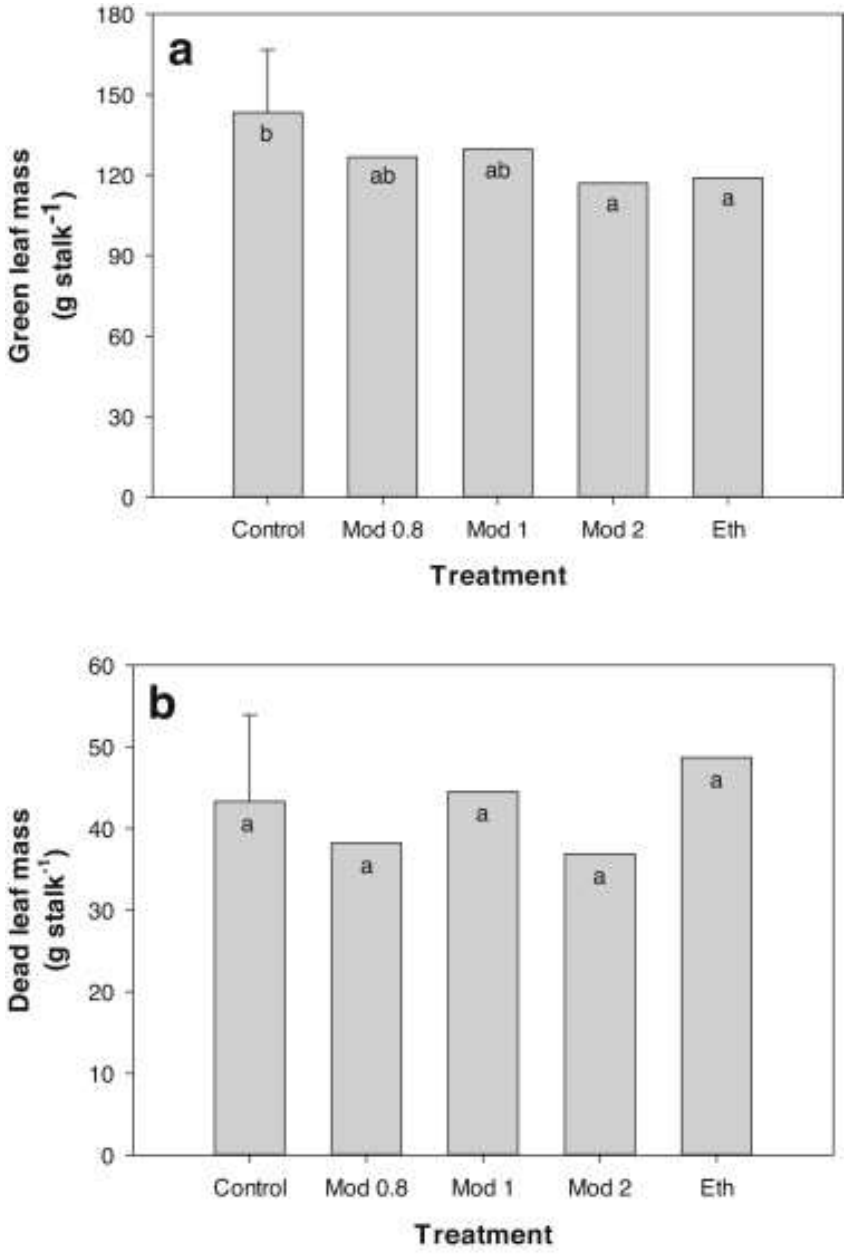

Figure 3 : The effects of three Moddus® application rates (Mod 0.8, Mod 1 and Mod 2) and Ethephon® on (a) green and (b) dead leaf mass measured at harvest. Values represent the mean of five replicates and error bars indicate $LSD_{0.05}$. Significant differences ($p < 0.05$) between control and treatment means are indicated by different letters.

3.5. Moddus® mode of action – effects on leaf growth

Fig. 3a illustrates the effect of Moddus® on green leaf mass per stalk as determined at harvest. The Mod 0.8 and Mod 1 treatments did not result in a significant reduction in green leaf mass. The Mod 2 treatment, however, caused a significant ($p < 0.05$) reduction in green leaf mass of 18% compared to the control. Consistent with its known mode of action, the Eth treatment also had a significant effect ($p < 0.05$) on green leaf mass of the same magnitude as the Mod 2 treatment. In order to assess if Moddus® and Ethephon® influenced the natural senescence rate of green leaves, the dead leaf mass per stalk was also determined at harvest. None of the treatments resulted in a significant change in dead leaf mass compared to the control (Fig. 3b).

4. Discussion

The results presented in this paper clearly demonstrated the high level of effectiveness of chemical ripeners in increasing cane quality (ERC%) and ERC yield (t ha^{-1}) in sugarcane. All four ripener treatments resulted in significant increases in ERC% and ERC yield averaged over the four crop cycles, making the extensive data set acquired in this long-term field experiment valuable for purposes of investigating the comparative efficacy of these treatments. Such a comparison between trinexapac-ethyl (Moddus®) and 2-chloroethyl phosphonic acid (e.g. Ethephon®) have not yet been reported in the scientific literature.

At all three application rates Moddus® resulted in similar increases in ERC% compared to Ethephon®. These results indicate that Moddus® is a very effective ripener, and that it does not cause adverse effects on the plant at high rates, in contrast to herbicidal mode-of-action ripeners such as glyphosate and fluazifop-p-butyl (Alexander, 1973; van Heerden et al., 2013). These results provided the impetus for the current application to the local authority to register Moddus® as a new ripener for the South African sugarcane industry, but should also provide impetus for exploration of the use of this chemical in other sugarcane producing countries across the world.

In order to reap the greatest economic benefit from chemical ripeners, increases in both cane quality (ERC%) and ERC yield (t ha^{-1}) must be achieved. As mentioned above all the ripener

treatments increased ERC% substantially, but their mode of action, namely suppression of stalk and/or leaf growth, could have also reduced millable cane yield. The results revealed that none of the ripener treatments resulted in a significant decrease in cane yield. The slightly lower values of between 3.6 – 5.8 t ha^{-1} (averaged over the four crop cycles) are in line with typical values reported in the scientific literature (Kingston and Rixon, 2007; Orgeron et al., 2013) and supports current thinking on sugarcane ripening, where slight suppression of cane yield can result in substantial increases in cane quality provided the crop is well-managed before and after ripener application (van Heerden et al., 2013).

An on-going, and often contentious, topic among sugarcane growers and millers is to what extent the loss (or perceived loss) of millable cane yield reduces the true economic benefit of chemical ripeners in terms of ERC yields achieved. The results obtained in this study convincingly showed that the substantial increase in cane quality in all four ripener treatments translated into ERC yield gains of between 1.8 – 2.9 t ha^{-1}, when averaged over the four crop cycles. The two lower Moddus® application rates (Mod 0.8 and Mod 1) resulted in similar increases in ERC yield compared to Ethephon®, but the highest application rate (Mod 2), significantly outperformed Ethephon® by 1.1 t ERC ha^{-1}.

The superior ERC yield performance of the Mod 2 treatment, coupled with the growth and biomass partitioning measurements done in these experiments, provided an ideal platform to gain new knowledge and to develop hypotheses guiding experimental design in future varietal screening trials with Moddus®.

Measurement of standing stalk height (Fig. 1) displayed inadequate resolution for revealing dose-dependent suppression of stalk elongation by Moddus®. We are of the opinion that this was largely an artefact of the way in which Moddus® compressed the new green leaves tightly into a whorl at the top of the stalk, irrespective of the dosage applied. This made it difficult for technical field staff to identify the youngest fully expanded leaves, used as point of reference for stalk height measurements, in the tall standing cane treated with Moddus®. In contrast, these measurements were successful in confirming the well-known fact that Ethephon® does not interfere with stalk elongation. As such, measurement of standing stalk height does not appear to be a sensitive indicator of how strong the response to Moddus® is manifested inside the plant at any given time. However, when the growth suppression by Moddus® was gradually relieved (through metabolism of the active ingredient

inside the plant tissue), stalk height measurements again became informative, because gradual resumption of stalk elongation could be accurately demonstrated in a significant ($p < 0.05$) dose-dependent fashion. In future varietal screening trials, measurement of standing stalk height could be used as a rapid and easy method to determine how quickly the active ingredient is broken down in different varieties, which is a factor to consider when attempting to explain varietal differences in ripening efficacy.

Direct measurement of the length of individual internodes, that were elongating after application of Moddus®, appears to be a sensitive indicator of chemical efficacy and can be done easily at harvest after stripping the green and dead leaves from harvested stalks. At the time of product application the number of the youngest fully expanded internode must be recorded, followed by length measurement of this internode, and all subsequent internodes, at harvest. These measurements revealed which internode was the first to be affected by Moddus®, to what extent the different dosages caused suppression of internode elongation, and for how long the maximum effect lasted after spraying (Fig. 2). The results also confirmed that Ethephon® did not inhibit internode elongation, and that the only way that this chemical can thus induce ripening, is through a reduction in green leaf size and mass. Since the suppression of internode elongation represents the principle mode of action of Moddus® (Resende et al., 2000; Rixon et al., 2007), the measuring method explained above could be employed to determine varietal sensitivity to the active ingredient, in terms of magnitude of response but also onset of response. The speed by which a variety responds to Moddus®, and the intensity of the response, are definitely factors to consider when attempting to explain varietal differences in ripening efficacy. An exciting hypothesis that remains to be tested is whether varietal differences in the levels of the four main biologically active gibberellins (GA_1, GA_3, GA_4, and GA_7) responsible for internode elongation in sugarcane (Souza et al., 2001) could explain some of these differences, since Moddus® inhibits only GA_1 synthesis (Rixon et al. 2007). If this is the case, the possibility of rapid screening of varieties for GA_1, GA_3, GA_4, and GA_7 composition, to predict responsiveness to Moddus®, exists.

A novel finding of this study was that, in terms of ripening stimulus, Moddus® ripened the variety chosen for this study through a dual mode of action above a certain concentration. At an application rate of 2 l ha^{-1}, Moddus® not only induced a ripening response through the lower sink

demand associated with reduced internode elongation (Fig. 2), but induced further ripening response through the lower sink demand associated with reduced green leaf mass (Fig. 3a). At this concentration, it thus sets Moddus® apart from Ethephon®, which relies solely on a reduction in green leaf mass to induce ripening. The dual mode of action observed in the Mod 2 treatment explains why this treatment significantly outperformed the Eth treatment in terms of ERC yields achieved. This observation is in line with findings by Inman-Bamber et al., (2008) who demonstrated how a reduction in plant extension rate (induced through deficit irrigation) resulted in reduced demand for photo-assimilate by both stalk fibre and green tops, thus triggering sucrose accumulation (ripening).

This dual contribution towards ripening by Moddus®, above a certain concentration, should not be ignored. It is entirely possible that this dual mode of action could activate at different dosages in different varieties. Although they did not quantified it through direct measurements and statistical analysis, Di Bella et al., (2007) did comment on the visual appearance of Moddus®-treated crops that had shorter leaves than the controls. In their experiments the product was applied at a much lower rate than 2 l ha^{-1}, but it is impossible to conclude if their observations was statistically significant or not. Nevertheless, it raises the pertinent point whether varietal differences exist regarding this dual mode of action.

Inman-Bamber et al., (2008) demonstrated that reduced growth of stalks and leaves, without a matching reduction in photosynthesis, led to increased sucrose accumulation and yield. A factor that could influence ripening response to Moddus® is the potential effect of this chemical on canopy photosynthesis. This follows from the observation that Moddus® reduced green leaf mass at the highest concentration. A reduction in photosynthesis by Ethephon® has been reported previously (Rostron, 1974), but the effects of Moddus® on photosynthesis have not been studied previously. If, for example, a certain variety shows a drastic reduction in green leaf area in response to Moddus®, the concomitant loss of canopy photosynthesis could reduce ripening efficacy.

In conclusion, the results presented in this paper demonstrated that Moddus® is a highly effective ripener of sugarcane. The dual mode of action of Moddus® on both stalk and leaf growth, above a certain concentration, contributes towards its efficacy. Characterisation of effects on individual internode elongation and mass of the green leaf canopy provide valuable insights to be

considered in future when attempting to explain varietal differences in response to this ripener. The easy-to-measure plant processes identified in this study as sensitive indicators of ripening efficacy, and the hypotheses proposed, might enable initial pot-based screening of large numbers of varieties for responsiveness to Moddus®, before embarking on more time-consuming and expensive field-based testing. The data acquired in this study could also be used in attempts to simulate Moddus®-induced ripening with mechanistic sugarcane crop models.

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