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Effects of ripeners on early season sugar production in sugar cane

Thesis submitted by

Terence Ernest Morgan B.Ag.Sc. Hons.

in March 2003

Thesis submitted for the research Degree of Masters of Science in Tropical Plant Sciences within the School of Tropical Biology at James Cook University

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Abstract

Ripening in sugar cane refers to an increase in sugar content on a fresh weight basis prior to commercial harvest. Certain chemicals are applied to cane in commercial fields in some countries to accelerate ripening and improve profitability of sugar production. However, responses have usually been reported to be variety and environment specific. We examined changes in the sucrose content in the juice extracted from 43 Australian sugar cane (Saccharum spp. hybrid) varieties in response to four chemicals in the Burdekin region in north Queensland over two years. The four chemicals used were ethephon (as $Ethrel^{(R)}$) + fluazifop-P butyl (as Fusilade®), glyphosate (as Weedmaster® Duo) and haloxyfop-R methyl (as Verdict[®]). These chemicals were applied in March/April each year. Of particular interest was to determine if economic responses are possible for Australian varieties harvested in the May and June period when sugar content in cane is usually low. Increases in sucrose (measured by pol) levels in cane juice were observed after combined application of $Ethrel^{(m)}$ + Fusilade^(m) (E+F) and after application of glyphosate. These results suggest opportunities exist in the Australian industry to improve the profitability of earlyharvested sugar cane crops, but further research is required to quantify effects on cane yield and responses in diverse environments.

A second component of this study looked at physiological traits associated with responsiveness of varieties to be chemically ripened. Three highly responsive (Q113, Q135 and Tellus^A) and 3 non-responsive varieties (Q167^A, Q179^A and Q186^A) were selected from 42 varieties tested to glyphosate in April 2000. These varieties were treated again in April 2001 and changes in brix, pol, fibre and dry matter were monitored in bottom, middle and top stem sections at T₀ (time of application), T₄ and T₈ weeks after application. Fresh weight pol results show that both response-type groups responded similarly to glyphosate at T₄ weeks but at T₈ weeks the responsive group had significant higher pol ($P \le 0.01$) relative to the non-responsive group. A pooled analysis of variance for all varieties showed glyphosate had not significantly affected either dry or fresh stalk weights by T₈ weeks.

Differences between the response-type groups were then examined at T_0 and it was found that the responsive type group had higher fibre ($P \le 0.01$) and less pol ($P \le 0.05$) in the bottom stem sections compared to the non-responsive group. It is speculated that the pol/DM ratio in the bottom stem sections is a useful measure for prioritising which early-harvested crops are suited to chemical ripening. It is suggested that potential to chemically ripen early harvested crops diminishes as this ratio increases in the bottom stem section. In this study, the pol/DM ratios in the bottom stem sections were 3.7 % higher in the non-responsive group at T₀. The responsive group also had on average more then eight green leaves at T₀. This confirms South African recommendations on the usefulness of this trait to predict the potential of early harvested crops for ripening.

Future research is required to confirm these results, particularly with other types of ripeners since glyphosate is known to adversely affect the yields of some varieties in the following ration crops.

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Statement of Sources Declaration

I declare that this thesis is my own work and has not been submitted in any form for another degree or diploma at any university or other institution of tertiary education. Information derived from the published or unpublished work of others has been acknowledged in the text and a list of references is given.

1. General Introduction

1.1 The need for chemical ripeners

Sugar is an important industry. It is Australia's fifth largest rural industry and Australian exports contributed to 14.4% of total global free sugar trade. Queensland was the largest world exporter of raw sugar for the first time in 1994-5 (Canegrowers 1997). The adoption of modern technology by growers has played an important role in achieving this. However there is increasing competition from other exporters overseas. Competitors like Thailand and Brazil are continually seeking ways through R&D to improve productivity and introduce product differentiation so that their sugar exports are more desirable to customers. These gains, plus relative values of currency are eroding the competitive advantage the Australian industry has enjoyed and the Brazilian sugar industry now produces sugar more cost-effectively then Australia. There is a need for the Australian sugar industry to conduct R&D in areas that will enable it to regain its market position.

The Centre for International Economics (2002) states the Australian sugar industry requires a 37% increase in productivity to restore profitability to the levels achieved in 1996/7. Growers are unlikely to see any short or medium term increase in the world sugar price so therefore future farm viability will depend mainly on productivity improvements made in the area of on-farm yields. The centre predicts most cane growing regions in Australia would not be viable producers by 2006-7 if no productivity gains are achieved and the world price remains static. This scenario would have a catastrophic impact on many coastal regional communities that have been founded on sugar.

Any large, future increases in productivity however will create some additional problems. Most Australian mills currently crush within a 21-22 week season that capitalises on the seasonal ripening of cane leading around the spring months. Additional cane from productivity increases will require either access to additional crushing capacity or an extended crushing season. Increasing crushing capacity is an uneconomic option for most mills in the current downturn. Frawley (1997) estimated that every one tonne per hour increase in milling capacity requires \$350,000 of investment capital. However, extending the current crushing season by initiating the crush earlier involves harvesting cane that is relatively immature and consequently of reduced sugar content and profitability. The impact of reduced sugar content could be reduced if ways could be found increase the maturity and profitability of any cane destined for early harvesting.

Chemical ripeners are one strategy that the industry may be able to use to improve its competitive advantage. The use of ripeners can provide gains in sucrose quality above those achieved by natural ripening (Rostron 1996, Eastwood and Davis 1998, Donaldson 1999, Resende *et al.* 2000; Millhollon and Legendre 2000). These gains can bring potential benefits to the primary (i.e., growers), secondary (i.e., millers) and tertiary (i.e., storage and marketing) industries involved with sugar. If the sugar content of sugar cane could be improved following the application of chemical ripeners the dollar return to growers could be increased. Transportation costs from the farm gate to the mill would also be reduced for the miller.

The successful introduction of ripener technology may facilitate the harvest of cane earlier in the season when it relatively immature. Early harvesting would also benefit millers by improving their returns on capital investment in milling infrastructure, by the use of existing milling capacity to process larger crops. Substantial savings are associated the avoidance of this opportunity cost on capital. The storage and marketing components of the industry might also benefit. If production was spread over a longer period, the demand for investment in storage facilities would be reduced and the industry could supply customers over a longer period of time. The costs savings and benefits for this last factor alone have been estimated to be worth just under US\$30 tonne (Mahony *et al.* 1997).

1.2 Knowledge gap constraints to the adoption of ripener technology in Australia

Although ripeners are currently used extensively overseas, this is not the case in Australia. Ripener research in Australia faltered after the late 1970's when a number of trials gave variable and inconsistent results. Ethrel[®] was registered in 1993 (Rhône-Poulenc 1996), but is not regarded by the sugar industry as a reliable ripening product under Australian conditions. Overseas research has now identified many of the factors required to obtain beneficial responses from ripeners. There are opportunities for the Australian industry to now take advantage of these observations. However, it is important to understand the extent of genotypic variation to chemical ripening before further study is undertaken to quantify environmental and management factors that are also known to affect the response. An understanding of genotypic variation in conjunction with better knowledge on how to predict which crops might respond to ripeners will contribute towards the eventual adoption of this technology by industry and growers.

1.3 Hypothesises and objectives

The original hypothesises for this study are that:

- There is genetic variation in response to chemical ripeners among Australian varieties and that some varieties can show an economic response to chemical ripeners; and
- 2. The prediction of response in responsive varieties can be improved by measuring both purity and growth rate at the time of ripener application.

The objectives are therefore to:

- 1. Identify cultivars that are responsive and non-responsive to various ripeners including combination treatments of ripeners; and
- 2. Identify physiological differences between the two genotype groups that might enable better response prediction to chemical ripening among different varieties in an immature, erect crop.

Chapter 2

A review of chemical ripening in sugar cane

2.1 Introduction

This review examines the present state of knowledge regarding the use of chemical ripeners in sugar cane. The first part provides some definitions, concepts and terminology. The second part focuses on key research that has been undertaken in South Africa, which has been at the forefront of ripener research for the last 30 years, and Australia where research stalled after initial results in the 1970's proved inconclusive. This approach allows one to understand the research strategies that have evolved over time as knowledge about chemical ripening in sugar cane has increased. Comments are made occasionally about the quality of the work conducted and how it is related to work that was done elsewhere. Important tables and figures from studies that added to important areas of our present knowledge base are also presented. The concluding section summarises what are the current knowledge gaps and how the proposed study will contribute to the existing knowledge base.

2.2 Concepts, Definitions and Terminology

2.2.1 The sugar cane crop

Sugar cane and sugar beet are the two crops that are used to commercially produce sucrose. World production of sugar in 1989/90 was 109 M tonnes, about 63% of which was produced from sugar cane (Cooke and Scott 1993). Sugar cane was one of the first tropical crops to be adapted to large-scale farming and it underpins the economies of many regions and in some cases, whole countries (Humbert 1983).

2.2.2 The composition of cane

On a weight basis, about three quarters of a sugar cane stem is water (Table 2.1).

Component	%	Details		
Water	74.50			
Ash	0.50	SiO ₂ , K ₂ O, Na ₂ O, others		
Fibre	10.00	cellulose, pentosans, lignin, others		
Sugars	14.00	sucrose, glucose, fructose		
Nitrogenous bodies	0.40	amino acids, amides, others		
Fat and wax	0.20			
Pectin (gums)	0.20			
Free and combined acids	0.20			
Total	100.00			

Table 2.1: The composition of sugar cane in Louisiana (Source: Spencer and Meade 1948).

Sucrose (O- β -D-fructofuranosyl-[2 \rightarrow 1]- α -D-glucopyranoside) is the commercially valuable component of sugar cane and it makes up about one half of sugar cane stem by weight after all water is removed from the stem.

2.2.3 Factors affecting sucrose yields

Sucrose yields vary because of effects on the sugar cane plant of soil fertility, irrigation, varieties, cultural practices, fertiliser use, weed, pest and disease control and many other factors including the length of the crushing season (Humber 1983). The optimum time to harvest sugar cane may occur for only for a few months each year. Maximum sucrose production would require very large milling capacity to process the crop in such a short time so many industries tend to harvest before and after this "optimum" period when sugar recovery is less. Australia has a relatively short crushing period (22 weeks) compared to South Africa (34 weeks) and Hawaii (over 44 weeks).

2.2.4 The measurement of sucrose yield

Sucrose yields per hectare (tonnes of sugar per hectare or TSH) are calculated by multiplying the cane yield per hectare (tonnes of cane per hectare or TCH) by the sucrose content of fresh weight cane. Growers are paid for sugar cane by varying formulas depending upon the country they are in. Some countries will pay growers by cane yield only but most mills now measure the quality of cane (primarily sucrose content) and use this in conjunction with measurements of cane fresh weight to calculate payments to

growers. Commercial cane sugar (CCS) is a commercial measure of sucrose content in fresh weight cane in the Australian industry. The South African equivalent is called estimated recoverable sugar (ERS). Both measures take into account the proportions of non-sucrose solids (i.e. other sugars, fibre) to sucrose in the estimation of commercially obtainable sucrose.

2.2.5 The relationship between sugar yield per hectare, sucrose content and grower returns.

Crops with the same sugar yields per hectare but varying sucrose contents provide different returns to growers in countries like Australia. The payment formula in Australia is structured such that a premium is paid for cane that has higher sucrose content. This is because the cost of producing sugar from cane with low sucrose content is higher than that for cane with high sucrose content. The additional costs are associated with harvesting, transport to the mill, milling and the processing of the juice. Table 2.2 shows a low sucrose content crop is worth only about 70% to the grower of the high sucrose content crop with the same amount of sugar. This is an important concept because short-term desiccation (eg, 3–8 weeks) of a sugar cane crop can result in little or no change in the sugar yield per hectare and should not be confused with a true ripening effect for reasons discussed later. However, both desiccation and ripening effects can be very profitable to the grower.

Table 2.2: A comparison of two crops with equivalent sugar yields but with different values to an Australian grower.

Attribute	Crop One	Crop Two
tonnes of sugar	80	80
tonnes of fibre	96	96
tonnes of water in cane	624	411
% sucrose fresh weight	10	13.6
total fresh weight (tonnes)	800	587
total value of crop $(A\$)^1$	6986	9875

¹ World sugar price = \$250 tonne, harvesting costs = \$5.30 tonne

2.2.6 What is ripening?

Van Dillewijn (1952) defines ripening simply as the "storage of sucrose in the stem". Ripening is best measured by increases in sucrose as percentage dry weight. Sucrose as a percentage fresh weight is not a good measure since apparent ripening can be due to just a desiccation effect. The example in Table 2.2 above could have arisen if a crop had dried out without any extra accumulation of sucrose. Although the sucrose content has increased on a fresh weight basis there has been no additional accumulation of sucrose as percentage dry weight. Therefore, by definition, no ripening had occurred.

2.2.7 The justification for using ripeners.

Crop ripeners may be defined as compounds applied to a crop before harvest so as to accelerate the natural maturation processes. This acceleration in maturation results in the crop returning a greater gross margin per hectare to the grower than what would have been achieved through natural processes.

Crop ripeners are used widely in agriculture. In some crops they are used to improve the quality characteristics of the end-product (e.g. colour, sweetness, etc.). In others they are used to synchronise ripening where only one attempt can be made at mechanical harvesting (e.g. unsupported tomato crops for processing).

In sugar cane, crop ripeners have been used to improve the accumulation of sucrose in harvested cane. This is important in situations where environmental or management factors limit the effectiveness of natural maturation processes. In many countries sugar cane is grown in climates that lack the declining temperatures and moisture that induce natural maturation of sugar cane. Some mill areas also require the harvesting of immature crops because milling capacity is insufficient to process the entire crop during the period at which it is fully mature. Harvesting immature cane is also practiced to reduce or avoid the impacts of frosts or insects (McCulloch 1989) on sugar yields late in the season. As a result, some of the crop has to be harvested when the percentage sucrose is less than ideal. During the 1998 Australian crushing season, 22% (8.73 MT) of the total crop was crushed before 25/7/98. Most of the harvested cane in this early part of the crushing season is still relatively immature and could potentially benefit from the use of chemical ripeners.

At a grower level, the use of ripeners in sugar cane can be justified when the resulting increase in percentage sucrose covers the expense of the compound and its application.

This break-even point may be as low as a quarter of one percent (Kingston *et al.* 1991). It is possible that some ripeners in some situations can incur environmental costs and collateral damage to neighbouring crops such as tomatoes and bananas (Rhône-Poulenc 1996) or even adjacent young cane. The exact risks are difficult to define but they should be taken in account whenever ripeners are used.

At an industry level, the use of ripeners can be justified from a number of perspectives. Firstly, increases in sucrose percentage directly decrease the unit cost of each tonne of sugar produced. This is achieved by cost reductions associated with cane transport and milling for each unit of sugar produced. Secondly, the resulting increase in overall sugar production helps improve the return on investment made by the industry outside the farm gate. Thirdly, ripeners have the potential to extend the crushing season and allow the milling and marketing sectors to realise the benefits mentioned in the introduction.

2.2.8 A brief history of sugar cane ripeners

The earliest investigation into of the use of chemical ripeners in sugar cane cites research done in Florida during the 1920's although no reference is made as to the success of this early work (Gilbert *et al.* 2002). Beauchamp reported in 1949 that the herbicide 2, 4-D was able to induce chemical ripening in sugar cane. The Australian sugar industry also began investigative work with ripeners from the mid 1950's (Skinner 1956).

Research intensified in the late sixties and early seventies when the compound N, N-bis [(dihydroxyphosphinyl) - methyl] - glycine (syn. Glyphosine, CP41845, MON-0845) showed some promise in early trials (Bieske 1970, Yates 1971). This compound was granted a temporary experimental permit in USA in 1972 and later trademarked under the name "Polaris". Early field trials in USA showed Polaris increased the sucrose percentage on average by 10 % if applied 4 to 10 weeks before harvest (Sellick *et al.*1974). Similar promising results were also observed in other countries (Pan and Lee 1974, Rostron 1974).

Glyphosine was phased out in USA during the 1985-86. It was replaced by the compound phosphonomethyl glyphosate (syn. glyphosate), which was marketed under the brand name "Polardo[®]" (syn. Mon 8000). Glyphosate proved to be more active than glyphosine when applied over a range of environments and varieties (Eastwood and Davis 1997). The increased activity associated with glyphosate also proved detrimental to the ratoon crops in

some situations when compared to Glyphosine (Rice *et al.* 1984). In the USA the use of glyphosate is now recommended with crops that not being rationed (i.e. ploughed out).

Another compound that showed promise in the late sixties and early seventies was the ethylene- producing compound (2-chloro-ethyl) phosphonic acid (syn. ethephon). This was marketed under the name "Ethrel[®]" and it proved to be successful on the variety NCo376 during early trials in South Africa (Rostron 1973). Rostron reported in 1975 that Ethrel[®] was the best chemical ripener for South African conditions (Rostron 1975), however, this has not been consistently observed overseas where glyphosate has proven to be more effective (Eastwood and Davis 1997).

The grass herbicide fluazifop-p-butyl (trade mark = Fusilade[®]) is the only other compound that has been registered for use as sugar cane ripener since glyphosate. In 1983 and 1984 Rostron (1985) reported that the effects of Fusilade[®] were similar to or better than those of Polardo[®] or Ethrel[®]. Fusilade[®] did not appear to harm the ratoons at the rates tested providing the crop was actively growing at the time of application.

The use of chemical ripeners in Australia has not been adopted as commercial practice. Research by the Bureau of Sugar Experiment Stations (BSES) in 1977 showed that, on average, the response to either Polaris[®] or Ethrel[®] was insignificant when applied in May/June for early season crops or October for late harvested crops (Kingston *et al.* 1978). The poor responses could be partly explained by the fact the sugar cane was already close to maturity at the time of application. High cost of application and low sugar prices also contributed to this work being deleted from BSES research plans. Later research demonstrated that larger gains with Ethrel[®] could be achieved when applied to relatively immature cane (Kingston 1988). In 1993, Ethrel[®] became the only chemical ripener registered for use in Australia.

Glyphosate, Ethrel[®] and Fusilade[®] are currently used widely as ripeners on commercial crops in South Africa (Donaldson 1999), Swaziland (Rostron 1996), Florida (Dusky *et al.* 1986), Hawaii (Bartholomew and Silva 2001), Mauritius (Soopaya and Naramuth 2001) and Guyana (Eastwood and Davis 1998). The South African industry has also registered another ripener (haloxyfop-R methyl ester) for commercial use (Donaldson 1999).

2.3 The types of ripeners

The following categories for grouping chemical ripeners used on sugar cane are shown below (Vlitos and Lawrie 1965). These include:

- 1. Defoliants
- 2. Desiccants
- 3. Plant growth regulators
- 4. Enzyme inhibitors

As mentioned in the last section, Ethrel[®], glyphosate and Fusilade[®] are the only three ripeners registered for use in sugar cane at present.

1. Defoliants

These result in the abscission of leaves.

2. Desiccants

These compounds cause rapid drying of leaves and include chemicals like paraquat and diquat. Both increases and decreases in sucrose content have been observed following the application of these compounds. Arvier (1965) reported decreases in sucrose content with paraquat. However, recent research by the BSES in the Burdekin showed CCS increases in cane strips that were treated with paraquat to reduce their attractiveness to greyback beetle (Cocco 1999).

3. Plant Growth Regulators

This group includes compounds that affect hormone-regulated growth processes in the plant, particularly auxin-regulated processes. The most commonly used growth regulator is the herbicide 2,4 D.

Vlitos and Lawrie (1965) observed variable responses (to mixtures of 2,3, 6trichlorobenzoic acid and 2-methyl-4-chlorophenoxyacetic acid ("Pesco 1815") and reported that researchers at the David North Plant Research Centre had encountered similar variable responses between glasshouse and field trials with the same mixture.

Ethylene is a plant growth regulator. Its effects on plant development and fruit ripening have been known for a long time. The Chinese noted that burning incense increased fruit

ripening and, in 1864, leaky gas lights in street lamps stunted nearby plants (Saupe 2002). Most plants make ethylene with the shoot apex and senescing tissues producing the highest concentrations. Ethylene causes a broad range of responses in a plant that include promoting fruit ripening, abscission, epinasty, thigmomorphogenesis, stimulation of germination and inhibition of flower senescence.

A commercial product, called "Ethrel[®]", produces ethylene within the sugar cane tissue. The mechanism by which ethylene causes ripening in sugar cane is not known. Recent work has shown that ethylene reduced the activity of extracellular invertase in *Chenopodium rubum* suspension culture cells (Roitsch *et al.* 1999). It is possible that ethylene also reduces the activity of extracellular invertase in sugar cane and therefore reduces the amount of sucrose that is catabolised in sink tissues.

A recently developed plant growth regulator that shows excellent potential for ripening sugar cane is trinexapac-ethyl (Primo[®] 250 EC. Syngenta, PO Box 249, Wentworthville NSW 2145). It appears to suppress, but not inhibit, growth in grasses by interfering with the biosynthesis of gibberellic acid (Bywater 2001). Researchers in Brazil reported that it increased the sucrose content of the 25 most important cane varieties by about 10% on average (Resende *et al.* 2001).

4. Enzyme Inhibitors

The herbicidal activities of glyphosate and Fusilade[®] are associated with enzyme inhibition.

Glyphosate inhibits 5-enolpyruvylshikimate 3-phosphate synthase (EPSP synthase). This inhibits the synthesis of EPSP from shikimate 3-phosphate and phosphoenolpyruvate and therefore affects the plant by inferring with aromatic amino acid biosynthesis (Su *et al.* 1992).

Fusilade[®] inhibits acetyl coenzyme-A carboxylase, an enzyme involved in the conversion of acetyl-CoA to malonyl-CoA. Fatty acid synthesis is thus disrupted and the formation of cellular membranes inhibited (Donaldson and Van Staden 1995).

2.4 The physiological responses of sugar cane to chemical ripeners

2.4.1 Natural ripening and sucrose accumulation - what is really happening?

Growers apply ripeners to sugar cane so that the percentage sucrose is increased above what would be achieved through natural processes at harvest. The physiological processes responsible for the accumulation of sucrose are poorly understood. This lack of mechanistic understanding and thus of how agronomic practice and environment actually influences the mechanism is consistent with the observation that a large number of trials report little or no response following an application of a ripener. Assuming correct application has occurred, researchers speculatively attribute many of these observations to various varietal, environmental, crop or management factors.

Does a ripening phase or phenomena occur? In sugar beet it was believed that a specific ripening phase or "sugaring-up" occurred. This ripening phase was triggered by falling night temperatures (Ulrich 1955). Later it was shown that sugar beet in England accumulates sugar to total root dry matter at relatively unchanged rate of 0.70 - 0.75 % after mid-August (Milford and Thorne 1973). However, the sucrose concentration in sugar beet on a fresh weight basis steadily increases from June to November. In contrast, to the sugar to root dry matter ratio, the maximum concentration reached on a fresh weight basis varies widely with season and location and is also affected by moisture availability and rainfall.

There are similarities between sugar beet and sugar cane ripening. Recent work by Inman-Bamber *et al.* (2002) suggests that maturation of a cane stalk could be defined by two phases that are similar to the pre and post mid-August phases given for sugar beet above. The first phase is associated with the sucrose content (on a stalk dry weight basis) of the basal internodes increasing. The second phase is associated with the basal internodes being fully ripened. The basal internodes reach maximum sucrose content of around 0.55 g/g of dry matter in the variety NCo376 when the total stalk dry weight approached 150 g. This data was obtained from a field experiment conducted in 1988 in South Africa. Work reported by Muchow *et al.* (1997) suggests that sucrose accumulation in sugar cane is a continuous process directly related to stalk growth, a process that is not triggered by low temperatures, water deficit or nitrogen stress. They found that in 3rd and 4th ratoons Q117 grown in fully irrigated conditions at Ayr, increased in stalk sucrose (g/g DW) to maximum of 0.48 to 0.50. These levels were maintained over the crushing season from July to November. However, this did not apply for sucrose (g/g FW), which steadily increased in both crops over the same period. This work suggests that:

- 1. The ratio of sucrose to dry matter in the stem does not change for mature cane over the crushing season.
- 2. The ratio of sucrose to total fresh weight does increase as sugar cane matures through the crushing season.
- 3. There is no ripening phase in mature sugar cane.
- 4. Natural maturation of sugar cane is directly associated with desiccation of the stalk.

The main conclusions from the partitioning work done in South Africa and Australia are similar and support the theory that sucrose content on a dry matter basis plateaus after a certain crop size has been achieved.

2.4.2 Chemical ripening and sucrose accumulation

The reasons why glyphosate or Fusilade[®] increase sucrose accumulation in the stalk when applied as a sub-lethal dose, appear to be complex and not fully understood.

Hawaiian researchers showed that glyphosate resulted in an increase in sucrose content in the stalk just five days after application (Su *et al.* 1992). A significant reduction in acid invertase activity was observed (about 80%) but they concluded glyphosate also affected sugar cane in other ways to account for the increase in sucrose content observed. One explanation suggested was glyphosate depressed auxin activity that in turn decreased acid invertase and shoot growth activity (i.e. there is also a glyphosate/auxin interaction). Osgood *et al.* (1981) showed glyphosate increased dry matter partitioning towards sucrose at the expense of fibre. It is possible that many of the physiological responses associated with sucrose accumulation following glyphosate application would be similar to those seen for Fusilade[®]. Although these ripeners have different modes of action, both result in the cessation of growth at the apical meristem. The removal of this important sink will therefore have similar physiological consequences regardless of the ripener used.

2.5 A review of chemical ripening research in South Africa

2.5.1 The early 1970's

The first information published on chemical ripeners in South Africa was in 1973 by Harry Rostron, a researcher with the South African Sugar Association Experiment Station (Rostron 1973). Rostron presented data from seven trials that were conducted in 1971 and 1972 in South Africa and Swaziland. NCo376 was the variety in all the trials except one, which contained N55/805. Four trials involved applications between the 21 March and the 29 April. The other three were applied to late harvested crops between the 20 September and the 30 December. A control and up to seven treatments were applied with various ripeners including Ethrel[®] and Polaris[®]. Rostron reported that Ethrel[®] and Polaris[®] produced a consistent improvement in sucrose content in NCo376. However, there was considerable variation in the responses seen in this variety. Polaris[®] reduced cane yield in some trials to an extent where improvements in ERS did not result in an improvement in TSH. Significant improvements in sugar yields were observed in trials using Ethrel[®] and Polaris[®] where the cane was immature at the time of application and which subsequently had good growing conditions until harvest. These same responses were not as pronounced in trials that contained cane that was more mature and had a higher purity. Rostron concluded that further work was required to determine the conditions that favoured the use of these ripeners. This first series of trials identified two important issues relating to the success of ripeners:

- 1. The use of ripeners on immature crops that are harvested early in the season.
- 2. Maintaining good growing conditions after the application of ripeners.

These two issues would be confirmed repeatedly over the next 30 years.

Rostron repeated field and pot trials in 1973 to confirm the conclusions of the previous experiments (Rostron 1974). It was demonstrated that both Polaris[®] and Ethrel[®] applied to NCo376 improved purity, sucrose concentration and sugar yield. These improvements occurred at 4 to 6 weeks and were maintained for up to 12 weeks after application. Other measurements showed that both chemicals reduced the uptake of CO₂ at high light intensities. Cane dry matter content was also significantly increased in the upper parts of the stalk. It appears that both ripeners induced water stress within the plant and modified the translocation and storage of dry matter within the plant. Rostron noted that the quality and yield gains seen in NCo376 contradicted the observations made with other varieties in

his previous work and that of others (Rostron 1973, Alexander and Montalvo-Zapata 1973). He also suggested that where good growing conditions were maintained after application, a second application of ripener could be beneficial one month after the first, especially where the juice purity was still low. This idea was tested with Ethrel[®] in later work (Rostron 1977b) and is now the basis of commercial practice in South Africa.

A review of the research (Rostron 1975) conducted over the previous six years concluded that:

- 1. Ethrel[®] and Polaris[®] will ripen cane under certain conditions in South Africa and Swaziland;
- 2. Ripening increases % sucrose and sugar yields per hectare;
- Millers can benefit from increased sucrose recovery at times of the year when cane quality is low;
- 4. Ripeners applied to early-harvested crops gave better gains compared to late-harvested crops;
- 5. Observed response to ripeners is dependent upon the ability of the crop to grow after it has been sprayed, particularly in relation to the amount of plant-available soil moisture;
- 6. There is an inverse relationship between the observed response and the juice purity at the time of ripener application (Figure 2.1).



Figure 2.1: The inverse relationship between juice purity at the time of application and the observed response in sugar yield for NCo376 (Rostron 1975)

The potential of six chemical ripeners including Ethrel[®] and Embark[®] (mefluidide) was examined in seven experiments in 1975 and 1976 (Rostron 1977c). Only Embark[®] and one

other compound produced ripening effects to warrant further testing. The results from various trials suggested that:

- 1. High early sugar varieties do not seem to respond as well as varieties with lower sucrose contents;
- 2. Spraying earlier in February is more effective than in April;
- 3. The observed response is more dependent on the physiological state of the crop at the time of spraying than the month it is sprayed;
- 4. Previous observations of an inverse relationship between the observed response and the juice purity at the time of Ethrel[®] application are correct.

So far, research in South Africa had reported no adverse effects of Ethrel[®] on cane yield. Trials conducted in 1976 investigated the effects of large, multiple rates of Ethrel[®] on the cane yield and quality of irrigated NCo376 (Rostron 1977b). The results of this work are shown in Figures 2.2: & 2.3.



Figure 2.2: Long term effects on the estimated recoverable sugar percent fresh mass (ERS %) to multiple rates of Ethrel[®] in NCo376 in Natal, South Africa (Rostron 1977b).



Figure 2.3: Long term effects on the sucrose percent cane dry mass to multiple rates of Ethrel[®] in NCo376 in Natal, South Africa (Rostron 1977b).

Rostron reported that:

- 1. Ethrel[®] did not affect cane yield in multiple applications despite a reduction in foliage mass. This was observed over six months of sampling using up to 6 L/ha of Ethrel[®];
- 2. The optimum time for a single application appeared to be 6 to 12 weeks but further applications could extend and further improve the magnitude of the response;
- 3. Multiple applications did not result in additive improvements in cane quality; there were diminishing gains with each application;
- 4. Treatments with multiple applications coped with later drought better than the untreated control.

A detailed review of chemical ripening with Ethrel[®] in Southern Africa gives an excellent overview of the research conducted over a five-year period (Rostron 1977a). Rostron points out that:

- 1. All varieties appear to be responsive to Ethrel[®] when applied to early harvested crops;
- 2. Some varieties were adversely affected when Ethrel[®] was applied to late harvested crops;

3. Some varieties (e.g. L76) are more responsive at lower rates (Figure 2.4)



Figure 2.4: The effect of Ethrel[®] rates on sucrose per cent fresh weight cane for different varieties 12 weeks after application (Rostron 1977a).

Rostron states that:

"...any adverse effect of Ethrel[®] on quality would therefore appear to be related more to the physiological state of the plant at the time of spraying and the rate of chemical applied than to the variety *per se*" (Rostron 1977a)

This statement possibly suggests that in previous studies in which response differences following application of the ripener were attributed to genetic variation of the cane, the responses were in fact more attributable to genetic variation determining the physiological state of the cane plant at the time of application (assuming all environmental factors are equal). If so, there is a need to measure the environmental and physiological variables at the time of application to understand what determines responsiveness to chemical ripeners. Knowing this, we may then predict that in certain environments or certain physiological points, some varieties will respond better or worse than others to the application of chemical ripeners.

2.5.2 The late 1970's

The first trials using Roundup[®] (glyphosate-isopropylammonium) were reported in 1978 (Clowes 1978). Clowes compared the responses of Roundup[®] with Ethrel[®], Embark[®] and Mon 8000 on five early harvested and three late harvested crops. In the early harvested crops, Roundup[®] and Mon 8000 significantly improved sucrose content even when juice purities were higher than 84% at the time of application. This did not occur for Ethrel[®] and Embark[®] where little response occurred when purities were above 80% at the time of application. Ethrel[®] and Embark[®] did not appear to reduce stalk mass but Roundup[®] and Mon 8000 did, particularly when applied to immature crops that were actively growing. However, Clowes stated that the significant improvements in juice quality more than offset adverse effects on cane yield.

In the late harvested crops, Roundup[®] and Mon 8000 significantly improved sucrose content at three and six weeks after application but this did not occur following application of Ethrel[®] or Embark[®]. The latter two ripeners were associated with small insignificant improvements in sucrose content although Ethrel[®] caused some increases in cane yield in some of the trials. This study reconfirmed that Ethrel[®] is best suited to application in actively growing immature crops that are harvested early in the season. It also suggested that there is a role for Roundup[®] in more mature crops that have high purities at the time of application. Roundup[®] did produce some chlorosis in the young ratoons up to three months of age but stunting only occurred at rates above 0.9 kg a.i./ha.

Clowes and Inman-Bamber (1980) investigated the ripening response to glyphosate when applied to various varieties, nitrogen fertiliser treatments and soil moisture regimes. A total of 30 trials were performed. The study showed that soil moisture levels had an important effect on the ripening response. Consistent, beneficial responses only occurred when adequate soil moisture was available before and after application up to the time of harvest. Glyphosate-treated cane that was not moisture stressed had similar sucrose contents but greater sugar yields compared to an untreated control that was ripened by moisture stress (Figure 2.5). Cane yields increased in the glyphosate-treated cane because moisture was available for growth up to the time of harvest. Soil nitrogen levels had little effect on ripening responses but significant differences in cane quality and yield associated with nitrogen levels were observed.



Figure 2.5: The effects of Roundup[®] on sucrose % fresh weight cane in NCo376 subjected to different nitrogen and irrigation treatments (Clowes and Inman-Bamber 1980).

In twenty rain-fed trials that were part of the above work, Clowes and Inman-Bamber (1980) observed that ripening responses to glyphosate were very dependent upon moisture availability and temperature (Figure 2.6). They stated that these two factors alone "overshadowed the effects of varying soil types, crop age and other management factors". These conclusions are very similar to those made the same year by a researcher investigating the efficacy of Roundup[®] as a herbicide for killing sugar cane (Turner 1980). Although many factors can affect the herbicidal ability of Roundup[®] in sugar cane, it is critical that cane is actively growing at the time of application because Roundup[®] inhibits the synthesis of amino acids that are incorporated during protein synthesis. Turner stated that winter applications were "definitely inferior" to summer spraying when temperatures and moisture availability are higher

Observations on the large-scale use of glyphosate to ripen commercial cane were made in 1978 and 1979 (Mills 1980). Commercial data suggested a substantial improvement in cane quality when glyphosate was applied at either ends of the milling season. The interval between application and harvest was six to ten weeks in early harvested crops and decreased to three to six weeks in late harvested crops. Mills (1980) states that these intervals generally coincide with the rate of side shoot emergence, which in turn is ruled by the amount of growth occurring. Other benefits included better burns, fewer tops and trash in the cane delivered and increased bin weights.



Figure 2.6: Changes in sucrose % cane fresh weight from control after applying glyphosate (bottom) to each of the 20 trials (Clowes and Inman-Bamber 1980).



Figure 2.7: The effects of Ethrel[®] on sucrose % cane and stalk mass on mature cane (purity>80% at application) for five South African varieties. An asterisk indicates that the difference was significant (P=0.05) (Clowes 1980).

Previous studies (Rostron 1977a, Kingston *et al.* 1978) showed that inconsistent and often adverse responses occurred when Ethrel[®] was applied to mature cane that was harvested late. Clowes (1980) conducted six trials that looked at the ripening responses in different
varieties that had Ethrel[®] applied to them when mature (i.e. juice purity of more than 80). Following an application of Ethrel[®], five of these trials showed significant adverse effects on cane quality whilst there were apparent gains in cane yield (Figure 2.7). No adverse effects were observed however in NCo376.

Concerns that ration stunting occurred following glyphosate applications had been expressed in early studies (Clowes 1978, Mills 1980). A review of data from trials of ration crops that were used to assess the ripening responses to glyphosate concluded that there was little effect of glyphosate on ration yields when glyphosate was applied to actively growing cane that had suffered no moisture stress before, at or after the application (Donaldson and Inman-Bamber 1982). However, ration yields were affected by glyphosate if the crop was not actively growing because of water stress. It was suggested that the same effect could occur if the cane was subjected to other stresses (e.g. water logging, disease or low temperatures). The conclusions suggested that glyphosate may only have a role as a ripener in plough-out crops where there is a significant chance of crop stress occurring just before, at or after the time of application.

2.5.3 The 1980's

Fusilade[®] (fluazifop-P butyl) was first registered for commercial release as a grass-specific herbicide in 1980 by ISK Biosciences (Agranova 2001). In eleven field experiments conducted in 1983 and 1984, the ripening responses of Fusilade[®] were compared with those of the standard ripeners Ethrel[®] and Polardo[®] (Rostron 1985). The trials demonstrated that Fusilade[®] produced consistent improvements in cane quality when applied to actively growing cane. Ripening responses to Fusilade[®] showed a mean sugar yield gain of 1.1 tonnes/ha. No adverse effects were observed in the subsequent rations at rates of up to 97 g a.i./ha. The optimum rates were found to occur between 38 and 50 g a.i./ha Fusilade[®] was an efficient ripener when compared on a weight for weight basis with other ripeners. Up to 8 and 14 times the amount of active ingredient was required for the optimum responses from Polardo[®] and Ethrel[®] respectively. This is an important factor when the cost of aerial application per hectare for ripeners is often more than the cost of the chemical. Rostron's report also contained the first data showing the beneficial responses obtained from "piggy back" applications of Ethrel[®] and Fusilade[®]. These involved spraving Ethrel[®] on the cane one month prior to the application of Fusilade[®] or Polardo[®]. Both these combination treatments significantly increased the sucrose content (as measured by ERS) 10 weeks after spraying, compared to the Ethrel treatment alone (Figure 2.8).



Figure 2.8: Changes in percentage estimated recoverable sugar (ERS % cane fresh weight) with time for various ripener and combination ripener treatments (Rostron 1985).

A further seven Fusilade[®] trials in 1984/85 confirmed the earlier work published in 1985 (Rostron *et al.* 1986). However, there was a poor response in three trials where crops

suffered from moisture stress after application. Data was presented that showed the dry matter % cane at the time of spraying and the observed response in sugar yield for Fusilade[®] (Figure 2.9). Although little data was reported, it appears that this attribute and to a lesser degree, juice purity, could be useful in predicting the ripening response in actively growing crops that are harvested early in the season.



Figure 2.9: The relationship between sugar yield response (ters/ha) and initial cane dry matter percentage for + = irrigated cane; • = non-irrigated cane; A = droughted cane; B = very mature cane; C = excluded from regression (Rostron *et al.* 1986).

Donaldson (1986) investigated the effect of drying-off on irrigated cane that had been treated with either glyphosate or Ethrel[®]. The results from four trials confirmed earlier observations that glyphosate had no residual effects on ratoons providing the right conditions existed at the time of application (Donaldson and Inman-Bamber 1982). The crop should be kept well irrigated after application to a period just before harvest when it should be dried off sufficiently to avoid soil compaction from harvesting equipment.

Donaldson (1986) also found that the ripening response from glyphosate was independent of the amount of nitrogen applied but there was an increased ripening response on the high nitrogen treatments when Ethrel[®] was applied. Cane quality and yield responses to Ethrel[®] were better than for glyphosate when cane was water stressed for 30 to 60 days after ripener application.

Sweet *et. al.* (1987) reported the effects of various ripeners that were used on Simunye sugar estate in Swaziland between 1982 and 1986. Most of responses recorded were for the variety NCo376 (Figure 2.10). This commercial data shows that the best ripening responses were obtained either early or late in the harvesting season. It also confirms an earlier report by Rostron (1985) that combination treatments of Ethrel[®] and Fusilade[®] could produce responses that were on many occasions were superior to Ethrel[®] alone.



Figure 2.10: Commercial ripening responses in mainly NCo376 to different types of ripeners ($\Box = \text{Ethrel}^{\circledast}$; $\blacktriangle = \text{Ethrel}^{\circledast} + \text{Fusilade}^{\circledast}$ vs. Ethrel[®]; $\blacksquare = \text{Fusilade}^{\circledast}$; $\circ = \text{Polardo}^{\circledast}$; $\bullet = \text{Ethrel}^{\circledast} + \text{Fusilade}^{\circledast}$) at Simunye Sugar Estate in Swaziland. (Sweet *et al.* 1987).

The use of Polardo[®] on late harvested crops at Simunye Sugar Estate was discontinued because of concerns on ration stunting in some crops. This problem was not encountered with Fusilade[®] when it was applied to similar crops.

The variety N14 often gave poor responses to Fusilade[®]. Donaldson (1989) conducted five ripener trials that looked at the effect of 300, 400 and 600 mL/ha of Fusilade[®] on sugar quality and sugar yield. The results suggested that the optimum rate was probably about 55 g a.i./ha (= 400 mL/ha Fusilade[®]). However, similar responses between the lowest and highest rates could be obtained by varying the time between application and harvest. For example, cane sprayed at the highest rate and harvested shortly thereafter gave a similar response to cane sprayed at the lowest rate and harvested much later.

Rostron (1989) investigated the opportunities for improving sugar yields with ripeners in early- harvested crops in the Natal Midlands before frost damage occurred. Three ripener treatments (Ethrel[®], Fusilade[®] and glyphosate) were applied to five different varieties between 1984 and 1986. There appeared to be no significant variety by ripener interaction but this may not have been detected because of limited replication. The variety N11 showed a consistent lack of response in 1984 and 1985 when the cane quality in the control plots for this variety was significantly better than the other four varieties. This confirms observations made that high, early-sugar varieties maybe relatively unresponsive to the use of ripeners. However, cane quality differences between this variety and the others in the control treatments were non significant in 1986 and it did appear that Ethrel® resulted in some beneficial response that year. Reasons for these observations could not be attributed to climatic variation or soil moisture availability. Rostron concluded that some variety by ripener interaction may exist and that new varieties need to be screened for responsiveness to different ripeners. This investigation also highlights the potential for growing cane in frost-prone areas of inland Australia that have access to irrigation water and are close to existing milling infrastructure on the coast. It could be feasible for new areas to grow cane providing transport costs are not prohibitive and that the industry agrees to schedule harvesting of cane in different regions within a mill area for optimal industry profitability.

The cane pest eldana borer (*Eldana saccharina* (Walker)) presented another situation in South Africa where it was considered profitable to harvest more immature cane before serious crop damage occurred. McCulloch (1989) investigated the optimal crop age for harvesting cane to maximise profits on the North coast of Natal following crop failures in older crops because of eldana borer. This investigation showed that profits were likely to be maximised by harvesting crops that are 10 - 12 months old (Figure 2.11) before serious pest damage resulted. The harvesting of relatively immature crops can benefit from chemical ripening. McCulloch found a mean beneficial gain of 1.3 tons sucrose/ha in 1988 from the application of Ethrel[®] when applied to crops of a relatively young crop age.



Figure 2.11: Sucrose/ha/month (kg) vs. age of cane at harvest (months) for rain fed, field crops harvested in Natal between 1983/84 and 1987/88 (McCulloch 1989).

2.5.4 The 1990's

A previous study by Clowes and Inman-Bamber (1980) had shown that the ripening responses to glyphosate were adversely affected by moisture stress. Donaldson and Van Staden (1992) conducted a similar investigation with Fusilade[®] in 1988 so find why the ripening responses in NCo376 to this chemical were affected by varying degrees of moisture stress. They reported that the mean sucrose yields (tons/ha) to Fusilade[®] in the stressed cane was 50% less than the well-irrigated control and later work confirmed the best responses from Fusilade[®] occurred in well-irrigated, unstressed cane (Donaldson and Van Staden 1993). No residual effects were observed in the following ratio crop even where

the cane had been severely stresses and treated with Fusilade[®]. Cane yields in the following ratoon crop after one year of growth were also unaffected.

Donaldson and Van Staten (1995) followed this work up with another trial in 1990 that looked at the effect Fusilade[®] had on the leaves and dry matter components of stressed and unstressed sugar cane. Stalks from both treatments were divided into five sections and analysed for brix, pol, dry matter, glucose and fructose. Fresh and dry leaf mass and leaf area were also measured fifty days after application of ripener. Fusilade[®] did not affect the total dry matter content for both the stressed and unstressed treatments. Moreover, Fusilade[®] significantly increased the sucrose content (% DM) in the top four sections of the unstressed cane. Significant gains were only observed in the very top section (200 mm) for the stressed cane. Donaldson and Van Staten (1995) showed that lower levels of glucose and fructose were measured with increases in sucrose (Figure 2.12).



Figure 2.12: Changes in the sucrose, glucose and fructose % dry mass in stalk segments of Fusilade[®] treated, unstressed cane (Donaldson and Van Staden 1995).

Fusilade[®] substantially reduced the emergence of new leaves in the unstressed and stressed treatments. It was postulated that Fusilade[®] (and also glyphosate) increases sucrose in the stem by:

- 1. The removal of a primary, competing sink (i.e. new leaves and stalk apex);
- 2. Reduction of substances in cell walls that prevent phloem downloading to storage parenchyma

The South African Sugar Association (2002a) published an information sheet for growers that outlined a number of crop factors needed to obtain an optimal ripening response. Growers could use these to help identify the best cane suitable for ripening. These factors included:

- Eight or more green leaves/stalk
- Long upper internodes
- No symptoms of diseases or stress
- Uniform stand and not lodged
- No arrowing

It was stated that if all these were present then a worthwhile response would only occur if conditions permitted vigorous growth following application. This meant that rain fed cane had to have sufficient moisture in the soil profile to maintain growth after application or else there would be a reduction in the response according the duration and degree of moisture stress occurring.

Further trials after 1977 confirmed the potential of combination applications of ripeners (Rostron 1985; Sweet *et al.* 1987). Donaldson (1994) conducted seven trials between 1991 and 1993 that compared response differences between Ethrel[®], Fusilade[®] and combination treatments of both these ripeners on different varieties. He also investigated the effect of varying the interval between the application time of Ethrel[®] and Fusilade[®] on the variety NCo376. This work showed that the optimum times for the application of Ethrel[®] and Fusilade[®] in early harvested NCo376 (purities at the time of application were between 59 - 66%) were about 12 and 8 weeks before harvesting respectively. The other trials showed that all the varieties grown under irrigation responded to at least one of the ripener treatments applied. However, the expected additive responses were not always observed, which was attributed the lack of response to moisture stress. The poor response in one of the trials was also thought to be due to crop lodging. This work indicates the importance of growing cane in ripener trials so no moisture stress occurs and the possible adverse effect of crop lodging on ripening response.

The benefits of combination treatments had been shown in the past mainly on the variety NCo310. Donaldson (1996a) investigated the ripening responses in three early maturing varieties and N12 to varying ripener treatments including an Ethrel[®]/Fusilade[®] combination.

Generally the varieties tested performed best to the combination treatment. However, in some instances the additional gains made from the second application were small. Donaldson stated that some varieties (e.g. N12) might not produce an economic response following a second application of chemical ripener.

A database was compiled of all ripener trial data collected by SASEX between 1981 and 1995 (Donaldson 1996b). A summary of the mean responses is shown in Table 2. 3. When all known factors known to adversely affect the responses to ripeners were removed, the mean sugar yield increase for 52 trials was 1.21 tons ERS/ha. The probability of cost recovery (estimated at 0.2 tons ERS/ha) is 82.7%. Donaldson states that in 1996, 28,134 ha were treated with ripeners. He estimates that this produced an additional R15.1 M (\equiv A\$5.59 M as of Jan. 1996) at a cost of R3.8 M (\equiv A\$1.41 M as of Jan. 1996). A trial repeated every year between 1991 and 1995 showed that large gains from combination treatments above single ripener treatments could be achieved with some varieties (e.g. N19) whereas little gain was made with others (e.g. N22, N12). The variety CP66-1043 appeared to respond best to single application of Fusilade[®] as opposed to the combination treatment. There is clearly a basic need to understand the reasons why these response differences occur. Until the reasons are understood, it will be necessary to screen varieties individually for responses to various chemical ripener treatments.

Donaldson published a more recent review on chemical ripeners in 1999. The South African crop area sprayed with chemical ripeners in 1997 had increased to 38,605 ha, nearly doubling that treated in 1995. The area treated in 1997 amounted to 37% of the irrigated crop area and 2% of the rain-fed area. The increased adoption of ripeners by South Africa growers may reflect the availability of better knowledge resulting from the research conducted by South African Sugar Association Experiment Station (SASEX) since the early 1970's.

Data	ERS	SE±	Cane	SE±	ERS	SE±	n	Prob.
	t/ha		t/ha		% cane			0.2t
1.All data	0.75	0.10	-2.91	0.58	0.86	0.07	105	0.70
Excluding	0.83	0.13	-3.11	0.77	0.94	0.10	92	0.69
2. Low rates								
3. >65 days	0.74	0.16	-3.45	0.90	0.92	0.12	71	0.63
4. >450 mL	0.79	0.17	-2.98	0.93	0.90	0.12	64	0.66
5. Dry	0.80	0.17	-3.11	0.93	0.91	0.12	63	0.67
6. Lodged	0.84	0.18	-4.28	0.94	1.04	0.13	52	0.67
7. N14 < 400	0.82	0.20	-4.52	0.94	1.05	0.13	50	0.68
mL								
8. Adding >65	0.97	0.18	-4.00	0.92	1.12	0.11	61	0.72
days								
9. Excluding	1.21	0.18	-2.84	0.95	1.13	0.12	52	0.83
late season								

Table 2.3: Responses and standard errors (SE \pm) of yield characteristics, number of treatments (n) and probability (prob) of recovering costs after eliminating factors that influence response to Fusilade[®] Super (Donaldson 1996b)

Notes

- 1. all data
- 2. exclude all trials with application rates below 37.5 g a.i./ha
- 3. exclude all trials with treatment harvest intervals >65 days
- 4. exclude all trials with application rates above 56.25 g a.i./ha
- 5. exclude all trials affected by moisture stress
- 6. exclude all trials affected by lodging
- 7. exclude all data for variety N14 that was treated at application rates <50 g a.i./ha
- 8. include all trials with treatment harvest intervals >65 days
- 9. exclude all trials treated late in the milling season

One of the weaknesses of past research in South Africa (and elsewhere) is that the control treatments have often not been dried-off as would occur in commercial practice. Donaldson (1999) questioned whether the actual differences between chemically ripened cane and drought-ripened cane are sufficient to justify the cost of application. Drought ripened cane has the advantage of withholding inputs and reducing costs by not irrigating as opposed to the chemical ripening which requires inputs and increases costs. This is an issue requiring a further investigation that quantifies the cost/benefits of each method and the associated probabilities of success. It is likely that the desirability of one method above another (or a combination of both) will change with rainfall probabilities, the potential for the crop to grow and the ability for the ground to support machinery.

Future research in South Africa (Donaldson 1999) is likely to focus on:

- Screening new varieties and ripeners (eg. haloxyfop-R methyl ester was recently registered in South Africa) for potential response;
- Linking growing degree-days to the use of ripeners in summer harvested crops;
- Better understanding the sugar accumulation and ripening processes.

2.6 A review of chemical ripening research conducted in Australia

BSES researchers reported in 1953 and 1955 that they were unable to repeat the ripening responses found with 2,4-D in Cuba (Skinner 1956). The first report of beneficial response to a chemical ripener in Australia was in 1955 when a 4% solution of maleic hydrazide applied in March resulted in a four unit CCS response seven weeks after application (Skinner 1956). However, applications made in May had no effect on sucrose content. The BSES did not further investigate this ripener, as there appeared to be little opportunity to improve the sucrose content within the normal harvesting period. In fact, BSES did not investigate chemical ripening again for 20 years. Since Skinner's data was obtained from unreplicated plots (with the exception of the control treatment), it is possible that there was a significant response in the May application but it could not be detected because of the poor trial design. For reasons unknown, these early findings were not investigated any further and there are no other reports in the literature of similar successes until the enzyme inhibitor glyphosine was made available fifteen years later.

The first reported study of chemical ripeners from the glyphosate family in Australia was performed in 1969 (Bieske 1970). Bieske, an employee of Fairymead Sugar Co. Ltd, conducted five trials on company properties in the Bundaberg area. Two ripeners were trialed, D.A./5 (Dupont) and C.P. 41845 (=glyphosine from Monsanto). These ripeners were mostly tested on the variety NCo310, a variety that was later shown to be responsive to chemical ripeners in South Africa. Bieske observed significant commercial cane sugar (CCS) increases to glyphosine when it was applied early and late in the season. The early season trials involved applications at various rates beginning as early as April 9th. In these trials, CCS gains of up to 1.71 units were observed for the earliest application times in combination with the highest application rates. The late season trials included the variety Q76 and CCS gains of 1.76 units were observed for the highest application rates five weeks after treatment on October 24th.

Bieske later attended a meeting with Monsanto at the 1971 ISSCT conference in Louisiana (Bieske and Wells 1971). Monsanto indicated at this meeting that they were "committed to the production of C.P.41845 for use as a ripener". The following interesting points were also raised at this meeting:

- Following effects were observed in young rations but these rarely persisted to final harvest;
- 2. Best results from this ripener occurred under high nitrogen applications;
- 3. Response varied with variety but high sucrose varieties appear best suited to ripener use.

The mid-1970's saw three investigations reported into the use of chemical ripeners.

Chapman and Kingston (1977) conducted eight trials in 1976 for the BSES, following overseas reports on the commercial use of glyphosine as a ripener. These trials, conducted at Babinda, Mackay, Bundaberg and Isis, tested eight ripeners including Ethrel[®] and glyphosate. Four trials were performed on early harvested crops and another four on late harvested crops. The varieties tested included NCo310, Q68, Q76, Q87, Q90 and Q86. The only varieties previously known to be responsive were NCo310 and Q76. Significant CCS increases were observed in six of the eight trials, with Ethrel[®] giving a 1.7 unit CCS advantage in an early harvested crop at a Mackay trial. The late harvested trials showed CCS gains of up to 2.2 units for the highest rates of glyphosate at a Mackay trial. The two

trials that did not show significant increases in CCS (Babinda and a Mackay trial) nevertheless showed a trend for an increase in CCS following some ripener treatments. The data also indicated that Ethrel[®] was not effective at halting CCS decline in late harvested crops. It is unfortunate that this work did not include common varieties across sites given that the varietal responsiveness for most of the cultivars used was unknown. For an example, it cannot be determined if the lack of response seen in the Babinda trial was due to use of the variety Q90, or site factors, or both.

Subsequent BSES trials conducted by Kingston et al. (1978) involved four phases of work. The first two phases examined the responses to Polaris[®] and Ethrel[®] from ground and aerial applications. The responses to the aerial applications of Polaris[®] and Ethrel[®] in NCo310 and Q87 at Mackay and Bundaberg were not outstanding. A mean CCS advantage of 0.2 and 0.1 units for Polaris[®] and Ethrel[®] respectively was observed for ten trials. These trials were unreplicated so errors associated with block variation may have made it difficult to observe real differences. The ground application trials were conducted at eight sites. These sites were located in the Northern, Central and Bundaberg regions and the trials were replicated. All treatments except one in the Bundaberg region were applied to early harvested crops. Only one trial at Bundaberg showed a significant 0.73 unit CCS increase following treatment with Polaris[®] to an early harvested crop. Particularly interesting is the observation that Ethrel[®] caused a significant decrease in CCS at three of the Northern trials. The decrease was up to 1.33 units at one site. Negative responses in CCS to Ethrel[®] in late harvested crops were also reported in South Africa by Rostron (1977a). The three Northern trials only had Q90 in them, which was the same variety in the 1976 trials that showed no response to ripeners.

The third phase screened six ripeners in small plot trials at six sites across the same three regions. The varieties tested included Q90, Q96 and NCo310. Polaris[®], Mon 8000 (syn. glyphosate) and XHH148 (unknown compound) caused significant ripening responses in four of the six trials. The fourth phase looked at the varietal responses in Q87, CP44-101 and NCo310 to Ethrel[®] at five and nine weeks following application. Significant CCS increases were observed in Q87 and CP44-10. The Q87 showed a peak response at five weeks of 1.37 units whilst the peak response of 1.30 units in CP44-101was not observed until nine weeks. It is interesting that the responsive variety NCo310 failed to show a significant increase in CCS. Kingston *et al.* (1978) concluded that it not possible to predict or obtain useful responses to Polaris[®] or Ethrel[®] at the sites tested. They attributed the

consistent lack of response to applied ripeners to the favourable conditions for natural ripening and the high base level of sucrose. These two factors are often absent where ripeners have proven successful overseas. In hindsight, these trials should have also included common varieties across sites with increased replication and the aerial spraying trial should have included at least one replication.

Hurney and Schmalzl (1978) conducted two series of trials in 1976 and 1977 that looked at the ripening responses to Polaris[®] when applied under commercial conditions. The 1976 trials were applied to nine fields in the Babinda and Mossman areas. A control and three treatment rates were applied but there was no replication apart from the control. All nine fields were early harvested crops of Q90 with the earliest application time being the 21st April. Observations of commercial mill data showed an average CCS increase of 0.92 units at the Babinda sites. The Mossman sites showed little CCS response and this was attributed to the favourable conditions for natural ripening that occurred there. The 1997 trials consisted of 14 trials in the Babinda area and 15 in the Mossman area. Only one rate of Polaris[®] was applied, based on the best response obtained in the 1976 trials. All 29 fields were early harvested crops mainly consisting of the variety Q90, which as mentioned earlier, was found to be largely unresponsive. The mean CCS response in both areas was about 0.77 units but a negative response was observed in treatments that suffered from yellow spot disease (Cercospora koepkei (Krüger)). It was also observed that the percentage increase in CCS was best for the first application date compared to the other ones.

The investigations performed in the mid-1970's showed the responses to ripeners to be variable and difficult to predict. Little was known about the responsiveness of the few varieties tested or the optimum conditions required for response (e.g. crop maturity at time of application, application time, crop erectness, etc.). The data obtained from the aerial application trials was inconclusive as it was unreplicated. It was calculated that more than 0.8 unit response was required at the then current low world prices to obtain an economic response from the use of chemical ripeners (Kingston *et al.* 1978). The data collected suggested this would not be achieved in most instances. It was also felt that the adoption of this technology at that time would be slow as growers generally were not familiar with spraying chemicals on crops. For all these reasons, no further work in Australia was undertaken or reported on chemical ripeners for the next ten years.

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Kingston (1988) conducted an economic re-evaluation of cane ripeners in the mid-80's. The need for this arose from improved sugar prices and South African research that indicated better gains from Ethrel[®] could be achieved when applied to immature cane in the March-April period. A 6 x 6 latin square trial was established in 1987 on a fertile, low-CCS site in the Moreton Mill area. Ethrel[®] was applied by ground application to H56-752. Four application times were used. The first application was on the 27 March 1987 and the others were every twenty days thereafter. Significant increases in purity were observed for all application dates for a sampling done on the 6 July 1987 (Table 2.4).

Table 2.4 : A summary of CCS responses to Ethrel [®]	in H56-752 treated in the Moreton Mill
area in 1987. (Kingston 1988).	

Application	Purity at application	Maximum response	Days after
date	date	(CCS)	application for
			max. CCS
			response
27/3/87	48.7	2.17	101
16/4/87	59.2	1.76	81
6/5/87	68.2	1.37	82
26/5/87	79.2	1.17	41

Some interesting trends emerged from these trials. These are:

- 1. Maximum responses occurred in cane in cane treated in March;
- 2. The responses diminished as the purity of the treated cane increased;
- 3. The time required for a maximum response decreased with increasing purity.

Treatments applied in the March/April period also achieved gains that persisted for a longer duration. It confirmed work done in South Africa between 1972 and 1976 (Rostron 1977a) that the best CCS gains with Ethrel[®] occurred when it was applied to immature crops around March or April. Until now, all previous research done with Ethrel[®] in Australia involved application dates on crops that were relatively mature. This could explain why

most previous work in the mid-70's had shown negligible responses in CCS to chemical ripeners.

The first Australian trial using Fusilade[®] was conducted by Robert Williams from the North Queensland Agricultural Research and Development Pty. Ltd in 1988 (Pers. Commun. Harry Townley – CropCare Townsville). This company had been contracted by ICI (now CropCare, a subsidiary company of Orica which was formerly ICI) to conduct three trials on commercial crops at different sites in the Herbert region with Fusilade[®]. Each site contained a different variety, these being Q117, Q119 and Cassius. Three application rates were used and these were applied to small, replicated plots between the 8 and 10 June 1998 at all sites. CCS was measured at 0, 4, 6, 8, 10 and 12 weeks after application. The responses seen in Q117 at one site are shown in Figure 2.13.



Figure 2.13: CCS response in Q117 to different rates ($\Box = \text{control}$; $\blacksquare = 250 \text{ mL/ha}$; $\blacktriangle = 350 \text{ mL/ha}$; $\bullet = 450 \text{ mL/ha}$) of Fusilade[®] (a.i. 212 g/l fluazifop-P butyl) after application in the Herbert region. Data kindly provided by Harry Townley – CropCare Townsville; from the work done by Robert Williams, North Queensland Agricultural research and Development Pty. Ltd.

The two other varieties at the other sites also expressed significant increases in CCS, 6 to 10 weeks after the application of Fusilade[®], at rates ranging from 250 to 450 mL/ha of 212 g/L fluazifop-P butyl. Overall there was no significant CCS response to the dose applied and Williams believed that lower rates could be as effective with fewer adverse effects on cane yield observed in some varieties at the higher rates. No significant increases in sugar yield per hectare were observed with any of the treatments when they were harvested at 10 to 12 weeks after treatment. However, significant differences could have been seen if the harvest had occurred between 6 to 8 weeks after harvest. The above CCS responses for Q117 are the greatest in this period. Fusilade[®] significantly increased the number of side shoots and significantly reduced the number of suckers seen in Q117. Williams concluded that further work was needed to identify:

- 1. Optimum rate range;
- 2. Varietal responses;
- 3. The effect on the response interval with CCS levels at the time of application.

The accurate data collected by Williams permitted some potentially important observations. These were:

- The consistent response in CCS increase with Fusilade[®] in this series of trials. This was not always the case with previous studies involving other ripeners (eg. Kinston *et al.* 1978);
- 2. A CCS response was obtained despite the high purities (93% for Q117) at the time of application;
- 3. That lower application rates might be possible compared to those being used in South Africa.

The study performed by Williams was unfortunately not published. Further investigations were not pursued because ICI felt that there would be environmental issues associated with the aerial spraying of Fusilade[®] and that the responses seen would be variety dependent. This would burden ICI with the responsibility of continuously testing varieties as they were released (Harry Townley, CropCare - pers. comm.).

Kingston *et al.*(1991) reported responses to Ethrel[®] when used to improve early season CCS between 1987 and 1990. Results from a total of 21 replicated small plot trials in the

Northern and Southern areas were collected. The varieties used in the small plot trials were Q96, Q115, Q117, Q119, Q124, Q137, CP44-101 and H56-752. Twelve commercial strip trials were also conducted in 1988 with Q96, Q107, Q115, Q117, Q122, Q123, Q128, Cassius and CP44-101. These strip trials were performed throughout Queensland. All of above trials were sprayed between mid-March and mid-April at 1.5 L/ha Ethrel[®] and some varieties were found more likely to respond than others (Table 2.5).

Variety	Probability of achieving
	a response of $\geq = 0.5$ units CCS
Q115	100
Q119	100
Q137	91
Н56-752	84
CP44-101	56
Q96 & Q124	50
Q117	37

Table 2.5: Probability of varietal responses to Ethrel[®] in the first six weeks of the harvest season (Kingston *et al.* 1991).

The poor response seen in Q117 may have been due to high purities that were never below 75% at the time of spraying. The above work showed that there was a 70% probability of a CCS response of 0.5 units of greater following the application of Ethrel[®]. There was a 20 % probability that the CCS response would be less that 0.25 units with a negative dollar return on investment. Probabilities could be improved if the cane had purities less than 75% at the time of application and that subsequent growth occurred in non-stressed conditions.

Willcox *et al.* (1999) conducted a trial that investigated the effect of three crop ripeners on CCS and yields of Q124 at Mackay in 1998. Ethrel[®], glyphosate and Fusilade[®] were applied on 10/3/98 to replicated small plots that were sampled 8, 10 and 12 weeks after application. The harvest of the trial occurred 15 weeks after application. Significant

increases were observed in CCS for all ripeners (Table 2.6). However, significant decreases in cane yield occurred for the Fusilade[®] and glyphosate treatments (Table 2.7). No significant reduction in cane yield was observed with Ethrel[®]. Stalk elongation stopped one week after the application of Fusilade[®] and never resumed. The application rate used for Fusilade[®] was 450 mL/ha (212 g/L fluazifop-P butyl). This was considered too high by Williams and is above the rates used by the South Africans except for their less responsive varieties like N14. However, the Fusilade[®] treatment still gave the best dollar return on investment despite the high rate used and the adverse effects on cane yield.

Treatment 5 May 18 May 1 June 24 June Control 9.3 8.8 8.2 12.7 Ethrel® 9.4 9.7 11.0 13.6 Roundup® 10.1 10.1 11.8 13.9 Fusilade® 12.5 13.5 14.4 16.2 0.95 0.79 0.39 l.s.d. (*P*≤0.05) 1.76

Table 2.6: CCS levels for each treatment at four dates after spraying (Willcox et al. 1999).

Table 2.7: Plot yield and CCS for each treatment and monetary return to grower after deducting \$7/t harvest cost with sugar at \$330/t (Willcox *et al.* 1999).

Treatment	Yield	CCS	Yield	Return to
	(t cane/ha)		(t sugar/ha)	grower (\$/ha)
Control	95.8	12.71	12.17	1862
Ethrel [®]	87.2	13.64	11.88	1868
Roundup [®]	83.7	13.88	11.61	1881
Fusilade®	70.5	16.16	11.38	2031
l.s.d. (<i>P</i> ≤0.05)	10.05	0.39	1.37	

Australian research in the area of chemical ripeners has been hampered by:

1. A knowledge gap in understanding the reasons for the variable responses of cane varieties to ripeners in early trials;

- 2. Low world prices at the time the time of investigation that indicated the probability of an economic response would be low;
- 3. Grower attitudes to spraying technology in the 70's and 80's;
- 4. Environmental concerns and resistance to aerial spraying near residences;
- 5. Possibility of ratoons being affected by chemical ripeners;
- 6. Limited registration of chemicals for ripening sugar cane in Australia.

The Australian sugar industry has currently made very little use of this technology to improve cane quality in some of the crops sent to mills.

2.7 Current commercial practice

The choice of ripener, rate and treatment harvest interval varies with country (Table 2.8). South Africa differs from other countries in that it practices combination ripener treatments.

2.8 Other Issues

The adoption of ripener technology by growers also needs to be considered in terms of effects on the whole farming system. For an example, it important that growers ensure adequate soil moisture or irrigation is available to chemically ripened crops if optimum benefits are to be achieved. The use of ripeners can also reduce the flexibility of the grower to manage the harvest sequence if unforseen circumstances occur just prior to harvest (eg. flooding rains, uncontrolled burns, etc). A block that has been managed for ripeners is probably less able to cope with harvesting machinery at harvesting if rainfall occurs as opposed to a block that has been "dried-down". However, ripeners can reduce the number of green leaves and trash at harvest time thereby minimising the difficulties cane harvesters have processing high yielding crops that are cut green.

Country	Ripener ¹	Rate	THI^2	Comments	Reference
Mainland USA	G	0.14 - 0.33 kg ai/ha	(days) 21 - 35	last ratoons only, applied to crops harvested in Oct & Nov., higher rates used with smaller THI	Millhollon and Legendre 1996; Gilbert <i>et al.</i> 2002.
Guyana	G	900 mL/ha of 480 g ai/l	28		Eastwood and Davis (1997, 1998)
Guyana Guyana	F T	425 mL/ha 700 mL/ha	42 28	higher rainfall areas	(c)) (c))
Australia	Е	1.5 l/ha	56 - 70	Apply Mar/Apr	Rhône- Poulenc 1996.
South Africa	Ε	1.5 l/ha	42 - 84	certain varieties, less than 75% purity, sufficient soil moisture, harvest before July, smaller THI for fast growing cane	South African Sugar Association 2002b.
South Africa	F	300 - 440 mL/ha	35 to 70	rate varies with variety, purity less than 85%, sufficient moisture to maintain growth for 35 days after application, THI depends on rate of cane growth, can be used for early and late harvested crops.	South African Sugar Association 1998.
South Africa	E + F	as for previous recommen dations	77 - 84 (E) 42 - 49 (F)	only early harvested crops	South African Sugar Association 2002b.

Table 2.8: Commercial application rates and treatment harvest intervals for various countries.

 $\frac{\text{dations}}{\text{G = glyphosate, T = Touchdown 4LC; F = Fusilade^{\text{®}} \text{ Super, Ethrel}^{\text{®}}}.$

² Treatment harvest interval.

2.9 Cost benefit analysis of using ripeners

Cost/benefit ratios in the literature show returns from ripening responses that range from \$3.50 for every dollar spent to returns as high as \$32 for every dollar spent (Table 2.9). The lowest returns were reported from Australia and these probably reflect the variable and inconsistent responses obtained from ripener trials conducted there.

Country	Ripener ¹	Cost/Ha	Expected	Cost/benefit	Reference
		(currency)	response	ratio	
Swaziland	Е	80 (E)	0.75 t sucrose/ha	1:6	Rostron 1996
Swaziland	F	35 (E)	0.75 t sucrose/ha	1:15.1	cc>>
Swaziland	E & F	115 (E)	0.75 t sucrose/ha	1:5.5	(())
Guyana	G or T or	11.50 (US)	0.35 - 0.75 t/ha	1:15 to 1:32	Eastwood
	F				and Davis
					1997
Guyana	G or T or	11.95 (US)	1.04 % (w/w	1:15 to 1:32	Eastwood
	F		cane)		and Davis
					1998
Australia	Е	90 (A)			Kingston et
					al. 1978
Australia	Е	56 (A)	0.5 % CCS		Rhône-
					Poulenc
					1996.
Australia	Е	70 (A)	0.8 % CCS	1:3.5 to 1:4.7	Kingston
					1988
South	F	80 Rand	1.2 t sucrose/ha	1:4	Donaldson
Africa			for irrigated cane		1996b
			and a 33% lower		
			response for dry		
			land cane		

Table 2.9: Some expected responses and cost/benefit ratios for different ripeners for some of research that has been conducted overseas.

 1 G = glyphosate, T = Touchdown 4LC; F = Fusilade[®] Super, Ethrel[®].

2.10 Environmental and safety issues pertaining to chemical ripeners

The use of ripeners can create potential problems with chemical residues in the harvested crop. Despite the relatively short withholding period (i.e. the time between spraying and harvesting) Donaldson (1990) states that residue problems are unlikely because:

- 1. Relatively low application rates are used for ripening compared with the recommended herbicidal rates for the same chemical;
- All the ripeners currently recommended for use in South Africa have extremely low mammalian toxicity. All have LD₅₀ ratings above 3000 mg/kg body weight;
- 3. The production process (crystallisation) tends to exclude nearly all impurities.

Aerial application of ripeners can also be managed so drift outside the crop area is minimised. Unlike herbicides, it is not necessary to apply ripeners to all areas of the field. Where necessary problems with spray drift from chemical ripeners can be minimised by leaving an unsprayed margin on the outsides of the field. Fusilade[®], for an example, is toxic to aquatic life, however its entry into waterways and riparian areas can be avoided by ensuring unsprayed margins are maintained between these areas to prevent entry into these sensitive environments.

Residue testing for fluazifop-p butyl performed for preliminary trials conducted at Kalamia in 1999 failed to detect any residues within the cane juice, four weeks after cane was treated with 300 mL/ha for Fusilade[®] (212 g/L a.i).

2.11 Conclusion

Chemical ripeners can increase the accumulation of sucrose in the sugar cane stem when applied under the right conditions. The conditions required for successful application have become clearer in recent years primarily through research in South Africa.

Much of the research undertaken shows that it is not easy to obtain meaningful results from ripener trials. The errors associated with trials are often greater than the small differences being tested for. It is particularly difficult to measure real differences in cane yields from small plots following the application of ripeners (Julien 1977). Considerable care needs to be taken with ripener trials to ensure that there adequate randomisation and replication exists whilst ensuring that the environmental influences across treatments are as uniform as possible. A great deal of past work has failed to achieve this.

Many knowledge gaps still exist despite a better understanding of the requirements for the successful use of ripeners. For an example, much of the past South African work makes reference to the importance of the physiological status of the sugar cane at the time of

application. An inverse relationship between purity at the time of application and the observed response has been shown for Ethrel[®] but this less apparent for other ripeners. Also, no work reports the correlation between growth rates before and after application with the observed response. This should be investigated given the number of reports that state this is an important determinant of the ripening response. Therefore, there is a need to accurately measure such growth on small time scales around application time and also correlate this with the observed response. The manufactures of ripeners that are also used as plant growth inhibitors strongly emphasise that herbicidal success of these ripeners is correlated with active growth at application. There are almost certainly linkages between the successful use of these chemicals for herbicidal and ripening purposes and these should be investigated.

Crop age and the time of application can determine the rate of growth when ripeners are applied. There is a need to investigate the response to ripeners in crops of differing ages at one application date. The effect of various application dates should also be investigated. The observed responses should be correlated back to physiological growth at the time of application as mentioned in the preceding paragraph.

Little is understood at a biochemical level about what signals are used by sugar cane to determine how photosynthate is allocated either to sucrose or non-sucrose production. The plant growth inhibitors (eg. glyphosate, fluazifop) inhibit all apical meristem activity and the removal of these primary sinks affects the internal sink/source relationships so there is a more favourable partitioning of photosynthate towards sucrose storage. However, it is speculated that each of these inhibitors may affect the plant in many other ways, which also increases the accumulation of sucrose. Further work needs to be done on investigating the message mechanisms that determine how photosynthate is finally utilised in sugar cane and where are these mechanisms located.

Another knowledge gap is the lack of information as to how important the genetic variation in sugar cane is for the observed response to ripeners. One weakness of past research is that it in most instances the gains from chemical ripening have not been fairly compared with a control that has been dried off to maximise sugar yields. Drying off has been shown to increase sugar accumulation and it is probably the most cost effective way for a grower to ripen a crop where it is possible to do so. Most control treatments in past ripener trials have been well irrigated because the ripener treatments require good growing conditions after

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application to maximise yields. Growers require data that shows differences between what they can currently attain with what is being investigated.

Before many of these knowledge gaps can be filled in, there is a need to first understand the extent of varietal responses to the ripeners used. It has been suggested that the variation may not be extensive but this needs to be verified by studies that assess the responses of cane to ripeners under controlled environments. Confirmation that the differences due to genetic variation are small would allow future research to focus more on understanding the differences due to environmental factors. It would also allow future researchers to avoid using varieties and varieties in certain environments that are known to be non-responsive to ripeners. An understanding of these factors will add value to any future research undertaken with this technology and avoid the pitfalls that plagued much of the past research in this area.

Chapter 3

The responsiveness of Australian sugar cane varieties to chemical ripeners early in the year

3.1 Introduction

Sugar content in cane on a fresh weight basis is an important determinant of the costs of production of sugar and hence industry profitability. This is because major variable costs, including harvesting, cane transport and some milling costs are strongly related to the amount of cane involved. Normally cane is only harvested in that part of the year when sugar content in cane is at its highest, and hence profitability is greatest. In Australia, sugar content in cane is normally lowest from mid-summer to late autumn, and the harvesting normally occurs between mid-winter and late spring (mid-June to November). There would be potential economic advantages in beginning the harvesting period earlier to obtain increased rates of return on harvesting and milling capital, but only if the sugar content in the cane was sufficiently high.

Although researchers in Australia have examined chemical ripeners since the mid-1950's (Skinner 1956; Bieske 1970; Kingston *et al.* 1978), the adoption of this technology by Australian industry has been limited. There are several reasons for limited adoption. First, in most years, the bulk of the cane in Australia is harvested after maturing under cool, dry conditions following May, and these conditions have been shown to be less conducive to producing responses in the presence of chemical ripeners (How 1976). Second, many trials conducted in the 1970's (Chapman and Kingston 1977; Kingston *et al.* 1978) resulted in responses that were variable and inconsistent, in part probably related to the timing of application, but also due to other reasons not fully understood. Some of the factors that affect response have since been identified including type of ripener or ripener combination (Rostron 1985), time of application and crop maturity (Clowes 1978), application rate (Donaldson 1989), and treatment harvest interval (Dusky and Alvarez 1986). A key factor is also genotype (Dusky *et al.* 1986), but little knowledge exists of the responsiveness of current Australian varieties to chemical ripeners

The aim of this study was to evaluate a large range of Australian sugar cane varieties for responses to chemical ripeners. These varieties were tested at a single site where a number of environmental and crop variables were managed to optimise the likelihood that the observed responses were due to mainly to genotypic responses. This study represents a key step towards understanding the interactions between ripener, variety and season, and to determine if and how chemical ripeners could be used to improve the sucrose content and profitability of early-harvested cane in the Australian industry.

3.2 Materials and Methods

3.2.1 Experimental design

The experimental design followed a split plot in space and time (Steel and Torrie 1980). Five chemical ripener treatments were applied to whole plots allocated at random to each of five blocks. Forty-three sugar cane cultivars were allocated at random to subplots within each whole plot. The varieties included commercially-grown cultivars and selections from advanced stage selection trials in breeding programs from the Northern, Burdekin and Central cane growing regions of Queensland (Table 3.1) and represented 85.9 % of all cane supplied to Australian mills in 2001 (Bureau of Sugar Experiment Stations 2002). The experiment was planted on 11 Aug. 1999 at Kalamia Estate, Ayr (147.412759E, -19.536277S). Each variety was planted into single row plots 4 m in length. There were 44 plots in each of the whole plot treatments, consisting of one plot of each variety and two plots of variety Q124.

The Fusilade[®] and Verdict[®] treatments included the non-ionic wetting agent Agral 60[®] at 350 mL/ha and 200 mL/ha of spray solution respectively. The combination treatment (E+F) involved the application of Ethrel[®] followed about four weeks later by an application of Fusilade[®]. The Ethrel[®] application was applied on 8 March 2000 and all the other ripeners were applied 30 days later on the 7 April 2000. All the ripeners were applied from the ground as a 3 m swath by a liquid petroleum gas (LPG) powered, overhead-spray boom. This operated at 100 kPa and the spray solution was applied at 100 L/ha. The average stem height to the top visible dewlap of all the varieties in three of the five replicates was 2.67 m on 23rd March 2000.

The ration crop was slashed to ground level on the 11 October 2000 to prevent lodging in 2001.

No.	Variety	Parent				
	2	Female	Male			
1	81N82	Q113	69N2915			
2	87A1413	Q135	61N1232			
3	Argos ^A	CP51-21	MQ68-79521			
4	CP74-2005	CP66-1043	CP63-588			
5	EOS	LF71-4738	MQ73-631			
6	H56-752	H49-118	Unknown			
7	Mida ^A	Q96	MQ79-1030			
8	Q113	NCO310	54N7096			
9	Q115	NCO310	54N7096			
10	Q117	Q77	58N829			
11	Q120	NCO310	54N7096			
12	Q124	NCO310	54N7096			
13	Q127	54N7096	H49-3666			
14	Q130	Q117	CP50-11			
15	Q133	58N829	CP49-50			
16	Q135	NCO310	54N7096			
17	Q136	NCO310	54N7096			
18	Q138	58N829	66N2008			
19	Q141	NCO310	54N7096			
20	Q152	71N814	CO440			
21	Q158	58N829	66N2008			
22	Q162	58N829	66N2008			
23	Q163 ^A	68N1797	Q96			
24	Q164	Q117	66N2008			
25	Q165 ^A	Q117	CP33-372			
26	Q166 ^A	58N829	66N2008			
27	Q167 ^A	58N829	66N2008			
28	Q171 ^A	64C386	Q121			
29	Q172 ^A	Q99	H49-3666			
30	Q173 ^A	68N1797	60S7540			
31	Q174 ^A	Q117	66N2008			
32	$O176^{A}$	Q117	67C444			
33	Q177 ^A	75N1675	Q121			
34	Q179 ^A	58N829	66N2008			
35	Q180 ^A	67N3184	CO1007			
36	Q181 ^A	75N1649	66N2008			
37	Q183 ^A	Q124	H56-752			
38	Q186 ^A	Q117	66N2008			
39	Q187 ^A	58N829	66N2008			
40	O189 ^A	Q117	CP56-59			
41	Q195 ^A	Q117	MEX59-1828			
42	Q96	Q63	Q68			
43	Tellus ^A	ROC-1	Unknown			

Table 3.1: List of varieties examined for their responses to chemical ripeners in 2000 and2001 and their parentage.

The treatments and rates of application are documented in Tables 3.2 and 3.3.

Treatment	Year	
	2000	2001
1	Control	Control
2	Ethrel [®] + Fusilade [®] (E+F)	Ethrel [®] + Fusilade [®] (E+F)
3	Fusilade®	Fusilade®
4	Glyphosate	Residual glyphosate from
		2000
5	Verdict [®]	Glyphosate

Table 3.2: Details of ripener treatments imposed on all varieties in 2000 and 2001

Table 3.3: Details of ripeners and rates used on all varieties during 2000 and 2001.

Ripener Treatment	Chemical Name and concentration of active	Rate
(inc, trivial name)	ingredient in the source product	(mL/ha)
Fusilade [®] (F)	212 g/L fluazifop-P butyl	200
$Ethrel^{\mathbb{R}} + Fusilade^{\mathbb{R}}$	480 g/L ethephon + 212 g/L fluazifop-P butyl	1500 + 200
(E+F)		
Glyphosate (applied	360 g/L glyphosate as the isopropylamine salt	1000
as Weedmaster [®] Duo)		
Verdict [®]	513 g/L haloxyfop-R methyl ester	37

Most ripener treatments were reapplied in 2001. However, some changes from procedures in 2000 were implemented. Firstly, samples were taken from all plots during March and April. Secondly, a carry-over effect adversely reducing CCS was identified in the plots treated with glyphosate in 2000 following the sampling on the 6 March 2001. As a consequence, it was decided not to treat these plots with glyphosate in 2001 in order to further investigate the carry-over effect, and to substitute the Verdict[®] treatment with a glyphosate treatment in 2001 since the responses to Verdict[®] in 2000 were not statistically significant. Thirdly, changes were made to improve the uptake of the crop ripeners. The crop oil Uptake[®] (582 g/L paraffinic oil, 208 g/L non-ionic surfactant) and LI 700 (350 g/L soyal phospholipids, 350 g/L propionic acid) were both added at 500 mL/100 L of spray mix to the Fusilade[®] application. The Ethrel[®] and Weedmaster[®] Duo applications were both applied with Activator Surfactant[®] (900 g/L non-ionic surfactant) at 100 mL/100 L of spray

mix. The decision to change the surfactants used in 2001 was based partly on advice received that year on the use of surfactants with crop ripeners (G. Kingston – Pers. Comm.) and the unexpected lack of response to the Fusilade[®] treatment. There is a possibility that these changes could have influenced the ripener x year interaction effects observed by improving the uptake of ripeners and hence the potential response. However, these changes were not associated with mean response improvements to any of the applied ripener treatments in 2001.

Ethrel[®] and Fusilade[®] were applied to the E+F treatment on the 9 March 2001 and 10 April 2001 respectively. The Weedmaster[®] Duo and Fusilade[®] alone treatments were both applied on the 11 April 2001.

The trial was then grown and fertilised according to local industry recommendations for commercial cane with irrigation maintained up to four weeks before machine harvesting on the 28 June 2000. Furrow irrigation was applied to the plant crop on 20 August 1999, 14 September 1999 and 29 March 2000. The first ration crop was furrow irrigated on the 8 August 2000, 13 March 2001, 28 March 2001, 12 April 2001 and 16 May 2001. The estimated plant available water content of the soil following each furrow irrigation in the plant and first ration crop was estimated to be 200 mm (G Imman-Bamber – Pers. Comm.).

3.2.2 Measurements

Sampling for brix and pol (as % juice) was undertaken on four occasions each year. Samples consisting of two stalks were taken from each control and E+F treatment plots on the 6 March 2000 and 7 April 2000 just prior to the application of ripener treatments. The same sampling procedure was applied again to every the plot in the trial when the treatment harvest interval (THI) was 4 (8 May 2000) and 8 (6 June 2000) weeks after application of the Fusilade[®], Weedmaster[®] Duo and Verdict[®] treatments. In 2001, samples from all plots in all treatments were taken on the 6 March 2001, 4 April 2001, 8 May 2001 and 5 June 2001.

The estimation of commercially extractable sucrose in cane juice involves measurement of brix and pol. Brix (in g solute per 100 g solution or %) is a measure of total dissolved solids in solution; pol (in g solute per 100 g solution or %) is an estimate of sucrose content in cane juice; apparent purity (hereafter referred to as purity) was determined from the ratio

of pol to brix (Bureau of Sugar Experiment Stations, 1984a). Brix was measured using an automatic refractometer (Index Instruments GPR 11-37) with units expressed as degrees (°) brix, which is the same as % brix. Pol was estimated using a Schmidt and Haensch NIR W2 polarimeter using the dry lead acetate method with undiluted solutions. The exact procedures used in this work for the measurement of brix and pol are described by the Queensland Sugar Corporation (1999) and Bureau of Sugar Experiment Stations (1984b) respectively. Laboratory conditions for the measurement of brix and pol % were maintained at 20°C. Cane juice was extracted from cane stalk samples using a fixed two-roll, small mill (manufacturer unknown).

The number of emerged shoots was counted in a 2 m section of plot for a subset of varieties; Q96, Q117, Q120, Q127, Q135, Q138, Q152 and Q158 on 12 September 2000 to determine the effect of the ripener treatments on shoot emergence in the following ratoon crop. The number of stalks in a 2 m section of plot in the same subset of varieties was also counted on the 6 March 2001

3.2.3 Weather conditions

Temperature, solar radiation and rainfall were measured at a weather station about 500 m from the experimental site (Figure 3.1). The 2001 season was drier and less cloudy during April and May compared with 2000 with the plots receiving 20 % more solar radiation in 2001. Daily minimum temperatures in May 2001 were 3.6°C cooler on average than in 2000. Maximum temperatures for both years were similar.



(b)





Figure 3.1: Weather data for 2000 (——) and 2001 (-----). (a) Solar radiation; (b) minimum temperatures; (c) cumulative rainfall and; (d) maximum temperatures. Ethrel[®] treatment applied on Julian Day (JD) 68 for both years. Other ripener treatments applied on JD 98 in 2000 and JD 100 in 2001.

3.2.4 Data analysis

Data was analysed using the PROC GLM procedure in the SAS statistical package (SAS ver. 8.00, SAS Institute Inc. Cary, NC 27513). The statistical model used for analysing each trait followed that of a split plot in space and time (Steel and Torrie 1980). The following linear additive model was used for the statistical analysis of each trait for each of the ripener treatments combined with the control treatment:

 $Yijkmn = \mu + bk + ti + (bt)ik + gj + (tg)ij + (gd)ijk + cm + dn + (cd)mn + (cd)mnk$ + (tc)im + (tc)in + (tcd)imn + (cd)mnki + (gc)jm + (gd)jn + (gdc)jnm + (gtc)jim + (gtdc)jinm + eijkmn

Where:

Y = observed yield (ie. brix, pol, purity, etc) of the ith treatment for the jth variety in the kth block in the mth year for the nth sampling date

 μ = the overall mean

bk = effect of kth block, k=1...5

ti = effect of the ith ripener treatment, i=1...2

(bt)ik = effect of the kth block within the ith treatment (error one)

gj = effect of the jth variety, j=1...43

(tg)ij = interaction effect between treatment and variety,

(gb)ijk = interaction effect between the jth variety and the ith treatment within the kth block (error 2)

cm = effect of the mth crop-year, m=1...2

dn = effect of the nth sampling-date, n=1...3 for E+F treatment, n=1...2 for glyphosate treatment

(cd)mn = interaction effect between the mth year and the nth sampling-date

(cd)mnk = interaction effect between the mth year and the nth sampling date within the kth block (error 3)

(tc)im = interaction effect between ith treatment and the mth year

(td)in = interaction effect between ith treatment and the nth sampling-date

(tcd)imn = interaction effect between ith treatment, the mth year and the nth samplingdate

(cd)mnki = interaction effect between the mth year and the nth sampling date within the kth block and ith treatment (error 4)

(gc)jm = interaction effect between jth variety and mth year

(gd)jn = interaction effect between jth variety and nth sampling date

(gdc)jnm = interaction effect between jth variety, the nth sampling date and the mth year

(gtc)jim = interaction effect between jth variety, the ith treatment and the mth year

(gtd)jin = interaction effect between jth variety, the ith treatment and the nth sampling date

(gtdc)jinm = interaction effect between jth variety, the ith treatment, the nth sampling

```
date and the mth year
eijkmn = random subplot error (error 5)
```

Formulae for the calculation of standard errors between means were derived from Steel and Torrie (1980) with modification of the divisor to account for time factors (i.e. sampling date, year) where appropriate.

3.3 Results

3.3.1 Seasonal differences between 2000 and 2001

Seasonal conditions for ripening in 2001 were better than in 2000 with the pol and purity in the control treatment both being higher in 2001 than in 2000 at all times of measurement (Table 3.4).

Table 3.4: Mean pol (%) and purity (%) across all varieties in the control treatment in 2000 and 2001.

Attribute	Year			Month		
		March	April	May	June	—
Pol (%)	2000	5.72	9.56	13.54	16.70	
	2001	8.67	11.75	17.05	19.84	
	SE	1.59	1.38	2.48	2.09	
Purity (%)	2000	52.71	69.53	81.12	87.72	
	2001	65.31	72.77	85.35	91.53	
	SE	4.83	5.38	4.15	1.58	

3.3.2 Main effects of ripeners

There were significant ($P \le 0.01$) overall effects on brix, pol and purity from the E+ F combination treatment and glyphosate treatment (Table 3.5). No significant effects of Fusilade[®] alone or the Verdict[®] treatment were observed. Further discussion therefore focuses on the results from the other treatments.

Table 3.5: Analyses for significance for various ripener treatment (R), variety (V), date (D) and year (Y) interactions. Analysis is based on data collected for the E+F and glyphosate treatments during the months of May and June for the years 2000 and 2001. May = treatment harvest interval (THI) of 4 weeks, June = THI of 8 weeks.

	Leve	el of Sig	gnificar	nce ¹				
	Ethr	el®+ Fu	silade®)	Glyph	osate		
Source of	d.f.	Brix	Pol	Purity	d.f.	Brix	Pol	Purity
Variation			(%)	(%)			(%)	(%)
Main Plots								
Ripener								
Treatment (R)	1	**	**	**	1	**	**	**
R x Year (Y)	1	ns	ns	ns	1	**	**	**
R x Date (D)	1	*	*	*	1	ns	ns	ns
R x Y x D	1	ns	ns	ns	1	ns	ns	ns
Sub Plots								
Variety (V)	41	**	**	**	42	**	**	**
V x R	41	**	**	**	42	ns	ns	ns
V x R x Y	41	*	ns†	*	42	*	**	*
V x R x D	41	ns	ns	ns	42	ns	ns	ns
V x R x D x Y	41	ns	ns	ns	42	ns	ns	ns

¹ ns = not significant, $* = P \le 0.05$, $** = P \le 0.01$, + P = 0.0515

3.3.3 Main effects of Ethrel[®] + Fusilade[®]

Averaged across all varieties and both years, the application of Ethrel[®] increased brix, pol and purity four weeks after the application (Figure 3.2). The difference between the ripener treatment and the control treatment increased further following the application of Fusilade[®] in April. By early May, the response increases relative to the control treatment were 1.12 % units for brix, 1.46 % units for pol and 2.78 % units for purity.




Figure 3. 2: Effects of combined Ethrel[®] and Fusilade[®] treatment ($-\infty$) relative to the control plots ($-\bullet$) averaged across years (2000 and 2001) for (a) brix, (b) pol and (c) purity. Capped vertical lines represent l.s.d. at *P*=0.05 for treatment comparisons within each month.

There was no significant treatment x year interaction found in an analysis of the E + F and control treatment plots for brix, pol or purity, despite the different weather and crop maturity from March to May between years. An analysis of the data for the months of May and June across years showed that significant treatment x date interaction ($P \le 0.05$) occurred for brix, pol and purity occurred (Table 3.5). The mean pol % response for all varieties in May was 1.47 units (l.s.d. (P=0.05) = 0.30 pol %) compared to 0.95 units for June. It is not known whether the increased response to the Ethrel[®] and Fusilade[®] treatment in May compared with June was due to the Ethrel[®] effects alone or the result of an additional effect of Fusilade[®] following its application.

3.3.4 Main effects of glyphosate

Averaged across years and varieties for May and June, the mean brix, pol and purity of the plots treated with glyphosate were 0.83, 0.93 and 1.14 % units higher respectively than the control plots. There was no ripener treatment x date interaction identified from an analysis of the glyphosate and control treatment plots for the months of May and June across both years, indicating similar responses at four-weeks and eight weeks after glyphosate

application. Unlike the E + F treatment, there was a significant ripener x year effect (Table 3.5). Averaged across all varieties, pol in the glyphosate treated plots was 1.37 % units higher than in the control plots in 2000 but in 2001 the gain in pol was only 0.52 % units (Figure 3.3).





Figure 3.3: Effects of glyphosate application (dotted columns) in 2000 and 2001 on (a) brix, (b) pol and (c) purity, averaged across all varieties and across the May and June samplings relative to the control (blank columns). Capped vertical lines represent l.s.d. at P=0.05 for treatment comparisons within each year.

3.3.5 Variation among varieties for response to ripeners

The analysis of variance results in Table 3.5 showed significant ($P \le 0.01$) differences among varieties for pol overall. The average control treatment pol % for May and June in 2000 and 2001 ranged from 14.63 for Q113 to 19.12 for Q176^A.

There were significant ($P \le < 0.05$) increases in pol for 31 of the 42 varieties tested to the Ethrel[®] + Fusilade[®] treatment (Figure 3.4). Varieties that were very responsive to this treatment included Q117 (2.31 pol % increase) and Q127 (2.01 pol % increase), the two commercially most important varieties in the Burdekin region which both have high CCS early in the harvesting season.



Figure 3.4: Pol % for varieties in control treatment and the Ethrel[®] + Fusilade[®] treatment, based on averages across May and June in 2000 and 2001. Numbers represent data points for varieties (Table 3.1). Capped vertical lines represent l.s.d. at P=0.05 for treatment comparisons between the variety data point and the corresponding point for the control given on the 1:1 ratio line (—).

No variety x ripener treatment interaction was observed from an analysis of the glyphosate and control treatment plots for May and June across years. However separate analysis for results from 2000 alone showed a significant ($P \le 0.05$) ripener x variety interaction. Twenty-five varieties showed significant pol increases to glyphosate in 2000 (Figure 3.5). No significant ripener x variety interaction for glyphosate occurred for the 42 varieties tested in 2001. No correlation was apparent between ripener response to E+F and the pol level for the untreated plots (Figure 3.4), and similarly with respect to glyphosate (Figure 3.5). This clearly suggests that in general, high pol varieties can respond equally as well as low pol varieties to these ripener treatments.



Figure 3.5: Pol % for glyphosate treated varieties averaged for May and June 2000. Numbers represent data points for varieties (Table 3.1). Solid line represents the 1:1 ratio. Capped vertical lines represent l.s.d. at P=0.05.



Figure 3.6: Varietal pol % responses for E+F treated varieties averaged for May and June in 2000 compared to those for glyphosate. Numbers represent data points for varieties (Table 3.1). Capped vertical and horizontal lines represent l.s.d. at P=0.05.

No relationship was evident between the response of varieties to E+F for pol and responses to glyphosate in 2000 (Figure 3.6). This suggests that the initial modes of action of the ripeners are not the same.

No significant interaction effects for variety x ripener x sampling date and variety x ripener x sampling date x year were observed for either E+F or glyphosate (Table 3.5).

3.3.6 Variation between years for relative response of varieties to ripeners

There was significant treatment x variety x year effects for pol % from an analysis of the E + F and control plots ($P \le 0.05$) and the glyphosate and control plots ($P \le 0.01$) (Table 3.5). The varieties Q162 and Q124 showed contrasting responses to the E+F treatment across seasons with significantly better ($P \le 0.05$) pol % responses in the drier 2001 season (Figure 3.7). However, most varieties performed reasonably consistently in response to the E+F treatment across years, particularly Q120, Q117, Q166^A, Q180^A, Q181^A, Q186^A and Tellus^A. The variety Q136 gave consistently poor pol % responses across years that were close to zero or negative whilst Q117 gave consistently good responses that were greater than 2.0 pol %.



Figure 3.7: Pol % responses of varieties to E + F treatment averaged across May and June in 2000 and in May and June 2001. Numbers represent data points for varieties (Table 3.1).

Most varieties responded less to glyphosate in 2001 compared with 2000 (Figure 3.8). While varieties Q138, Q162, Q163^A and Q186^A responded similarly across years to glyphosate, there were 13 varieties for which responses were significantly less ($P \le 0.05$) in the 2001 season, which was characterised by seasonal conditions favouring natural ripening. The varieties Q96, Q141, Q166^A, Q180^A and Tellus^A all showed significantly reduced responses ($P \le 0.01$) of more then 2.0 pol % in the 2001 season. The variety Q163^A gave consistently low responses of 0.3 pol % across years whilst Q135 gave consistently high pol % responses of 2.81 units in 2000 and 1.68 units in 2001.



Figure 3.8: Average varietal pol % responses to glyphosate treatment for May and June in 2000 compared to the responses seen in 2001. Numbers represent data points for varieties (Table 3.1).

3.3.7 The residual effect of glyphosate on the following ratoon crop

Counts of shoot emergence on the 12 September 2000 and stalk density on the 6 March 2001 found on average, ripener treatments did not significantly affect shoot or stalk numbers. However, there was a significant ripener treatment x variety interaction ($P \le 0.05$) for an analysis of glyphosate and control treatment plots, indicating that the extent of the carry-over effects on the following ratoon crop was variety dependent (Figure 3.9). Glyphosate applied in the plant crop reduced the mean shoot/stalk numbers for the variety Q152 in the ratoon crop, but had no significant impact on Q158, Q135 or Q96. Stalk numbers were less for the other varieties in the ratoon crop following application of glyphosate but large variations with the stalk count data across replicates meant this experiment lacked power to show statistically significant differences for moderate differences in stalk numbers.

Stalk count data also showed a significant variety by treatment by date interaction ($P \le 0.05$) indicating that the effect of glyphosate on the rate of ratooning of different varieties was not consistent across time. It was decided to monitor the residual effects from the 2000 glyphosate treatment in the 2001 season. Plots treated with glyphosate the previous year were sampled in March, April, May and June 2001. The mean pol for all varieties (Q113 was omitted since it failed to emerge in some of the ratoon plots) in plots previously treated with glyphosate was significantly reduced ($P \le 0.05$) to 13.93 % compared to 14.34 pol % in the control treatment. A highly significant ($P \le 0.01$) variety x ripener treatment interaction for brix, pol and purity indicated that some varieties were more adversely affected than others by glyphosate applied in the previous season (Table 3.6). Adverse carry-over effects on the pol of varieties like Mida^A and Tellus^A were detected whilst similar effects were negligible for varieties like O141 and O195^A (Figure 3.10). This suggests it may be possible to select varieties that can tolerate annual applications of glyphosate, provided that the effects on population and pol are consistent within varieties. The variety x ripener treatment x date interactions for brix and pol were non-significant but significant ($P \le 0.05$) for purity, suggesting that the carry-over effects on purity in some varieties varies with time.



Figure 3. 9: Mean shoot and stalk numbers for varieties in the glyphosate (dotted columns) and control (blank columns) treatment. Counts were done for a two-metre section of each plot in the first ration crop on the 12 Sep. 2000 and 6 March 2001 for a subset of 7 varieties (Q96, Q117, Q120, Q127, Q135, Q152 and Q158). Capped vertical lines represent l.s.d. at P=0.05 for treatment comparisons within each variety.

Table 3.6: Analyses for significance for ripener treatment (R), variety (V) and date (D) interactions related to the carry-over effects of glyphosate on the brix, pol and purity of varieties in the following season. Analysis is based on data collected for the 2000 glyphosate treatment during the months of March, April, May and June for 2001.

	Level of s	ignificance ¹				
	Glyphosate carry-over effect in 2001					
Source of Variation	d.f.	Brix	Pol	Purity		
			(%)	(%)		
Main Plots						
Ripener Treatment (R)	1	ns	*	**		
R x Date (D)	3	ns	ns	ns		
Sub Plots						
Variety (V)	41	**	**	**		
VxR	41	**	**	**		
V x R x D	123	ns	ns	*		

¹ $\overline{\text{ns} = \text{not significant}}, * = P \le 0.05, ** = P \le 0.01$



Figure 3.10: The effect of glyphosate (dotted columns) applied in 2000 on the mean pol for samplings in March, April, May and June 2001 compared to the control treatment (blank columns). A highly significant ($P \le 0.01$) variety by treatment interaction was identified and some contrasting varietal responses are shown. Capped vertical lines represent l.s.d. at P=0.05

3.4 Discussion

The purpose of the research was to evaluate the responses of varieties to ripeners across seasons and to examine the interactions between ripener, variety and season. Although the literature highlights large variations in ripening responses across environments, this study was deliberately designed to test varieties in environmental conditions and physiological states that maximize the likelihood that the responses observed were due mainly to genotypic differences. For this reason, multiple sites or environments were not considered necessary. The study also focused mostly on the responses observed in the cane juice and no attempt was made to measure concurrent effects on cane yield. The starting hypothesis that some Australian sugar cane varieties are responsive to ripeners was clearly supported by the results for two of the four ripener treatments tested. The results showed that either E+F or glyphosate have potential to improve the quality of the cane juice in cane destined for harvesting in May or June in many Australian sugar cane varieties. Variety, the type of ripener, season and treatment-harvest interval were all found to have affected, either individually or in interaction with each other, the responses seen for brix, pol and purity. In

most instances, the responses would generate financially attractive returns to Australian growers, provided there were not large negative impacts on cane yield. The responses suggest a potential commercial role for these ripeners in north Queensland and support further research using any of a wide range of varieties to investigate the repeatability of the effects on CCS, and effects on cane yield, across a range of environments.

The E+F treatment provided the most consistent responses in across years, but some varieties responded better than others (Figure 3.4). Averaged across both years, 31 of the 42 sugar cane varieties tested showed responses in brix, pol and purity that were significant ($P \le 0.05$) and potentially economically important. The five most responsive varieties to this treatment showed a mean pol increase for May and June across years of 2.04 % and included two major Burdekin varieties, Q117 and Q127 (Table 3.7). Both these varieties already have high CCS and are suited to early harvest. Table 3.6 also shows that the mean cost benefit ratio for these five varieties to this ripener treatment was around 1:5.0, indicating substantial opportunities are available for growers to increase the profitability of some early harvested crops. These estimates assume that ripeners have no significant, adverse effect on cane yield, a reasonable assumption that is supported by South African findings providing the treatment-harvest is not excessively delayed.

The optimum treatment harvest interval for all varieties to E+F was about 4 weeks after the application of the Fusilade[®] in April. The mean pol response in all varieties decreased if harvesting was delayed to 8 weeks after application of Fusilade[®] (Figure 3.2(b)). Whilst most varieties responded consistently to this ripener treatment, some did not. Q124 and Q162 recorded significantly better ($P \le 0.05$) responses in 2001 compared to 2000 (Figure 3.7). The reasons for the poor responses of these varieties in 2000 may be related to the susceptibility of these varieties to orange rust disease (*Puccinia kuehnii*) and the high incidence of the disease in 2000 when environmental conditions favoured this pathogen. Q124 is highly susceptible to orange rust disease whilst Q162 is moderately susceptible. Diseases have been reported to adversely affect ripener responses (Martin 1980; Soopramanien *et al.* 1984).

Glyphosate gave similar overall responses in pol as the E+F treatment in 2000, but again some varieties responded better then others. The results showed that across years however, the mean overall response to glyphosate varied significantly (Figure 3.8), in contrast to the more consistent overall responses to the E+F treatment (Figure 3.7). The overall glyphosate responses were smaller in 2001 when the conditions better favoured natural ripening. The responses of 13 varieties in 2001 were significantly less ($P \le 0.05$) compared to those seen for the same varieties the previous year. This suggests the usefulness of this ripener may be limited to some varieties in some years. The overall mean pol response for the five most responsive varieties in May and June across years was 1.79 % below that for the E+F treatment (Table 3.7), but potentially still financially attractive (depending on cane yield effects) to growers given that the application costs are nearly half that for the E+F treatment. Unlike the E+F treatment, the mean responses of all varieties to glyphosate at 4 and 8 weeks after application were similar with no significant interaction between ripener treatment and date (Table 3.5). This suggests an optimum treatment harvest interval does not generally apply for glyphosate if the crop is to be harvested at either 4 or 8 weeks following application in early April. It is possible that an optimum treatment harvest interval exists between these two dates but we were unable to ascertain this.

Treatment	Variety	Estimated	Pol (%)	CCS^1	Net profit ²	Cost: Benefit
		Cost/ha (\$A)			(A\$/ha)	ratio
E + F	Q117	\$100	+2.31	+2.12	572	1:5.7
دد	Q173 ^A	دد	+2.08	+1.92	518	1:5.2
دد	Q127	دد	+2.01	+1.81	489	1:4.9
٠٠	Q135	دد	+1.93	+1.79	483	1:4.8
"	Q195 ^A	دد	+1.88	+1.68	453	1:4.5
"	All varieties	**	+1.21	+1.12	302	1:3.0
Glyphosate	Q135	\$40	+2.25	+2.08	561	1:14
"	Tellus ^A	دد	+1.91	+1.65	446	1:11
"	Q117	دد	+1.72	+1.53	413	1:10
دد	Q113	دد	+1.57	+1.37	370	1.9.2
دد	CP74-2005	دد	+1.52	+1.34	362	1:9.0
	All varieties	٠٠	+0.96	+0.84	227	1:5.7

Table 3. 7: Estimated cost: benefit ratios found for the top 5 responsive varieties in 2000 and 2001 to ripener treatments.

¹ Commercial cane sugar, assuming a fibre value of 11%

² Assumes sugar price to grower = A\$250 tonne, harvesting costs = \$6.00 tonne, assorted levies = \$1.10 tonne, crop yield is unaffected at 120 tonnes per hectare and the CCS of untreated cane is 12 units.

This study found glyphosate had an adverse carry-over effect on the pol and purity of varieties in the 2001 ration crop following treatment in the 2000 plant crop. Negative impacts on the stalk weights and populations of some varieties following the application of

glyphosate in the previous ratoon have also been reported in the USA (Millhollon and Legendre 2000). However, unlike this study, they did not find any adverse carry-over effects on sugar quality, possibly because of the small number of cultivars examined. The USDA recommends that glyphosate be only used on crops that are not going to be ratooned (Gilbert *et al.* 2002). Our study also showed that the negative carry-over effect of glyphosate were variety specific, with some varieties not affected at all by the application of glyphosate in the previous ratoon crop. Other researchers have also reported that the carry-over effects of glyphosate were variety specific (Clowes and Inman-Bamber 1980; Millhollon and Legendre 2000). Glyphosate could therefore be potentially recommended for use on tolerant varieties in all crop classes or just on crops to be ploughed out in the case of varieties that showed negative carry-over effects.

The results found in this study on Australian sugar cane varieties are generally consistent with South African reports regarding E+F as being the best, consistent treatment (Donaldson 1994) and USA work on the inconsistency of the responses seen to glyphosate with season (Dusky et al. 1986; Legendre and Finger 1988). Research in Hawaii suggested that maximum responses to glyphosate occurred when minimum temperatures were above 15.5°C and 50 to 100 mm of rainfall occurred during the ripening period (How 1976). These conditions occurred in April and May 2000 but not for the same period in 2001. Our study indicates that decisions on the type of ripener to use could depend on the type of season likely to occur during the two to three months before harvest. Climate prediction data using cluster analysis of SOI data to predict the probability of rainfall in the March to May quarter leading up to harvest is available from "The Long Paddock" website (DPI/DNR 2000) and it would have been effective in predicting the best type of ripener to use in the Burdekin during 2000 and 2001 seasons. For an example, the probability of exceeding the median rainfall in the Burdekin region during the March to May quarter in 2000 was predicted at 60-80% whilst for the drier 2001 season, it was predicted to be around 50%. Whilst the SOI predicts rainfall probabilities, it is unlikely rainfall is the driving factor affecting natural ripening in the Burdekin in the March to May quarter since the crop is grown is grown under irrigation. More likely, it is the associated temperature with rainfall (or lack of) that determines the amount of natural ripening occurring. For an example, dry periods in the March to May period in the Burdekin are often associated with cooler temperatures, particularly at night. The converse is true for wetter seasons.

We were surprised not to find any significant increases in brix, pol or purity after Fusilade[®] treatment in 2000 or 2001 given Fusilade[®] was applied at the rate of 200 mL/ha as recommended by the South African Industry (South African Sugar Association 1998). However, significant responses were obtained in an adjacent experiment that assessed the responses of 20 varieties treated with Fusilade[®] in March 2000 at a higher rate of 300 mL/ha (McDonald *et al.* 2001). This finding suggests that the optimum rate of Fusilade[®] alone on most Australian varieties will be higher then 200 mL/ha.

There were three limitations with the research reported in this chapter, which should be addressed in future research. Firstly, the results reported are only for one location over two years. It is possible that the responses recorded would not be repeated or be of different magnitudes under different environmental conditions. However, under conditions considered to be favourable for chemical ripener responses we were able to show that most Australian varieties have a potential to respond to ripener application. Important commercial varieties identified in this study as showing the biggest responses to ripeners would be candidates for future research to evaluate the repeatability of responses across different environments. Secondly, no measurements were made on the concurrent effects of these ripeners on cane yield. This must be investigated in further work before recommendations for commercial use could be considered. Overseas work however suggests that adverse effects on cane yield are negligible and offset by the overall gains in sugar yield in tonnes per hectare providing harvesting occurs within the optimum treatmentharvest interval. Thirdly, this work was unable to discern between the responses to either Ethrel[®] or Fusilade[®] in the E+F treatment. Future work should include an Ethrel[®] alone treatment in addition to Fusilade[®] alone so the individual contributions of each ripener could be ascertained.

Chapter 4

Physiological traits associated with ripening

4.1 Introduction

Most previous work with chemical ripeners in sugar cane has not examined physiological traits that could be useful predicting crop types that are likely to give a response. This is surprising given the prevalence in the literature of marginal and inconsistent responses associated with use of chemical ripeners. Studies in Australia in the 1970's with glyphosate and Ethrel[®] encountered such problems (Kingston *et al.* 1978). Consequently, the adoption of this technology in Australia has been relatively small compared to other countries where continued research has identified crop factors associated with chemical ripening responses.

Extensive South African research since the 1970's identified a number of crop conditions that adversely affect ripening responses (Donaldson 1996b). Factors such as crop age, disease, and lodging have all been shown to affect responses. Other reports have also shown linkages between purity at application and response (Rostron 1973, Kingston 1988). Green leaf numbers at application have also been mentioned as another factor (South African Sugar Association 2002a, James 1999) but no quantitative data has been found in the literature to substantiate this claim.

The aim of this research presented in this chapter is to identify trait differences among varieties that could predict their relative response to glyphosate.

4.2 Method

4.2.1 Experimental design

The study described in this section was conducted between April and June 2001. The same experimental trial was used as described in the previous chapter for 2001. Six varieties were chosen, comprising three responsive and three non-responsive, selected on the basis of data obtained in the 2000 plant crop to glyphosate. The three responsive varieties were Q113, Q135 and TellusA. The non-responsive varieties were Q167A, Q179A and Q186A. Common parentages were shared by Q113 and Q135 and also by Q167A and Q179A (Table 3.1).

4.2.2 Measurements

Samples consisting of four stalks were taken from each plot of the control and glyphosate treatments on the 2nd April 2001, just prior to the application of glyphosate. These, and later samples, were in addition to those described in the previous chapter. Total dead leaf and green leaf node numbers were counted for each stalk and an average count for four stalks was obtained for each sample. Green leaf numbers were obtained by counting down the stem from the spindle leaf (spindle leaf = leaf number 1; Clements and Ghotb 1969) to the last visible green leaf. Dead leaf numbers were obtained by counting the total remaining node numbers down to where the stem was cut at ground level. The leaf node ratio (LNR) was calculated by dividing the green leaf node number by the dead leaf node number. Cane stalks were then topped at the natural breaking point and then measured and an average length for four stalks estimated. Each sample was then separated into bottom, middle and top stem sections. The top and bottom stem sections consisted of 50 cm of cane taken from each respective end. The middle stem section consisted of all stem material in between the bottom and top stem sections. Each stem section sample was then measured for brix, pol, fibre, dry matter and total weight. Data collected from the stem partition samples was used to calculated weighted averages for whole stalk values. Dry matter was measured by taking a 200 g sub-sample and drying it in an 850 mL aluminium tray in a forced draft oven for one week at 60°C. The same sampling procedure was applied again to the same plots in the trial when the treatment harvest interval (THI) was four (10 May 2001) and eight (7 June 2001) weeks after application of the glyphosate.

The estimation of commercially extractable sucrose in cane juice involves measurement of brix and pol is described in the previous chapter. Both were measured on a % juice basis. Cane juice was obtained from cane stalk samples by processing the stem sections through a

Jeffco cutter grinder (Model 265B, Jeffress Engineering Pty. Ltd. <u>http://www.jeffress.com.au/</u>). Cane juice was then extracted from a 500 g fibrated sub-sample, which was squeezed in a Carver press (Model M, Carver Inc. <u>www.carverpress.com</u>) at 15.7 MPa for sixty seconds. The remaining pressed fibre or "biscuit" was then weighed, oven-dried at 60°C for one week and reweighed. The values, along with the recorded brix, were used to estimate the percentage fibre according to the formulae:

 $((100 \times DryBiscuitWt) - (WetBiscuitWt \times Brix)) \div ((FibreWtBefore Press \div 100) \times (100 - Brix))$

The pol/DM ratio was calculated by dividing pol (% juice) by the dry matter %.

Stem elongation data measured in-situ in 2000 was collected by tagging two stalks in each plot for all the treatments for 3 of the 5 replicates. Stalk length was then measured on the 23 March 2000, 7 April 2000 and 4 May 2000 to give a before and after application estimate of stalk elongation for each variety.

4.2.3 Data analysis

Data was analysed using the PROC GLM procedure in the SAS statistical package (SAS ver. 8.00, SAS Institute Inc. Cary, NC 27513). The statistical model used for analysing each trait followed that of a split plot in space and time (Steel and Torrie 1980).

Formulae for the calculation of standard errors between means were derived from Steel and Torrie (1980 Table 16.2).

4.3 Results

4.3.1 The selection of ripener type

Both glyphosate and the E+F treatment had significant variety by treatment effects for pol in 2000 (c.f. chapter 3). In addition, varieties tested in 2000 showed a greater range of responses to glyphosate compared to the E+F treatment (Figures 3.7 and 3.8). The mean pol response for all varieties in May and June to glyphosate in 2000 was also slightly higher at 1.39 % units compared to 1.17 % units for E+F treatment. On the basis of this information, it was decided to use glyphosate in 2001 as a ripener to test two varietal populations that had contrasting responses to this ripener in 2000.

4.3.2 The selection of responsive and non-responsive varieties from small mill data obtained in 2000.

The responses for pol of the three varieties chosen to represent the responsive group, (shown in red) and the three varieties chosen represent the non-responsive group (shown in blue) to glyphosate in 2000 are indicated in Figure 4.1. The responsive varieties included Q113 (8), Q135 (16) and Tellus^A (43) whilst the non-responsive varieties included Q167^A(27), Q179^A(34) and Q186^A(38).



Figure 4.1: Pol % for glyphosate treated varieties averaged for May and June 2000 showing the selected responsive varieties in red and the non-responsive varieties in blue. Numbers represent data points for glyphosate treated varieties (Table 3.1). The solid line is the 1:1 ratio; Capped vertical line represents l.s.d. at P=0.05 for the varietal response to glyphosate.

4.3.3 Small-mill, whole stalk, pol responses seen in 2001 compared to 2000

The pol responses to glyphosate treatment in the cooler 2001 season were not as great as they were the previous year. Each group ranked as expected based on the 2000 data, but the only varieties in each of the response-type groups that differed significantly were Q167^A and Q135 (Figure 4.2).



Figure 4.2: A comparison between years of the small mill pol responses for whole stalk samples of the selected responsive (red) and non-responsive (blue) varieties. Capped vertical line represents l.s.d. at P=0.05 for treatment responses between varieties in 2001. Capped horizontal line represents l.s.d. at P=0.05 for treatment responses between varieties in 2000.

4.3.4 A comparison of the small mill and carver press data obtained in 2001

The small mill method was used to measure pol in the varieties tested for responsiveness to glyphosate in 2000. This method is fast and economical compared to the Carver press method so it was used to process the large number of samples involved (1150 per sampling occasion) in 2000. The six responsive and non-responsive varieties were then identified and tested in 2001 using the slower, more resource intensive Carver press method that provides fibre and dry matter estimates in addition to pol. However, it was necessary to confirm that the two techniques used had not confounded the data by significantly altering the relative pol of the varieties measured.

An analysis of variance for values collected in April, May and June showed that pol estimates using the small mill method were biased upwards compared to the Carver press method (Figure 4.3). However, no significant variety by process interaction occurred (Table 4.1). This means that the processes used had no significant effect on the relative rankings of the varieties tested for pol and it suggests the small mill method used in 2000 was an appropriate process for screening varieties for further testing in 2001 using the carver press method.



Figure 4.3: Process comparison of hydraulic press (closed symbol) vs. small mill (open symbol) of the average whole stalk pol % for all varieties in the control and glyphosate-treated plots for April, May and June. Capped vertical lines represent l.s.d. at P=0.05 for process comparisons within each month.

Data discussed below (with the exception of section 4.3.10 - "Other traits and glyphosate response") is associated with the research associated with or done via the Carver press method.

4.3.5 Plant growth responses to glyphosate

An analysis of all six varieties showed glyphosate did not affect either the average, whole stalk fresh or dry weights compared to the untreated control. However, treatment differences were seen for stalk length and stalk fresh weight when the data was analysed according to the response type groups.

			Mean squares			
Treatment	Source df		April	May	June	
Control	Process	1	20.11	17.72*	41.48**	
"	Process (block)	8	4.40**	2.16*	1.77	
"	Variety	5	18.75**	12.05**	5.92**	
"	Variety x Process	5	1.35	0.22	1.92	
"	Error 2	40	1.26	0.73	1.27	
Glyphosate	Process	1	18.11	3.72	18.24**	
"	Process (block)	8	3.88*	1.22	1.37*	
"	Variety	5	16.93**	15.4**	11.97**	
دد	Variety x Process	5	0.84	1.11	0.83	
"	Error 2	39 ¹	1.55	1.06	0.48	

Table 4.1: Mean square estimates for pol in April, May and June 2001. Process refers to

 either the small mill method or the Carver press method.

¹ 40 for the May sampling, $* = P \le 0.05$, $** = P \le 0.01$

4.3.5 (a) Stalk length differences between treatments

Average stalk length across all varieties in the glyphosate treated plots did not differ significantly from the untreated plots (Figure 4.4). Both treatments made around 30 cm of growth between April and May but stalk elongation ceased between May and June, probably as a result of lower temperatures experienced (Figure 3.1).



Figure 4.4: Stalk length differences between the untreated plots (closed symbol) and glyphosate treated plots (open symbol) for all varieties following application in April. Capped vertical lines represent l.s.d. at P=0.05 for treatment comparisons within each month.

4.3.5 (b) Effect of ripener application on stalk length

By June, glyphosate had greatly reduced (P=0.05) the stalk length in the responsive group of varieties compared to non-responsive varieties (Figure 4.5). The average difference in stalk length between the control and glyphosate treated plots for the non- responsive group was 22.4 cm greater then that for the responsive group.



Figure 4.5: Stalk length differences between the untreated and glyphosate treated plots for the non-responsive group (closed symbol) and responsive group (open symbol) following glyphosate application in April. Capped vertical lines represent l.s.d. at P=0.05 for treatment comparisons within each month.

4.3.5(c) Fresh and dry weight differences between treatments

Figures 4.6 and 4.7 show that growth rates for the glyphosate treated plots, as measured by stalk fresh weight and stalk dry weight respectively, did not differ significantly from the untreated plots. These Figures also show that stalk desiccation occurred between May and June in both the treated and untreated plots. Analysis not presented shows increases in stalk fresh weight for both treatments between April and May were significant but this was not the case for April and June. However, stalk dry matters for June were significantly higher compared to the April average.



Figure 4.6: Stalk fresh weight of the untreated plots (closed symbol) and glyphosate treated plots (open symbol) averaged across all varieties. Capped vertical lines represent l.s.d. at P=0.05 for treatment comparisons within each month.



Figure 4.7: Stalk dry weights of the untreated plots (closed symbol) and glyphosate treated plots (open symbol) averaged across all varieties. Capped vertical lines represent l.s.d. at P=0.05 for treatment comparisons within each month.

4.3.5 (d) Stalk fresh and dry weight differences between response-type groups

Glyphosate usually reduces growth so it was important to see, within the time frame examined, whether the two response type groups differed in terms of their fresh and dry weight responses to this treatment. Generally, no differences between the groups were seen for either fresh weight (Figure 4.8) or dry weight (Figure 4.9) suggesting glyphosate affected both groups similarly. However, there was one exception. Fresh weight responses in April were found to differ ($P \le 0.05$) between the two response type groups. The only explanation that can be given for this difference, since no treatment had been imposed at this point in time, is that errors were involved, either with the measurement or the sampling of the plots in April. Similar trends were seen for stalk dry weights although the differences in this instance were not significant.



Figure 4.8: Stalk fresh weight responses between the untreated and glyphosate treated plots (ie. response = glyphosate plot – control plot) for the non-responsive group (closed symbol) and responsive group (open symbol) following glyphosate application in April. Capped vertical lines represent l.s.d. at P=0.05 for treatment comparisons within each month.



Figure 4.9: Stalk dry weight responses between the untreated and glyphosate treated plots (ie. response = glyphosate plot – control plot) for the non-responsive group (closed symbol) and responsive group (open symbol) following glyphosate application in April. Capped vertical lines represent l.s.d. at P=0.05 for treatment comparisons within each month.

4.3.6 Whole stalk pol differences between treatments for different response-type groups

Differences between the response-type groups did not become apparent until June, eight weeks after the application of the glyphosate. Figures 4.10 and 4.11 show that glyphosate had significantly improved the pol for both the responsive and non-responsive groups at the four-week, treatment-harvest interval in May. Both groups also showed similar increases in pol at May of around 1.1 units relative the control treatment for each population. However, the sampling at the eight-week, treatment-harvest interval in June showed contrasting differences between the responses seen for pol in each of the response type groups. By June, the non-responsive group no longer showed a significant increase in pol for the glyphosate treated plots above the control. The responsive group however, now showed highly significant ($P \le 0.01$) gains of 2.10 % units pol for the glyphosate treated plots above the control.



Figure 4.10: Pol % changes with time for (a) the non-responsive group (Q167^A,Q179^A and Q186^A) and; (b) the responsive group (Q113, Q135 and Tellus^A) following the application of glyphosate in April. Glyphosate-treated plots (open symbol) are compared to untreated plots (closed symbol). Capped vertical lines represent l.s.d. at P=0.05 for treatment comparisons within each month.

Further analysis of the data now focuses on the data collected in June when contrasting response differences in pol occurred between the two groups.

4.3.7 Were the pol increases in June a sugar accumulation or desiccation effect?

Whole stalk responses in June for DM showed no significant differences between the two response type groups following glyphosate application. However, Table 4.2 below indicates that differences in the responses for pol/DM between the groups were highly significant. On average, the responsive group had a greater ($P \le 0.01$) whole stalk pol/DM ratio by 3.5 % units compared to 0.5 % units for the non-responsive group (Table 4.3). This suggests that if dry matter has remained relatively unaffected between treatments then no desiccation effect has occurred and pol has increased as a result of increased sucrose accumulation and not by desiccation.

Table 4.2: Mean squares for whole stalk responses between treatments for pol, dry matter(DM) and pol/DM in June 2001.

Source	Df	Mean Squares				
		Pol	DM	Pol/DM		
Block	4	3.47	5.57	0.0005		
Group	1	14.88**	7.29	0.0062**		
Variety(Group)	4	0.40	0.04	0.0005		
Error	19	1.80	3.00	0.0007		

 $* = P \le 0.05, ** = P \le 0.01$

4.3.8 The spatial contribution of different stem sections to the pol responses seen for each response-type group

The responsive group differed from the non-responsive group by the pol responses seen in the top and middle stem sections for the June sampling, eight weeks after glyphosate was applied in April (Figures 14.12a, b and c). Pol increases of 1.86 and 4.52 % units pol were observed in the mid and top stem sections respectively for the responsive group in June (Table 4.3). These increases were significant ($P \le 0.05$) when compared to the increases seen in the non-responsive group for the same sampling. No differences in pol responses were seen between the two response-type groups for the bottom stem section in April, May or June (Figures 14.12a, b and c). However, in June all the responsive varieties showed significantly higher pol in the top stem section in contrast to the smaller, non-significant responses seen for all the non-responsive varieties (Figure 4.13).

Table 4.3: Average responses¹ between the control and treated plots for stem partition data comparing traits between non-responsive group (NR) and the responsive (R) group varieties in June, eight weeks after glyphosate application.

Trait	Botto	m stem	Mide	lle stem	Top stem		Whole stalk	
	NR	R	NR	R	NR	R	NR	R
Pol (%)	0.49	0.57	0.43	1.86*	1.15	4.52**	0.06	2.04**
Fibre (%)	0.43	0.03	0.20	-0.21	0.62	0.24	0.32	-0.06
Purity (%)	0.39	-0.49	0.76	0.95	3.22	8.23	1.15	1.99
DM (%)	0.81	0.86	0.66	1.50	1.03	3.89*	0.79	1.80
Pol/DM (units)	0.000	0.002	0.002	0.030*	0.023	0.091**	0.005	0.035**

¹ Response = glyphosate treatment – control; * = $P \le 0.05$, ** = $P \le 0.01$

(a)





Figure 4.11: Partitioning data showing the pol % response (ie. response = glyphosate plot – control plot) for each section of the response type groups (closed symbols represent non-responsive varieties, open symbols represent responsive varieties). Capped vertical lines represent l.s.d. at P=0.05 for response group comparisons within each month. (a) Top stem section, (b) Middle stem section, and (c) bottom stem section.

(b)



Figure 4.12: Pol differences in the top stem section between the control (blank columns) and glyphosate treatments (dotted columns) for the individual varieties (3 left-hand side varieties = responsive group, 3 right-hand side varieties = non-responsive group). Capped vertical lines represent l.s.d. at P=0.05.

4.3.9 Traits associated with response-type groups at application time in April

The results in the previous section showed that the response-type groups differed significantly in response to glyphosate for pol in the mid and top stem sections in June. Plant measurements made in April were further examined in attempt to characterise differences between the two groups. The groups were found to differ in fibre and pol/DM in the bottom stem section (Table 4.4) and green leaf numbers (Table 4.5). Similar trends were seen for pol/DM and fibre in the middle stem section but pol/DM was the only trait for this stem section that differed significantly ($P \le 0.05$) between the two response type groups. No differences in purity or dry matter for any of the stem sections were found between the response-type groups.

Trait	Botto	Bottom stem		Middle stem		Top stem		Whole stalk	
	NR	R	NR	R	NR	R	NR	R	
Pol	13.75	12.80*	10.05	9.70	3.40	3.71	9.69	9.23	
Fibre	11.72	12.57**	10.64	10.88 ¹	8.29	8.51	10.44	10.80**	
Purity	78.5	77.0	68.2	67.3	35.9	37.7	64.5	63.5	
DM	26.81	26.86	23.23	23.32	15.78	16.10	19.91	20.71	
Pol/DM	0.511	0.474**	0.431	0.413*	0.209	0.226	0.408	0.389*	

Table 4.4: Least square means for stem partition data comparing traits between nonresponsive group (NR) and the responsive (R) group varieties in April at the time of glyphosate application.

¹ $P \le 0.06$, * = $P \le 0.05$, ** = $P \le 0.01$

Table 4.5: Node count data showing least square means between the non-responsive group (NR) and the responsive (R) group varieties in April at the time glyphosate.

Trait	LS means	l.s.d.	
	NR	R	<i>P</i> ≤0.01
Green leaf nodes	7.89	9.37	0.54
Leaf Node ratio	0.88	1.08	0.10
(green/dead)			

4.3.10 Other traits and glyphosate response

Small mill data from previous chapter was examined to see if pol at application time in April 2000 (Figure 4.14) and 2001 (Figure 4.15) showed any correlation with the responses seen later. No relationships were observed. Likewise, stalk elongation data collected before (Figure 4.16) and after (Figure 4.17) showed no relationship with the responses, although there is a suggestion (with one exception) that varieties which continued to elongate more than 10 cm after application, did not respond well. There is also a suggestion that varieties did not respond well if they made less then 20 cm growth in the period before application.



Figure 4.13: Small-mill data from 2000 comparing the initial pol at application time in April with the average pol response seen in May and June 2000. Each number represents a variety (Table 3.1).



Figure 4.14: Small-mill data from 2001 comparing the initial pol at application time in April with the average pol response seen in May and June 2001. Each number represents a variety.



Figure 4.15: Stalk elongations before the application of glyphosate (23 March – 7 April 2000) compared to the average pol response seen in May and June 2000. Each number represents a variety. Blue lines show non-responding varieties in the bottom left hand side corner with less than 20 cm growth before application of glyphosate.



Figure 4.16: Stalk elongations after the application of glyphosate (7 April – 4 May 2000) compared to the average pol response seen in May and June 2000. Each number represents a variety. Blue lines show non-responding varieties in the bottom right hand side corner with more than 10 cm growth after application of glyphosate.

4.4 Discussion

The objective of this work was to identify possible physiological traits that may help predict the potential of cane varieties be chemically ripened by glyphosate. We found that traits other than purity at application time may be better at predicting response of varieties to ripeners. These traits are associated with green and dead leaf node numbers and the pol, fibre and pol/DM levels in the bottom and middle stem sections.

It was found that on average varieties from the responsive group had 1.48 more green leaves compared to varieties from the less responsive group. The ratio of green to dead leaf nodes showed responsive varieties had a ratio greater then one at the time of application compared to unresponsive varieties, which were generally less than one. This measurement also has the advantage of probably gauging the physiological age or maturity of the crop compared to just a measurement of green leaves alone. Growers can easily measure either trait in the field when identifying blocks suitable for chemical ripening. Node counts done on samples of 20 stalks would give a good indication of the potential for a crop to be chemically ripened. The results from this work confirm recommendations (James 1999, South African Sugar Association 2002a) that eight or more green leaves are required to obtain a likely response and provide the first quantitative data supporting this recommendation.

Other traits that may be useful in predicting the ripening response of crops are more difficult to measure and require access to laboratory equipment. This study found that the fibre percentage and pol in the bottom stem section differed significantly between the two response-type groups at application time. Whilst both groups had similar dry matter contents (c. 26.8%) for the bottom sections, the less responsive varieties had on average 0.85 % less fibre ($P \le 0.01$)) and 0.95 % more pol ($P \le 0.05$) then the responsive varieties. Consequently, we found that the non-responsive varieties had average, significantly higher pol/DM ratios at the time of application, in the whole stalk ($P \le 0.05$) and bottom ($P \le 0.01$) and middle ($P \le 0.5$) stem sections, compared to the responsive group of varieties.

Cane varieties vary in the rates at which they acquire sucrose by fresh weight and fibre with time (Lingle and Irvine 1994). However, current Australian varieties seem to have a relatively narrow range of between 0.45- 0.55 g stalk sucrose/g DM when 12-month-old crops approach maturity between July and November. Mature crops of Q117 typically had relatively constant values of between 0.48 - 0.50 g stalk sucrose/g DM although large
variations in fresh weight sucrose and tonnes sucrose per hectare were associated with such crops (Muchow *et al.* 1997).

The pol/DM ratio along the stem could be a useful yardstick for the determination of cane maturity and its potential for chemical ripening. It is speculated that the more responsive crop types for crop ripening are those where pol/DM ratios in the bottom and middle stem sections have not yet attained that 0.45 - 0.55 g stalk sucrose/g DM ceiling or that maturation limit associated with the equilibrium that is eventually reached between competing sinks (sucrose storage and stem fibre). This hypothesis may also account for the finding that the ripening response appeared to be independent of whole-stalk pol at application time. It is also consistent with finding by Rostron *et al.* (1986) that the initial cane dry matters at application is also useful in possibly predicting the ripening response, giving a slightly better correlation then purity at application

The observations also suggest that shorter optimum treatment harvest intervals may apply to varieties of sugar cane that are relatively non-responsive to glyphosate. The pol responses seen for the non-responsive group were very similar to those for the highly responsive group at four weeks after application. We were not able to differentiate between the responses for the two groups until the eight-week, post-treatment sampling. The pooled data in the previous chapter did not identify a ripener by variety by sampling-date interaction with glyphosate. Although the responses seen in for both response type groups was not relatively large, it still may be commercially important and suggests that even non-responsive varieties may lend themselves to chemically ripening if the treatment-harvest interval is managed correctly.

Chapter 5

General Discussion

5.1 Summary of results

This work established for the first time, that most modern early-harvested Australian sugar varieties are responsive to either glyphosate or a combination treatment of Ethrel[®] and Fusilade[®]. About three quarters of the varieties tested to the combination treatment of Ethrel[®] and Fusilade[®] across two years showed statistically significant and potentially commercially important gains in sucrose content. Assuming no adverse impacts on cane yields, the cost benefit ratios of using this combination treatment ranged between 1:3 (average for all varieties) to 1:5.7 for Q117, a major variety in the Burdekin, which is similar to the return given for Swaziland of 1:5.5 (Table 2.9).

The observations were a little different for glyphosate. While there was an overall effect of glyphosate on pol, no statistically significant genetic variation in responses was found to ripeners when the data was pooled for the two years study. However, genetic variation was found to exist in the 2000 data for pol when relatively large responses were obtained. Large variations in responses were also seen between years for this ripener. Although the main treatment effects for pol seen in both years were significant, it was greatly reduced in 2001 when seasonal conditions favoured natural ripening. This contrasted with the responses seen for the E+F treatment that were a lot more consistent across years. However, the glyphosate did give more attractive cost/benefit ratios. These ranged from 1:5.7, the average for all varieties, to 1:14 for the best responding variety Q135. The cost/benefit ratio of 1:14 for Q135 compares similarly to the bottom end of returns given for Guyana (Table 2.9). The better cost/benefit ratios for glyphosate in relation to the E+F treatments are a reflection of the lower costs associated with glyphosate, as it requires only a single aerial application. Whilst the cost/benefit ratio for glyphosate may be more attractive, growers would also need to consider the risk that glyphosate could affect cane yield and quality in the following ratoon, depending on the variety being treated. Consequently, the industry might initially adopt a similar recommendation to the American industry and recommend that it only applied to plough-out crops that are not being ratooned. Although some ripener treatments like Fusilade[®] alone or Verdict[®] did not elicit the response expected, it is possible that the rates used in this work were insufficient.

The second hypothesis in this study that "the prediction of response in responsive varieties can be improved by measuring both purity and growth rate at the time of ripener application" was not found to be generally true for glyphosate. Other traits such as pol/DM and green leaf numbers at application time could be better predictors of crops that are likely to respond. The use of pol/DM to measure maturity in cane is particularly promising, especially in relation to the bottom stem section of early harvested crops. Since sucrose is progressively loaded into the bottom stem internodes, it makes sense to measure how much loading has occurred in the area of this active sink if one is to obtain an estimate of the capacity remaining for a ripener to add further increases in sucrose. The importance of green leaf numbers at application time also makes sense in terms of a crop being able to respond to a ripener. It is speculated that crops with fewer leaves are least likely to respond because less photosynthate is being produced for storage. Fewer green leaves also means less ripener can be absorbed by the cane at application time.

5.2 Practical implications

The opportunities identified in this work suggests chemical ripeners will play an important role in the Australian industry as it restructures itself to improve it's competitiveness with countries like Brazil. In order to become more competitive, the industry will continue the trend towards earlier harvesting as Australian growers and millers both seek to improve their return on capital. The main hurdle to early season harvesting at present is the low sucrose content of cane crops before June. This hurdle can bypassed with the use of ripeners but, in the meantime, the Australian industry needs to convince the agrochemical companies who own these compounds to have them registered for commercial use. These companies have been somewhat tardy in their enthusiasm to have these compounds registered as ripeners in cane, as evidenced by the fact that only the ripener Ethrel[®] is currently available for commercial use in Australia. Some of the reasons given for the lack of enthusiasm to have additional ripeners registered are valid but others are not. Agrochemical companies are justifiably concerned that any compound registered for commercial use should be accompanied by creditable advice for the consumer on what circumstances are required in a crop to elicit a response. For example, the recommendations that accompany Ethrel[®] recommend that it only applied to crop below a certain level of purity. However, there are no available guidelines for agrochemical companies to provide growers with on the use of other types of ripeners like glyphosate. In addition to identifying responsive varieties, this work has shown that there are other traits like pol/DM in the bottom stem section and green leaf numbers that may be more useful

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predictors on the relative responsiveness of a crop. The use of the observations from this study should assist agrochemical companies with this area of concern. The other issues agrochemical companies have are in the environmental area but these seem to reflect an ignorance of how this technology is applied (ie. Relatively low rates combined with the ability to selectively apply to only areas were risks are minimal – unlike herbicide applications). The use of ripener technology in countries like mainland U.S.A. and Hawaii has not been thwarted by environmental concerns expressed by some of the agrochemical representatives here in Australia. However, it would also be fair to say that product registration by Australian agribusiness today is a far more difficult process compared to that experienced 20-30 years ago when many ripener products were released overseas and environmental standards and litigation issues were of less concern.

5.3 Future research

Future work should now investigate the environmental and management factors that affect the ripening traits in the responsive cultivars identified in this study. It should investigate the effects of these ripeners on the cane yields of these varieties so a quantitative estimate of the changes in sugar yield can be made. There is also a need to identify varietal tolerances to annual applications of glyphosate so this ripener can be used on plant and ratoon crops not being ploughed out. An understanding of these factors will eliminate important knowledge gaps that have made it difficult for Australian growers to predict responses and thus hindered the uptake of this technology by local industry.

This study also identified physiological traits that may assist growers to identify crops that are responsive to ripeners like glyphosate. Further work should attempt to confirm these findings, particularly the use of green leaf numbers and pol/DM ratios in stem sections. Confirmation that pol/DM ratios in stem sections are useful in predicting crops to be chemically ripened could create further opportunities measuring maturity in cane crops. For an example, it could enable industry to better define the maturity potential for any given cane variety at a particular time in the season and this could be used to give feedback to growers on the maturity of the crops they have consigned to the mill

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