"IMPROVEMENT OF YIELD IN SUGAR CANE THROUGH INCREASED SUCROSE ACCUMULATION"

WORKSHOP REPORT

Sponsored by:

Sugar Research and Development Corporation

Organised by:

CSIRO Division of Tropical Crops and Pastures

Held at Bardon Professional Development Centre
7-8th October 1992

IMPROVEMENT OF YIELD IN SUGAR CANE THROUGH INCREASED SUCROSE ACCUMULATION - WORKSHOP REPORT

Edited by

J. R. Wilson

CSIRO Division of Tropical Crops & Pastures

Brisbane, Australia

Published by CSIRO
Division of Tropical Crops and Pastures
Brisbane, Australia

1992

Workshop sponsored and funded by

Sugar Research and Development Corporation

Brisbane, Australia

The National Library of Australia Cataloguing-in-Publication Data:

Improvement of yield in sugar cane through increased sucrose accumulation.

ISBN 0 643 05450 2.

1. Sugarcane industry - Australia - Congresses. 2. Sugarcane - Australia - Congresses. 3. Sucrose - Congresses. 4. Sugarcane Australia - Yield - Congresses. I. Wilson, John R. (John Richard), 1936- . II. CSIRO. Division of Tropical Crops and Pastures.

338.17361580994

CONTENTS

Foreword

This workshop has been sponsored by the Sugar Research and Development Corporation (SRDC) and organised by the CSIRO's Division of Tropical Crops and Pastures. The SRDC regard the workshop as an important event helping our planning for funding and integrating future strategic research for the sugar industry.

The Corporation has identified four areas of R&D as requiring special attention - in particular as being of such significance to the future prosperity of the sugar industry that they need greater continuity of attention from the research community than may be possible with conventional project funding.

These areas are:

> *Sugar accumulation - the industry significance of CCS content will be discussed at the start of this workshop by David Rutledge of the Queensland Sugar Corporation, and by Dr Owen Crees who will present the view of the Sugar Research Institute.*
>
> *Yield decline - the fact that sugar yields per harvested hectare have at best reached a plateau since the early 1970's is a great handicap to canegrowers. No farmer can cope with the continuing cost:price squeeze without productivity increase, and is fighting with one hand behind his or her back if the only available response is to cut costs. There is a definite link between the trend over the years in CCS and in total sugar yield, and this workshop rightly concentrates on sugar content as a research objective.*
>
> *Farm business management - this is another topic being developed strategically by the SRDC.*
>
> *Design networking - this area is aimed at improving sugar milling operations.*

The first two of these planning areas fall squarely within the topic of this workshop. I do not need to remind this audience that the physiology and biochemistry of sugar cane were the subject of research for a considerable time at the CSR Company's David North Laboraory here in Brisbane. That research was oustandingly successful in its contributions to scientific understanding, but we would not be here with today's agenda if it had been equally successful in benefiting the sugar cane industry.

It is a bold move on the part of the SRDC and the researchers to, in a sense, start again on strategic research aimed at improving sugar accumulation in cane. Although, by analogy with what has been achieved

with various grain crops over the last 50-100 years in improving the proportion of a harvestable product, it is an obvious strategy. But is there a fundamental difference between fruits and seeds on the one hand and the metabolic product, sucrose, on the other?

The chances of success must be much better now than they were even 15-20 years ago. There have been major advances in plant biotechnology, that show every sign of continuing for some time. I would like to think that there have been similar advances towards a realistic understanding of the complex relationships between advances in science and technology and useful applications to industry.

1 congratulate the organisers on setting up a program for the next two days that augers well for the fixture of this area of R&D within Australia. The program brings together a remarkable array of talent from around Australia and overseas. 1 want to make special mention of our visitors from The Hawaiian Sugar Planters' Association at Aiea in Hawaii. We welcome you and reiterate our interest in international cooperation in important strategic areas of sugar cane research - such as sugar accumulation. It is pleasing also that Chris Grof the new CSIRO appointee to work on sugar accumulation, has been able to return to Australia for this workshop.

I note that the workshop covers a variety of approaches to improve CCS in sugar cane, spanning most of the time spectrum of possible payoffs. It is extremely important for the industry, as I mentioned earlier, that it is provided with productivity improvements in both the short and long terms.

You will notice, when you see the SRDC's new Five-Year Plan in the near future, that it mentions increased sugar content of varieties but stops short of specifying goals for sugar accumulation during the period 1992-1997. We confidently expect to have a much better idea of what might be possible when the information gathered by this workshop has been assimilated. The interests and capacities of the various institutions for research on sugar accumulation will also be more clearly defined, and the potential for synergistic collaboration will be enhanced.

As Chairman of the SRDC, I welcome the participants to the workshop, and we all look forward with great interest to the proceedings of this two day meeting.

E.F. Henzell
Chairman
Sugar Research and Development Corporation

Summary of Outcomes

IMPROVEMENT OF YIELD IN SUGAR CANE THROUGH HIGHER SUCROSE ACCUMULATION

The workshop targetted the potential for strategic research to increase sucrose (CCS) levels in cane to raise sugar production per hectare to a higher level. Speakers presenting an industry perspective indicated that Australia needed to continue to introduce new technologies to increase its economic and competitive efficiency to maintain export markets. Also, from the milling viewpoint, a higher level of sucrose accumulation to increase sugar production per hectare was a desirable objective and posed no problems for the milling operations if overall CCS of crops was raised to the maximum levels of 16-18% measured in some crops. Dr Henzell, Chairman of SRDC emphasised the need for a balance of short-term and long-term research in sugar accumulation.

Research priorities

The following research opportunities to increase sugar accumulation were defined from the workshop group discussions:

Short to mid-term research

A. Continuation of current breeding programs for higher early season CCS
B. Identification of improved crop management procedures to maximise harvested sugar yield in different growing regions through development of quantitative response functions for climatic and nutritional influences on sugar accumulation and their use in a sugar cane crop simulation model.
C. Investigation of current sugar cane germplasm for low respiration lines and the presence of "wasteful* respiration pathways, such as the "cyanide-resistant" pathway.
D. Quantification of the interaction of current recommended ripeners and climatic factors so that their effectiveness and reliability is improved.

Longer term research

A. Molecular modification of key sucrose metabolism enzymes and the development of new genetically transformed cultivars of sugar cane with higher sucrose accumulation.
B. Breeding programs for higher CCS based on new introductions to Australia of a wider range of *S. officinarum* germplasm.
C. Reduction of fibre content, e.g. lignin, by interference with the lignin synthesis pathway.

Collaborative opportunities

Possibilities for collaborative research interaction between BSES, CSIRO, UQ and the SRDC were covered. Special attention was given at the workshop, and in a follow-up meeting, to plan an integrated program and collaboration on molecular biology between the Australian and the Hawaiian Sugar Planters' Association research groups.

Strategic capability building

Pursuing the research priorities listed above would require strategic inputs for:-

A. Personnel to quantify the climatic and nutritional influences on sugar accumulation, to incorporate this information into the cane growth model, and to develop management options for evaluation.
B. Laboratory equipment for routine analysis of sugars in the above program.
C. Increased quarantine facilities for expansion of cane germplasm introductions to Australia.
D. Large equipment items for use in the molecular biology program, such as an FPLC for protein isolation and purification or automated DNA sequencing aparatus.

PART I

INTRODUCTORY SESSION

Background and Nature of the Workshop

J.R. WILSON
CSIRO Division of Tropical Crops and Pastures
306 Carmody Rd., Sr Lucia, QLD 4067

Sugar is an important domestic and export industry for Australia. The industry is highly efficient but needs to continuously upgrade its production and milling efficiency to maintain and expand markets.

There is evidence that cane yields per hectare in Australia have tended to plateau in recent years (Table 1), and that the relative increase in cane yield (e.g. in Hawaii) over the past 35 years has been considerably lower than in other major field crops (Table 2).

Table 1. Queensland sugar cane production per hectare 1984-1991

	1984	1985	1986	1987	1988	1989	1990	1991
Yield (t/ha)	80.4	79.5	81.3	79.5	87.5	83.2	74,5	61.3

Adapted from SRDC Five-Year Plan 1992-7.

Some caution must be expressed in interpreting these trends. In recent years the cane areas in Australia have suffered severe droughts and flooding, perhaps resulting in unusual yield restrictions. Whilst, in comparing cane with other crops, we must remember that cane starts from a highly productive base, and is probably the most efficient of all major crops in conversion of sunlight into product (Moore 1989). Nevertheless, even with these provisos, there is a widespread belief that Australia needs to commence some new areas of strategic research to keep sugar production per hectare and the cost efficiency of production on a longterm upcurve.

The CSIRO Division of Tropical Crops and Pastures (DTCP) as a result of discussions with the industry and the Bureau of Sugar Experiment Stations (BSES) recently initiated a project to investigate physiological, biochemical

and molecular means to increase the efficiency of sucrose accumulation in cane. New National Priorities money from CSIRO was provided for a biochemist/molecular biologist to work on enzymes of the sucrose pathway. DTCP has matched these new resources, and has made a long term committment to this research.

Table 2. Percentage (%) increase in yield of major field crops since 1950

Crop	1970	1985
Maize	119	194
Rice	111	122
Wheat	65	106
Soybean	31	49
Sugar cane (Hawaii)	32	32

Adapted from Moore (1989).

Much of this new strategic research will build on the excellent groundwork of understanding of sucrose metabolism in cane developed by the CSR David North Plant Research Centre in the 1960's and 70's (Glasziou and Bull 1980). This work ceased in 1977 with the closure of the CSR Laboratory. The advent of molecular biology now provides the tools to translate the biochemical understanding gained into genetic modification of plants to under- or over-express enzymes of sucrose metabolism to provide genotypes, and eventually new cultivars, with greater maximum sucrose concentration and higher total accumulation. Impetus to commence this work in Australia has been provided by the recent research in this area made by the scientists at the Hawaiian Sugar Planters' Research Station at Aiea in Hawaii. Their advances in biochemical understanding of sucrose accumulation and in gene cloning in sugar cane offer exciting opportunities. So also does the pioneering work and success of the group in the Botany Department at the University of Queensland in developing gene transfer and plant regeneration systems for sugar cane.

Research of this nature is long term and high risk, but is seen as having a high potential return. Consequently, we also see a need to complement this work with shorter term physiological and biochemical research aimed at other methods to increase sucrose production and spread its peak of maximum accumulation in cane. There appears to be a need for quantitative relationships between sucrose accumulation and the climatic and nutritional environment to help develop better cane management procedures. There is perhaps potential for more effective use of chemical ripeners based on a better understanding of the interaction of environment with sugar accumulation. Other physiological modifications associated with reducing

respiration losses or fibre production to provide more carbon skeletons for conversion to sucrose could be considered.

This workshop was organised by CSIRO DTCP to discuss these opportunities provided by new physiological, biochemical and molecular approaches to increasing sucrose yield.

The Sugar Research and Development Corporation sponsored the workshop to bring researchers from the institutions involved in sugar cane research together with experts in the physiology and biochemistry of sugar metabolism and several of the senior scientists from the Hawaiian group. The workshop aimed to:

> Define the more promising research areas and their time frames for success
>
> Set priorities so that scarce resources can be put to best use
>
> Provide a forum for developing collaboration between Institutes and individual scientists
>
> Identify needs for possible strategic input of industry funds for key personnel and large equipment items.

The outcome from the workshop will aid in strategic planning by SRDC, CSIRO and other stakeholders for this research area.

The workshop is organised into three main sessions. Part 1 covers industry views of the needs and benefits of higher sucrose accumulation and longer harvest season. Part 2 provides the technical sessions on our state of knowledge of physiology, biochemistry and molecular biology of sugar metabolism. Part 3 summarises the collation sessions which bring together the aims described above under three sections : A) Environmental and management factors, B) Molecular biology approaches, and C) Other physiological approaches.

References

Glasziou, K.T., and Bull, T.A. (1980) A review of biochemical and physiological research at David North Plant Research Centre 1961 to 1977. (Bureau of Sugar Experiment Stations: Brisbane, Australia).

Moore, P.H. (1989) Physiological basis for varietal improvement in sugarcane. *In* "Sugarcane varietal improvement". (Eds K.M. Naidu, T.V. Sreenivasan, and M.N. Premachandran) pp 19-55 (Sugarcane Breeding Institute: Coimbatore).

Implications to Industry of an Increase in Sugar Yield Through Higher Sugar Content*

DJ.S. RUTLEDGE
Queensland Sugar Corporation
P.O. Box 981
Brisbane, Qld. 4001

The success of Australia's sugar industry has been built on its ability to compete in a highly corrupted world market. This relative efficiency has been achieved through the continued development and implementation of new technologies both on the farm and in the factory. However, past achievements are not enough, our competitors continue to improve their efficiency. It is imperative that the rate of productivity growth in the Australian industry eclipses that achieved by our competitors. Research is an important factor in reaching this objective. Nevertheless, if the output from research is to achieve this objective then the new technology or management practices must lower the cost of production and be implemented by the industry. Therefore, it is important to consider the economic consequences of research rather than focussing solely on technical matters.

* This paper was prepared by the Queensland Sugar Corporation's Principal Economist, Mr Warren Males and Chief Executive, Dr David Rutledge. The views expressed are those of the authors. They do not necessarily represent the views of the Queensland Sugar Corporation.

Introduction

Mr Chairman, thank you for the invitation to address this workshop on the *Accumulation of Sugar in Sugar Cane* and to present my views on the implications for the Queensland sugar industry of an increase in yield through higher sugar content as an opening perspective. I would like to note at the outset that the success of Australia's sugar industry has been built on its ability to compete in a highly corrupted world market. Australia is generally regarded as one of the most efficient raw sugar producers in the world. This relative efficiency has been achieved through the continued development and implementation of new technologies both on the farm and in the factory. However, past achievements are not enough. Our competitors continue to improve their efficiency, many benefit from government subsidies. In contrast, the Australian industry receives little assistance. Therefore, it is imperative that the rate of technical development in the Australian raw sugar industry eclipses that achieved by our competitors. Today's workshop by examining the technical issues aimed at improving the sugar content of cane deals with a very important issue for the continued competitiveness of the Australian sugar industry.

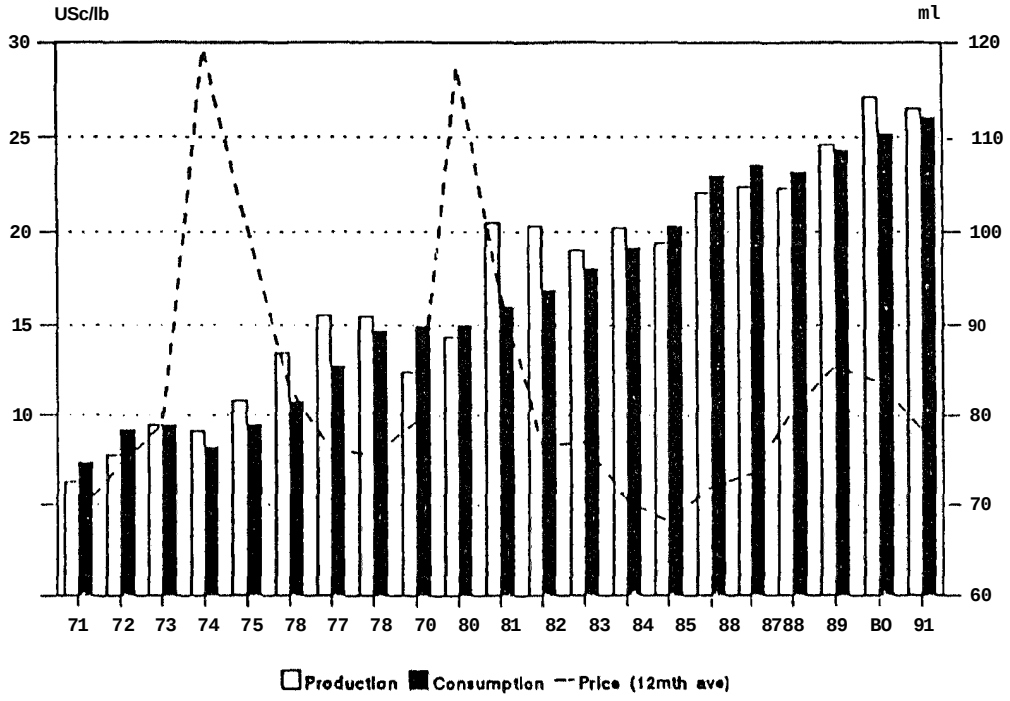
I would like to provide an industry perspective for this workshop by focusing on some of the challenges confronting the sugar industry and as a consequence both cane growers and mill owners. In this presentation I will highlight the importance of the Queensland sugar industry maintaining its competitive edge both on the farm and in the factory. This need arises largely as a result of the progress made by our competitors and of the corrupt nature of the world market. Therefore, I will begin by outlining the nature of the marketing environment confronting the Queensland sugar industry. Then I will briefly review the response to these challenges made by the Queensland Sugar Corporation and its predecessor organisation the Sugar Board — the development of an efficient marketing package for the industry. The theme I will attempt to develop throughout this presentation is that the industry needs to focus on improving its economic efficiency not simply its technical efficiency. Much of what I have to say is equally relevant to both farm and factory management.

Economic Influences in the Industry

Prospects for the Queensland sugar industry are linked to the outcome of two key forces — the climatic conditions which face the industry and the economics of the world sugar market. The industry is only now recovering from the adverse weather patterns during the 1991 growing season. The poor weather conditions in Queensland caused production to fall from a forecast 3.5 million tonnes to an actual production of 2.8 million tonnes for the 1991 season. This reduced income to Queensland's sugar producers by some 20 per cent or about $250 million in 1991. There is little that individual cane growers can do to offset the adverse effect of these climatic events. This is particularly true for those cane growers who do not have access to irrigation.

Leaving weather patterns aside, the key factor for the economic outlook for the sugar industry is the export market. This is because around 80 per cent of Queensland's sugar production is exported. Moreover, since the replacement of the import embargo with a tariff in 1989 and the subsequent reductions in the level of the tariff, all revenue from sales of raw sugar is dependent on world sugar price movements now more than ever. The government in no other sugar producing country has exposed its domestic producers and consumers to the vagaries of the world sugar market to the same extent. The world sugar market is characterised by government intervention and volatile world prices (Figure 1). In the past movements in the world sugar price have been essentially cyclical with a broad pattern of high prices for one or two years, followed by a long period of low or relatively low prices. This pattern occurs because sugar production tends to expand rapidly in response to high prices but is much less responsive when prices fall.

Figure 1: The world sugar market



For the future, it is uncertain that the GATT negotiations will reach a successful conclusion. Even if an agreement is reached, the ensuing reductions in assistance to sugar production world-wide are likely to be small. Therefore, while it is possible that world sugar prices will be more stable than previously, overall I expect the world sugar market to continue to be characterised by high levels of government intervention and volatile prices.

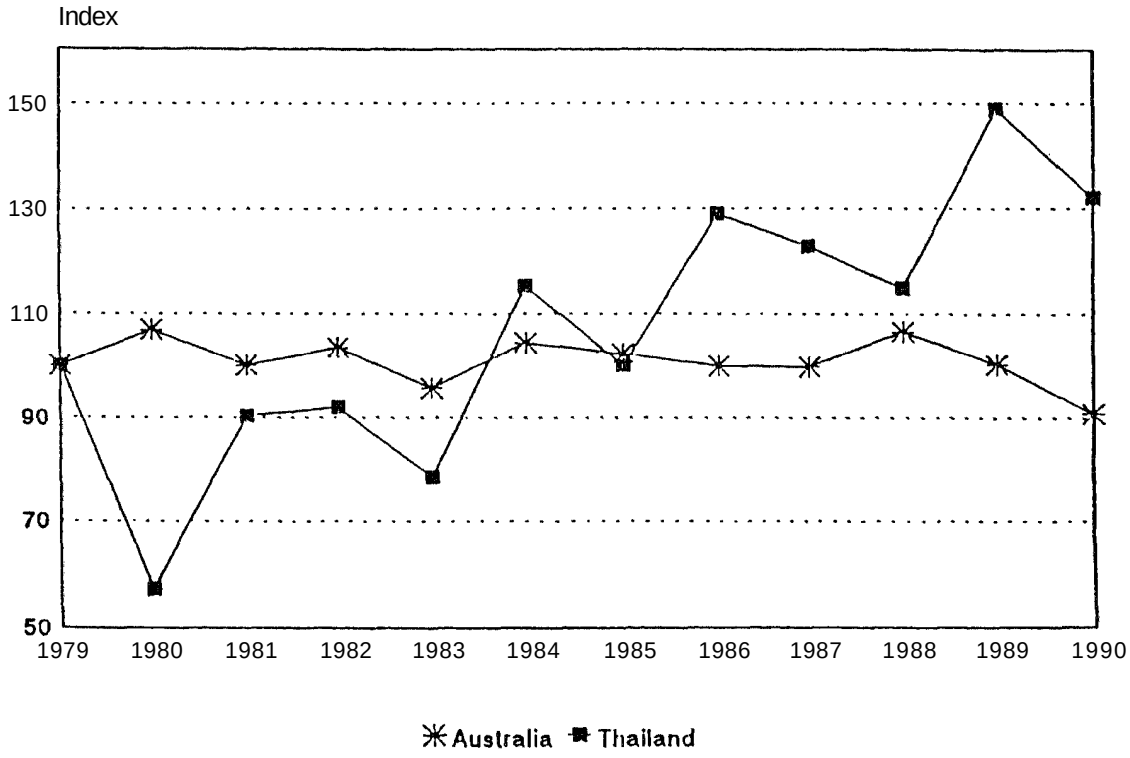
Unlike Australian sugar producers, producers in Thailand, the European Community and South Africa have the benefit of a protected domestic market with returns from this market insulated from movements in the world price. Currently, the domestic price for sugar is around US22c per pound in Thailand and around US28c per pound in the European Community and South Africa. At these price levels sugar producers cover a large portion of their fixed costs of production with the relatively high returns from their domestic markets. Under these circumstances, when prevailing world prices cover the variable costs of production, industry expansion is profitable.

The Europeans and the Thais have been successful in using this industry development strategy. The European Community was a net importer of sugar in 1975. Since then, production has increased dramatically and the EC now exports around 5 million tonnes of sugar annually. In Thailand, sugar production has more than doubled over the past decade. Thai sugar exports eclipsed Australian exports for the first time in 1990 when Thailand became the world's third largest sugar exporter.

There is little doubt that much of the development of the Thai sugar industry has been aided by high domestic prices. However as I said at the outset, our competitors continue to improve their efficiency. Nowhere is this more evident than in Thailand, our major competitor in the Asia-Pacific region. Landell Mills Commodity Studies (1991) estimate that the costs of sugar production in Thailand declined by 28 per cent on average between the first half of the 1980s and the second half of the 1980s. By comparison, Landell Mills estimates that costs of production in the Australian sugar industry declined by just 6.2 per cent between the same two periods. In addition, as is illustrated in Figure 2, a simple comparison between Thailand and Australia of sugar yields per hectare lends further support to the proposition that the relative technical efficiency of the Thai sugar industry is rising more rapidly than that of the Australian sugar industry.

Taken together, these estimates suggest that the rate of productivity growth or improvement in economic efficiency is occurring faster in the Thai sugar industry than in the Australian sugar industry. I visited Thailand in 1991 and saw no reason to dispute this conclusion. The main point to be drawn from this analysis is that the sugar industry in Thailand is positioning itself to be a competitive low cost sugar producer for the longer term. I expect this to be the case even if domestic support is reduced. The challenge being thrown out by Thai sugar producers is one which the Australian sugar industry must accept if it is to remain profitable in the longer term.

Figure 2: Sugar yield per hectare — Australia and Thailand



Despite the rapid increase in efficiency in the Thailand sugar industry, Australian sugar remains very competitive in the market place. This is due in large part to the low cost nature of the marketing package offered by the Queensland Sugar Corporation. The centrepiece of this marketing package is the integrated management and control of the bulk sugar terminal activities and the logistics of the associated transport operations. In contrast to Australia, sugar mills in Thailand are located a long way from the port and the sugar for export often needs to be trucked either through or around Bangkok. In addition, Thai ports, particularly Bangkok, are very congested and sugar is loaded onto ships from lighters,

The Queensland Sugar Corporation is continually reviewing its marketing activities and strategy. For example, the bulk sugar terminal infrastructures which were originally designed to receive sugar at 250 tonnes per hour have been gradually upgraded using common technology and management experience. Consequently, present day receiving rates are about 1000 tonnes per hour. Similarly, ship loading rates have been progressively improved. Originally, ships were loaded at 750 tonnes per hour. Average loading rates are now around 1600 tonnes per hour. These changes have significantly reduced labour and operating costs. Moreover, faster turnaround time for ships at all terminals and the stability and expertise developed through the integrated management of the separate terminals have established Queensland as the world leader in the technical and economic efficiency of bulk storage, handling and marketing of raw sugar. These developments have helped the Queensland raw sugar industry to maintain a competitive advantage in the world market despite the high levels of assistance and rapid growth in production efficiency being achieved by overseas competitors.

My intention in making these comments on the marketing environment and the activities of our competitors is simply to illustrate the fact that the imperative for the Australian sugar industry to continually improve its competitiveness is not likely to slacken even if there is a positive outcome in the current round of GATT negotiations.

Capture of Benefits from Operational or Technical Developments

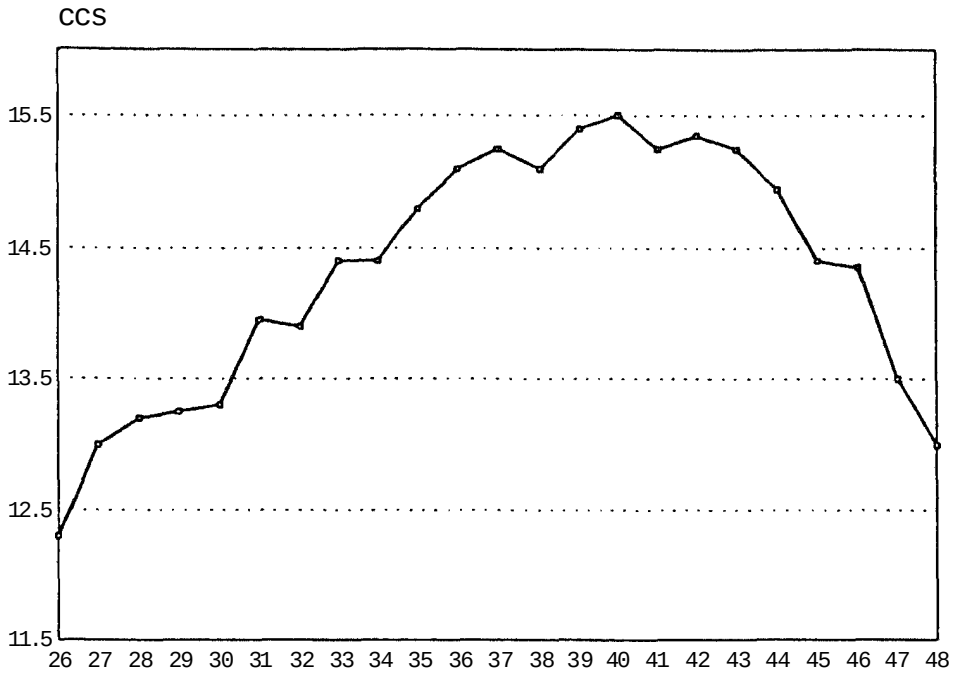
One of the methods by which the Australian sugar industry can achieve the required productivity improvements is by improving the sugar content of cane. Another is of course improving the recovery of sugar from the cane. These pursuits are important and success will earn the sugar industry and the economy as a whole significantly higher export income. Nevertheless, if the output from research is to achieve this objective then the new technology or management practices must be implemented by the industry.

In general, I expect that cane growers would only implement a particular strategy to increase yields if the benefits from adopting the strategy exceed the costs and that the net benefits of the proposed strategy exceed the net benefits from alternative strategies. These critera apply equally to technical changes which may be introduced in the factory. In other words, I expect producers to adopt new technology only if it improves the economic efficiency of their activities. If the economic efficiency criteria are satisfied there is little doubt that the sugar industry will benefit from either higher CCS in sugar cane or other technical improvements which might be available.

I would like to illustrate the nature of this cost-benefit trade off by discussing the importance of improving the economic efficiency of activities as opposed to focussing on technical matters. As is illustrated in Figure 3, a typical pattern for a mill area of CCS in sugar cane during the Queensland crushing season is for it to rise rapidly until mid-way through the season and then fall sharply thereafter. If the objective is simply to maximise the

sugar content of cane then an obvious strategy would be to dramatically increase harvesting and mill capacity and crush all the cane produced during the week in which CCS is highest. Such a radical solution, obviously would fail to take into account the economic issues associated with the integrated nature of farming, harvesting and milling operations. It is only after the economic considerations of both farming and milling have been taken into account that agreements over the season length in Queensland are reached.

Figure 3: Typical pattern for a mill area of ccs in sugar cane during the Queensland crushing season



It is worth noting that as cane production has risen following recent expansions in land assignments in some mill areas continuous crushing agreements have been negotiated. These agreements recognise the benefits to cane growers of maintaining the current season length and the benefits to mill owners of a greater utilisation of existing mill capacity. The net result of the arrangements has been to increase the economic efficiency of both cane growing and sugar milling activities. In addition to increasing the average throughput of sugar mills, retaining the existing season length, in effect, has increased the annual average CCS of cane in those mill areas. The continuous crushing agreements recognise these improved efficiencies and the benefits are shared between cane growers and mill owners through agreed changes to cane payment arrangements.

The continuous crushing agreements illustrate quite simply that improvements in the economic efficiency of the sugar industry can be achieved without recourse to new technology. There may be other areas of activity within the industry where similar gains could be achieved. For example, there may be changes in farm activities which might lead to improvements in factory efficiency. I suspect that such changes would occur only if the economic benefit captured by the mill owners from these improvements is shared with the cane growers. If such improvements are possible they should be pursued.

Assessment of Potential Economic Benefits

When making these points about the importance of pursuing gains which lead to improvements in economic efficiency, I do not wish to understate the importance of improving technical efficiency. Ultimately it is technical progress which determines the boundaries of efficient production within the industry. The economic benefits which arise from these technical developments can be assessed. For example, to assess the benefits from improving the level of CCS in sugar cane production, a simple application of a model developed cooperatively by CSIRO and the Australian Bureau of Agricultural and Resource Economics to evaluate the returns from research undertaken by the CSIRO Institute of Plant Production and Processing could be made (Johnston *et al.* 1992). A description of this model is contained in the paper titled *Rural Research — The Pay-off*. I would commend this model to you as an important tool for identifying the nature of the economic gains which will flow from the cost savings generated by the successful implementation of the findings from individual research projects.

I am not in a position which allows me to assess the costs of various strategies to improve the sugar content of cane which might be discussed in the more technical sessions of this workshop. However, it is relatively easy to discuss the nature of any benefits which will accrue to the industry and the economy generally if strategies are implemented which satisfy the broad economic criteria I have identified. Nevertheless, these benefits should be balanced against any costs.

Using the framework of the CSIRO-ABARE model, improvements in the CCS of sugar cane can be translated directly into greater quantities of raw sugar produced at a given level of recovery by mills. For example, raising the level of CCS from 13.5 to 13.7 on average or by about 1.5 per cent will lead to an equivalent increase in the quantity of raw sugar produced. To illustrate this point, in a typical season each 1 per cent increase in CCS achieved allows the Queensland Sugar Corporation to sell about two additional shipments of raw sugar to our export customers and earn around $10 million in additional export revenue. Through the existing cane payment arrangements it is the cane growers who receive the bulk of the additional benefit from higher sugar content in cane.

I would like to end this presentation by summarising what I see as the main conclusions of the joint CSIRO-ABARE paper as they effect sugar technologists. First, the key questions which need to be addressed are 'how much will this research benefit the industry?' or 'what will be the cost of production savings to the industry arising from the research?' The second conclusion drawn is that the research process will be most effective if the research results are disseminated quickly in a readily useable form. In order to ensure this will be the case appropriate technology transfer mechanisms should be established early in the research process or developed as part of the research project. In other words, while recognising the importance of research into various aspects of sugar production including an increase in sugar yield the real benefits from research will not be realised until the new knowledge has been applied successfully.

References

Landell Mills Commodity Studies (1991). *A World Survey of Sugar and HFCS Production Costs 1979-80 to 1988-89*, Oxford, England.

Johnston, B., Healy, T., I'ons, J. and McGregor, M. (1992). *Rural Research — the Pay-Off*, CSIRO Occasional Paper No 7, a co-operative study by CSIRO and ABARE, Canberra.

Factory Perspectives of Higher CCS and Longer Seasons

O.L. CREES
Sugar Research Institute
P.O. Box 5611
Mackay, Qld. 4741

Introduction

From a milling perspective, there are very few negative effects of longer seasons and higher CCS on mills. There could be some short term limitations due to possible changes in the amounts of sugar and impurities entering the factory if a change in CCS occurred rapidly. There may also be some limitations imposed by the effects of a longer season on harvesting and cane supply. However, there should be no technical problems within the mill other than those which might exist at present.

Higher CCS

Even from the simplest perspective, higher CCS must be beneficial to a mill since it will have to crush less cane and evaporate less water to produce a tonne of sugar.

There are already wide variations in CCS among mill areas (Figure 1), throughout the season and from year to year. In many mill areas, a substantial increase in CCS would simply bring them up to the level of the current highest yield regions.

Milling train

The extraction process, whether milling or diffusion, should not be significantly affected mechanically by an increase in CCS. If maceration rates remained unchanged, the absolute level of sucrose in final bagasse would almost certainly increase, although the percentage of sugar lost in bagasse should still be lower. An increase in maceration rates would further improve recovery.

Milling and diffusion should see only benefits from higher CCS.

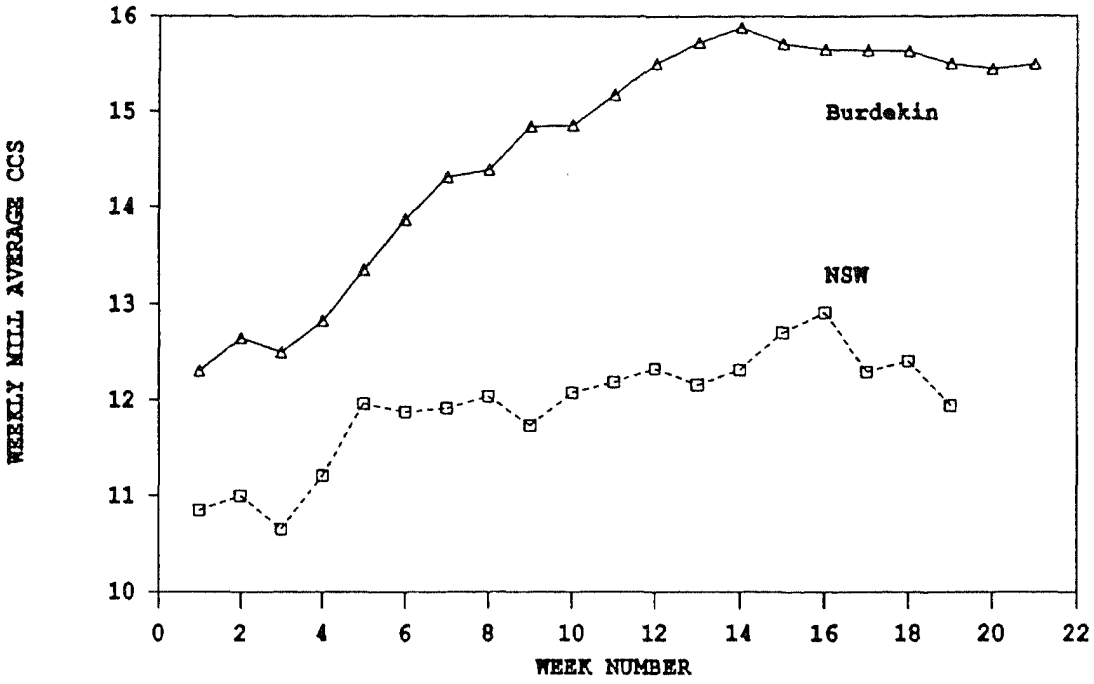


Figure 1. Weekly CCS profiles for Burdekin and NSW Mills

Processing

Processing operations are controlled by the separation of sucrose from impurities. Higher CCS generally means higher purity in juice and syrup so almost all aspects of processing should benefit.

Pan and fugal stations in individual mills are generally sized to cope with a particular balance of sugar and impurities and the change in that balance through the season (Figure 2). Within reason, crushing rates can also be adjusted to match the limitations of other parts of the factory.

An increase in average or peak CCS may require some additional pan and fugal capacities. Some additional sugar storage capacity may also be needed. However, the changes in CCS would almost certainly occur slowly enough for the factory modifications to be easily accommodated.

Other aspects

Existing boiler stations should be adequate as there would be no increase in the quantities of water to be evaporated. Similarly, effluent treatment requirements should not increase.

There may be some effect on raw sugar quality, particularly if juice purities increased in parallel with CCS. Under current marketing arrangements, raw sugar is produced with a fixed level of impurities. As purities increase, the relative proportions of the various impurities may change. For example,

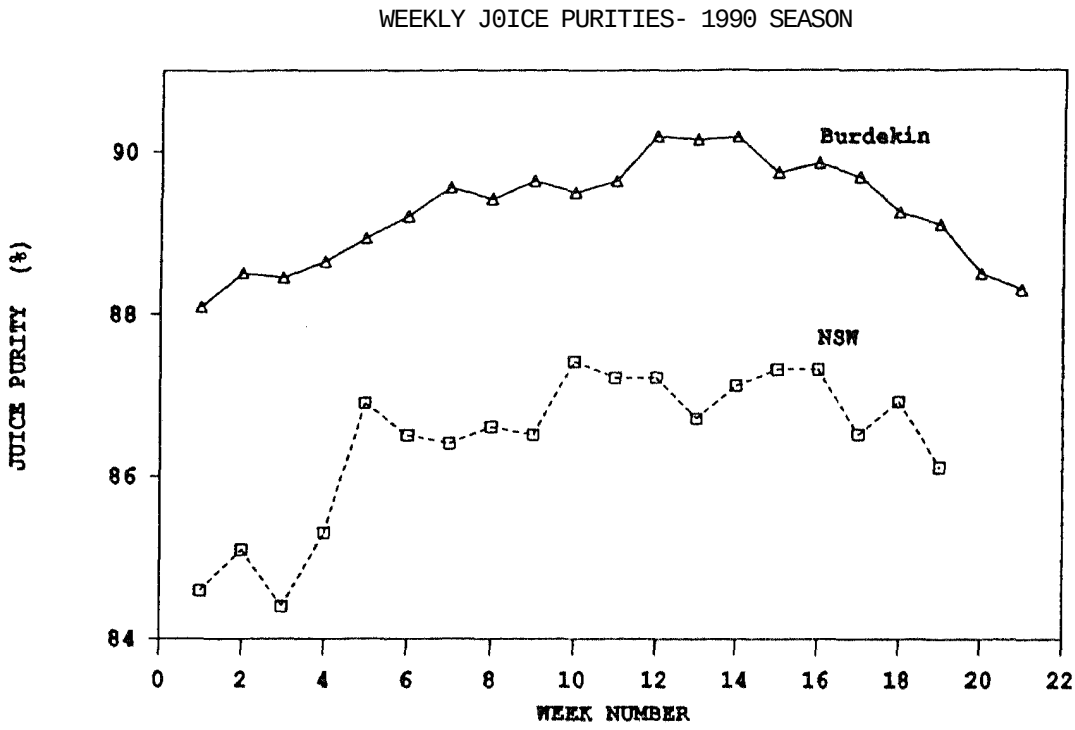


Figure 2. Weekly juice purity profiles for Burdekin and NSW Mills.

as the ratio of colour to other impurities increases, the absolute colour of raw sugar also increases.

Overall, the capital investment in factories may increase but productivity would aiso increase with a substantial net benefit. If juice purities also increased, there may be opportunities to introduce new technologies which are not economically viable under present conditions.

Longer Crushing Seasons

There is always a concern that a mill represents a very high capital investment which is idle for more than 50% of every year. Longer crushing seasons immediately increase the utilisation of existing plant and equipment.

Some improvements to current maintenance practices may be required to cope with longer operating cycles. Many mills are already beginning to address this problem with improved maintenance practices, condition monitoring and scheduled maintenance schemes. Equipment maintenance should therefore not pose any impediment to extended crushing seasons.

Cane supply

Increases in season length could have significant effects on the quality of cane supplies. With crushing starting earlier and/or finishing later, the risks of having to harvest under wet conditions would almost certainly increase.

Levels of dirt and other extraneous matter in the cane supply would increase unless harvesting technology improved considerably (Fueling, 1979; Crees *et al,* 1978). Higher dirt levels invariably mean higher maintenance costs and higher sugar losses (Clarke *et al,* 1988; Muller *et al*$_t$ 1982).

Mills in the northern wet belt already experience substantial processing problems due to dirt in cane supplies from time to time, particularly early in the season (Figure 3). These problems would be expected to be more widespread with longer seasons unless solutions were found through improvements in cane harvesting and/or factory based cane cleaning technologies.

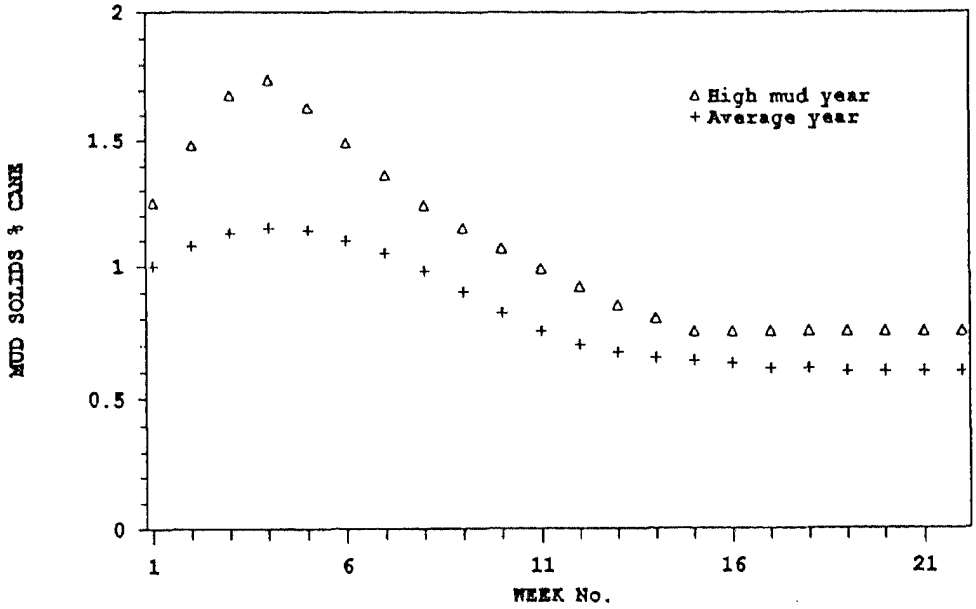


Figure 3. Weekly mud solids loadings for wet and normal seasons.

Extending the crushing further into the summer months increases the risks of processing and sugar quality problems associated with dextran. The risk of interruptions to harvesting operations due to the onset of summer storms increases. With the warmer weather, any increase in burn-to cut and cut-to-crush delays increases the levels of dextran in cane supplies (Foster *et al,* 1976; Wells and James, 1976). Many mills have first hand experience with the processing difficulties which result. Again, the northern areas are generally at greater risk.

The addition of dextranase enzyme to help to control dextran levels in juice is an accepted practice (Inkerman and James, 1976; Fulcher and Inkerman, 1978) but it regarded as an emergency measure because of the high cost. The move to green cane harvesting, already well established in the north, reduces the magnitude of this problem, particularly when harvesting is delayed by wet weather. However, while dextran is produced much more rapidly in burnt cane, it is still produced in green cane. Careful control of cane harvesting and transport schedules therefore remain essential and will increase in importance with extended crushing seasons.

Processing

The potential problems identified above are real and, if not addressed, could pose serious problems for mills. Beyond them, however, extended seasons should pose few processing problems. As with increased CCS, there may be a need to adjust the capacities of some plant items to cope with a wider range of purities but again, there is already a very wide range throughout the industry.

References

Clarke, W.B., Player, MR. and Weiss, G.H. (1988). Effect of extraneous matter on growers and millers costs. *Proc. 1988 Conf. Aust. Soc. Sugar Cane Technol,* p. 39.

Crees, G.L., Hale, D.J. and Whayman, E. (1978). The effects of mud solids loading on clarifier capacity. *Proc. 1978 Conf. Aust. Soc. Sugar Cane Technol.,* p. 191.

Inkerman, P.A. and James, G.P. (1976). Dextranase II. Practical application of the enzyme to sugar mills. *Proc. 1976 Conf. Aust. Soc. Sugar Cane Technol.,* p. 307.

Foster, D.H., Ivin, P.C. and King, J.H. (1976). Measurements of the deterioration of burnt mechanically harvested cane. *Proc. 1976 Conf Aust. Soc. Sugar Cane Technol.,* p. 37.

Fulcher, R.P. and Inkerman, P.A. (1978). Dextranase IV. The effect of cane dextran on the filterability of sugar. *Proc. 1978 Conf Aust. Soc. Sugar Cane Technol.,* p. 111.

Fuelling, T.G. (1979). Cane transport from wet cane fields. *Proc. 1979 Conf Aust. Soc. Sugar Cane Technol.,* p. 81.

Muller, R.A., Player, M.R. and Wise, M.B. (1982). An examination of input, deposition and effect of dirt in Queensland sugar mills. *Proc. 1982 Conf. Aust. Soc. Sugar Cane Technol.,* p. 1.

Wells, W.D. and James, G.P. (1976). Rapid dextran formation in stale cane and its processing consequences. *Proc. 1976 Conf Aust Soc. Sugar Cane Technol.,* p. 287.

Discussion summary
(by E.S. Wallis)

Four presentations were made to the introductory session of the workshop. Dr Henzell (Chairman SRDC) outlined SRDC's interest in the topic of the workshop and its interest in the outcome of the workshop to guide its R & D investment in this area. John Wilson (CSIRO, DTCP) outlined the program for the workshop and the process to be followed. David Rutledge (Chief Executive, Queensland Sugar Corporation) and Owen Crees (Manager, Process Research; Sugar Research Institute) gave papers designed to provide an overview of the impact of a potentially increased sugar content on the marketing and manufacturing segment of the industry.

David Rutledge observed that the success of the Australian Sugar Industry was built on its ability to compete on a corrupt world market. Technological breakthroughs are important to maintain a competitive edge. Higher cane yield levels may lead to lower CCS unless the current negative correlation of these factors can be modified. Another important issue raised in discussion related to potentially longer season lengths if CCS levels could be raised early and/or maintained at high levels late in the season. Earlier peak CCS was perceived to be a useful aim. However, there was concern expressed, supported by Ross Chapman from Canegrowers, that later harvesting may have adverse implications for the productivity of the succeeding crop. An holistic approach to the resolution of the balance between CCS level and season lengths was advocated.

Owen Crees concluded that increased CCS levels held few disadvantages for sugar mills, especially if the increases remained in the current CCS range 11 - 16. The mill would be more efficient at higher CCS levels. One other issue raised was the apparent advantage of longer season lengths to better utilise the capital investment in mills now only operating for six months of the year. This advantage is discounted because of the need to maintain low impurity levels, which are higher for early season harvesting, and the problems of harvesting later after wet periods with increased extraneous matter (dirt) levels.

The session reached the conclusion that if a technological breakthrough could be achieved to raise CCS levels, to the upper end of the current range of CCS levels, that this would be a desirable outcome for the industry.

PART 2

TECHNICAL

SESSIONS

Current Approaches to Increased Sugar Yield

M.C. COX
BSES
P. *0. Box 651*
Bundaberg, Qld. 4670

Introduction

The major objective of most sugarcane improvement programs is increased sugar yield. In the BSES cane breeding program we use net merit grade (NMG) as our main selection criterion. This is a measure of the relative economic worth of clones, including an adjustment for the bonus paid to growers for CCS above mill average. Clones with low CCS (eg 1-1.5 units less than standard varieties) are discarded from the selection program.

In addition to objectives related to varietal development, population improvement is another important aspect of sugarcane breeding, particularly in the longer-term. This means that the collection of clones used as parents must be dynamic, with older, unproductive parents being discarded and newer, productive clones being infused. As a result of a plant breeding review in 1990, BSES is intensifying efforts in this area. Selected clones are being recycled as parents more rapidly (shorter generation interval) and more emphasis is being placed on "experimental" crosses (as opposed to "proven" crosses). Population improvement methods for specific characters have been implemented and a recurrent selection program for early CCS content will be described.

Improvements in sugar yield due to breeding are difficult to quantify. Changes in cultural practices (eg mechanical harvesting), expansion on to more marginal soils, and other confounding factors complicate such estimates. It is our aim to have productivity increases of 1-2 per cent per annum, and we are monitoring this at all stages of selection with performance indicators. Hogarth (1976) calculated that the Queensland sugar industry improved yields by 1.9% per annum from 1948 to 1975 and plant breeding was likely to have contributed about one-half of the increase.

There are good productivity data available in Bundaberg (and other areas) from block recording schemes. In 1991, the two "new" varieties CP51-21 and Q141 returned $374/ha more than the older varieties CP44-101 and QUO, based on first and second ratoon crops only (Table 1). Cane yield was 12% higher, CCS was 0.9 units higher, and sugar yield was almost 20% higher. Varietal composition changes show how rapidly farmers have adopted these new varieties which have increased from less than 9% of the crop in 1989 to almost 40% in 1991 (Table 2). Progress in Bundaberg in recent times has been rapid but does follow a fairly static period after the outbreak of Fiji disease in the late 1960s.

Table 1. Productivity of "Old" vs "New" Varieties

		Productivity*			
	Area (ha)	Cane Yield (t/ha)	CCS (t/ha)	Sugar Yield (t/ha)	Value ($/ha)
Old CQ44/Q110)	1,579	68	14.4	9.8	1,510
New (CQ51/Q141)	3,749	76	15.3	11.7	1,884

* Based on 1st ratoon and 2nd ratoon crops only

Table 2. Composition changes from old to new varieties between 1989 and 1991

Varieties	Category	% of Crop	
		1989	1991
CQ44/Q110	Old	55	31
CQ51/Q141	New	9	40

However, the more important question to address is "what are the prospects for the future"? In the short-term, there is evidence of continued gains, e.g. with a more recent selection 8IS1880 compared to the "new" varieties described above (Table 3). In the longer-term, we believe that a greater emphasis on population improvement and recent implementations of more efficient and effective selection methods (eg weighing machines, family selection) will ensure sugar yields continue to increase. Currently a major limitation to breeding progress is the unpredictable nature of flowering-

Only 40 per cent of the parent population flowers in any one year and in some years (eg 1992) it is as low as 17 per cent. Encouraging results are emanating from flowering research in the photoperiod facility at Meringa and this could dramatically enhance our ability to make genetic gains.

Table 3. Prospects for further short-term gains in sugar yield with a promising line compared to the "new" variety CQ51/Q141

Variety	Productivity*			
	Cane Yield (t/ha)	CCS (%)	Sugar Yield (t/ha)	Value ($/ha)
CQ51/Q141	80	14.9	11.8	1,881
8IS1880	86	15.7	13.6	2,255

* Based on plant and 1st ratoon crops - 4 Bundaberg trials

Research has shown that there is great potential for improving cane yield through traditional plant breeding. Recent improvements (eg Q138 in the north, Q117 in the Burdekin, Q124 in Mackay, and a number of examples in the south) are expected to continue. However, there are concerns about making continued genetic gain for CCS. The prospects are likely to be different in different regions and will vary according to time of season. For instance, there is evidence of good prospects of improving CCS in New South Wales where levels are low.

Studies by Hogarth (1977) and Cox (unpublished) have shown that narrow-sense heritability for CCS is high at all times of the season. This means that selection of parents is effective in predicting progeny performance as there is little non-additive genetic variance. Genetic gain through selection is dependent on heritability and genetic variance as well as intensity of selection. Studies in Australia (Cox *et al*, 1989, Cox, unpublished) and overseas (Tai, 1985) have consistently shown that genetic variation for CCS is greatest early and decreases throughout the season. This means that the potential for genetic gain is highest early and may be quite limited at the end of the season.

Legendre (pers. comm.) demonstrated a 31 % increase in sucrose content of varieties obtained in Louisiana through five recurrent selection breeding cycles. The magnitude of this increase lessened with each successive cycle, indicating that further progress may be more difficult. However, there would be few documented cases where breeders have "run out" of genetic variation.

One approach to increase genetic variability for sucrose content is to re-examine the contribution of *S. officinarurn* to sugarcane hybrids. Of 107 commercial clones grown from 1940 to 1964, fewer than 19 *S. officinarurn* clones were involved (Arceneaux, 1967). As the major source of "sucrose

genes", it is unlikely that this small number included the best clones for sucrose content. A proposal to import 5. *officinarum* germplasm and screen for sucrose content is being prepared. Assuming higher levels of CCS than in hybrid material, a breeding program would commence to produce adapted hybrids, either with other species (5. *spontaneum, Erianthus arundinaceus)* or hybrids. This would be a long-term breeding approach to increased sugar yield.

REFERENCES

Arceneaux, G. (1967). Cultivated sugarcanes of the world and their botanical derivation. *Proc. Inter. Soc. Sugar Cane Technol.* 12: 844-854.

Cox, M.C., Hogarth, D.M, and Mullins, R.T. (1989). Clonal evaluation of early sugar content. *Proc. Aust. Soc. Sugar Cane TechnoL* 13: 90-98.

Hogarth, D.M. (1976). New varieties lift sugar production. *Producers Review.* 66: 21-22.

Hogarth, D.M. (1977). Quantitative inheritance studies in sugarcane III. The effect of competition and violation of genetic assumptions on estimation of genetic variance components. *Aust. J. Agric. Res.* 28: 257-268.

Tai, P.Y.P. (1985). Maturity differences among non-flowering clones of sugar cane. *Proc. Am. Soc. Sugar Cane TechnoL* 4: 119.

Discussion summary
(by DM. Hogarth)

Dr Henzell commented that he was convinced that plant breeding had been successful in maintaining yields and improving genetic potential He suggested that more research should be devoted to the major factors preventing the realisation of genetic gain.

Dr Henzell queried whether there were any trade-offs when selection was practiced for high early CCS. Owen Cox replied that he did not think so and discussed the results of current research that shows a high genetic correlation between sugar yield per hectare and CCS at the beginning of the crushing season. This correlation declines as the season progresses. For tonnes of cane per hectare and sugar yield per hectare, the correlation is low at the beginning of the season but increases during the season.

Merv Ludlow asked about the highest CCS obtained. CSR scientists calculated a theoretical maximum of 27%. Owen Crees could recall maximum values of 20% in Mackay, and Mac Hogarth recalled a value of 21.2% for Q117 in a trial plot in Ingham.

Paul Moore requested that Mike Cox make high x low crosses for high early CCS as well as high x high crosses. The progeny would be useful for genetic mapping.

Robert Furbank queried why CCS in NSW was so low. The major factors suggested were low temperatures, varieties, and the short growing season.

Russell Muchow questioned the importance of genotype x environment (GxE) interactions for CCS. Mike Cox replied that GxE effects were much lower for CCS than for tonnes cane per hectare. Philip Jackson pointed out that GxE effects may be higher for early CCS, but data are limited.

Quantification of the Environmental and Nutritional Effects on Sugar Accumulation

M M LUDLOW[A], R C MUCHOW[A], AND G KINGSTON[B]

[A] *CSIRO, Division of Tropical Crops and Pastures, 306 Carmody Road, St. Lucia, Brisbane, Qld. 4067*

[B] *BSES, P.O. Box 651, Bundaberg, Qld. 4670.*

Introduction

Yield of sugar can be increased by breeding varieties that partition more of their total biomass to sugar, either early in the season or throughout the season. It can also be increased by improved management practices that facilitate sugar accumulating to the genetic potential of a particular variety. Environmental stresses, such as water stress, nutrient stress, and low temperature, and chemical ripeners promote accumulation of sugar. Chemical ripeners are discussed in the following paper. In order to utilise the effect of environmental stress on sugar accumulation to develop improved management practices, quantitative relationships between environmental stresses and sugar accumulation need to be determined and incorporated into models that simulate sugar yields under a wide range of conditions. These models can then be used in conjunction with focussed field experiments to develop improved management practices, based on responses to environmental stresses (such as water and nitrogen management, and planting and harvest dates), and other agronomic variables (such as planting density and arrangement).

Various crop management strategies have been developed based on empirical relationships determined by experimentation in the field (Gosnell, 1970; Leverington *et al*, 1970; Kingston, 1972). Some are very effective, such as irrigation schedules based on Et/Eo = 0.85 to maximise sugar yield have been developed in Hawaii (Robinson *et al*, 1963). However, such empirical relationships are often limited to particular regions, varieties and current crop husbandry practices. Thus, they may be less effective with new varieties, new husbandry practices such as trash blankets and no-till, and as climate changes as a result of the greenhouse effect.

While we know that low temperature, water stress and nitrogen stress favour sugar accumulation (see review of Liu and Kingston, 1992), there are no quantitative relationships between these stresses and sugar accumulation (Bull and Glasziou, 1975). Consequently, models such as

AUSCANE are forced to estimate sucrose accumulation indirectly by allowing particular stresses to raise the fraction of dry weight increase partitioned to sugar (Jones *et al*, 1989), As a result of the lack of understanding of the physiology of sugar accumulation, simulation of CCS and sugar yield is unsatisfactory under most conditions (Wegener *et al*, 1988). The quest to increase sugar accumulation by improved management practices is not able to utilise the power of modern technology by combining simulation modelling with highly focussed field experiments. The only current option is further extensive empirical field experimentation, which the industry cannot afford.

This paper will discuss the process of sugar accumulation at the whole plant level, and summarise the effects of environmental and nutritional stresses on sugar accumulation. In addition, it will suggest the need for both field and controlled environment experimentation to obtain quantitative relations between sugar accumulation and environmental and nutritional stresses, so that simulation models can be developed to define improved management practices to increase sugar yield via greater levels of sugar accumulation.

Whole Plant Physiology of Sugar Accumulation

When carbon is fixed by photosynthesis, it can either be consumed in growth to produce leaves, roots and fibre and in respiration, or it can be stored as sucrose, mostly in the stem (Figure 1). The amount of storage will depend upon the balance between the production and consumption of the fixed carbon; accumulation occurring when production exceeds consumption. The efficacy of various environmental stresses, and of chemical ripeners, in promoting sucrose accumulation will depend, therefore, upon their relative effect on the production of carbon by photosynthesis and its consumption in growth and respiration (Figure 1); accumulation only occurring when the stress has a bigger effect on consumption than on production of fixed carbon. Bull (1980) shows, that between February and May in Brisbane, the use of fixed carbon to produce structural materials, such as fibre, and the storage of sucrose are usually balanced, and consequently sucrose concentration rarely exceeds 10% of fresh weight (Figure 2). Large accumulation of sucrose only occurred from May to October when low temperature, water stress and nutrition stress restricted stem elongation more than photosynthesis. However, if the stress becomes too severe, photosynthesis will also be impaired and sucrose accumulation will decline to zero. In the worst cases, sucrose can be removed from storage to meet respiratory costs (Figure 1). The rate and extent of sugar accumulation will depend upon the relative shape of the relationships between stress and consumption and stress and production of fixed carbon. This further supports the imperative to determine such relationships for sugar cane.

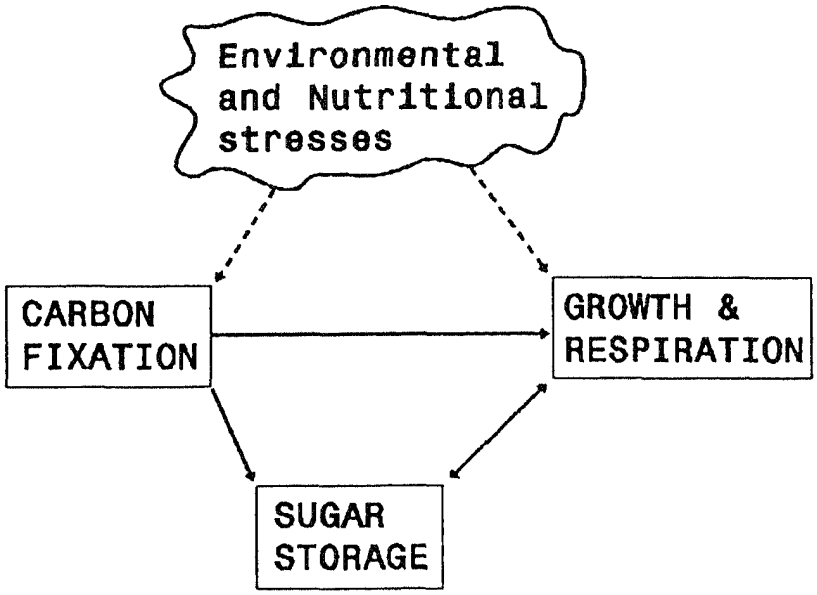


Figure 1 Schematic relationships between carbon fixation, growth and respiration, and sugar storage, and the impact of environmental and nutritional stresses.

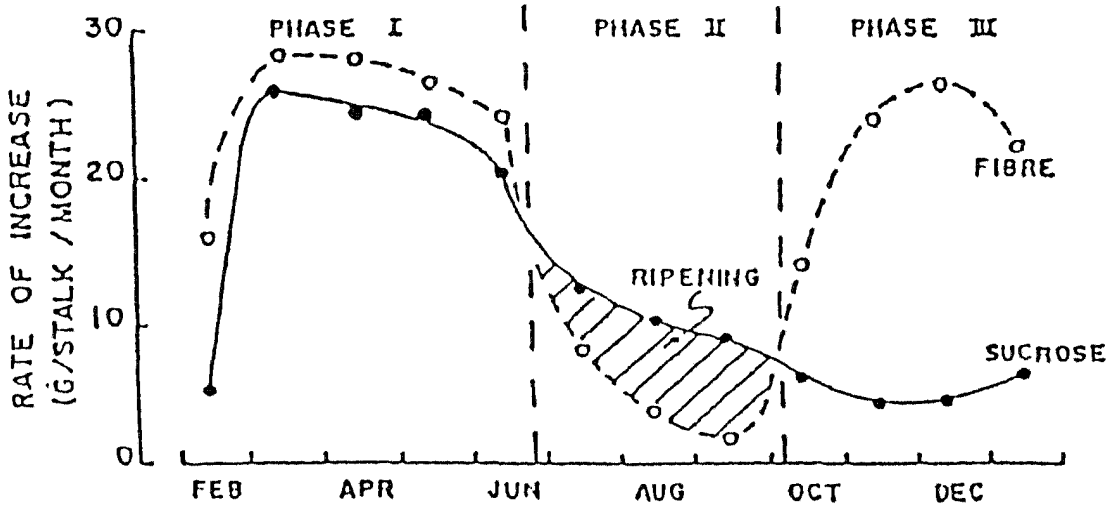


Figure 2 Seasonal changes in the rate of increase in fibre (non-sugar dry matter) and sucrose in stalks of Pindar growing in the field at Brisbane (T.A. Bull, unpublished data)

Quantitative Relationships between Environmental and Nutritional Stresses and Sugar Accumulation

Temperature

Low temperature is probably the main environmental factor causing sugar accumulation in Australia, South Africa, Brazil and the southern USA. The absence of low temperatures is the main reason why the Hawaiian industry have to ripen their cane with chemicals.

It is generally believed that low temperature promotes sugar accumulation because it reduces growth, and hence consumption of carbon, more than it reduces production of carbon (Wilson, 1975a). The critical mean daily temperature to initiate sugar accumulation in cane is thought to be between 20 and 24°C (Yates, 1983). This is illustrated by data of Bull (1980), where stalk elongation rates are more sensitive to decreasing temperature at temperatures below 25°C than is photosynthesis of a single leaf (Figure 3). Unfortunately, these data are from different experiments, and probably different varieties. There is no information on genotypic variation in these responses. Moreover, the effect of respiration is not included, and there are no data for leaf elongation. The effect of temperature on leaf and stem elongation, respiration and photosynthesis needs to be determined for a range of genotypes. If genotypic variability exists, separate relationships will need to be developed for each genotype.

Such relationships are best established by a combination of controlled environment experiments, and field experiments where leaf growth is examined at different times of the year when temperatures are different.

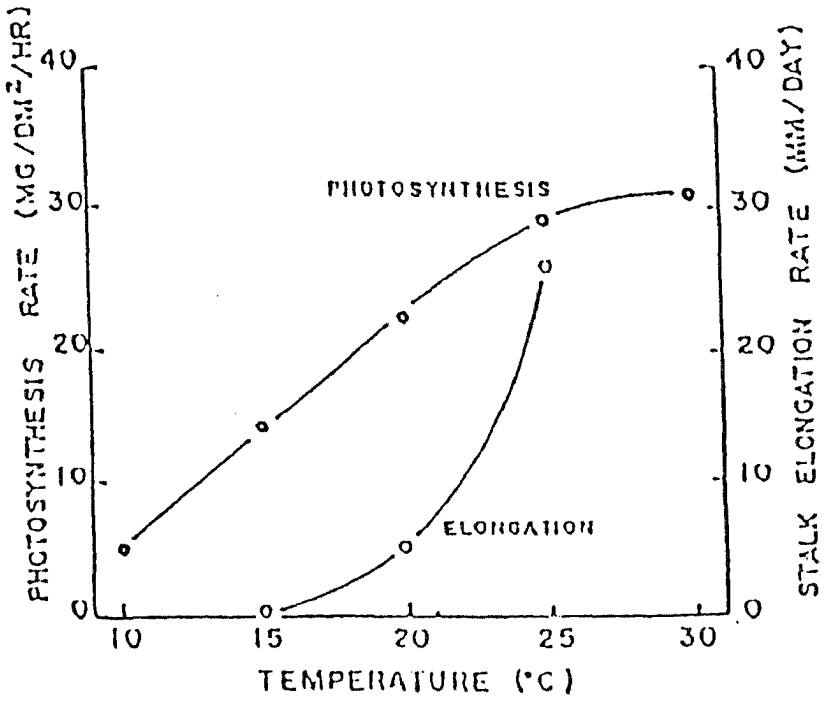


Figure 3 Effects of leaf or root temperature on photosynthetic rate and stalk elongation rate, respectively (Bull, 1980).

Sugar accumulation is not favoured by temperatures below 2.5°C (Liu and Kingston, 1992), and losses of sugar occur if cane experiences frost or chilling temperatures. Terminal buds and leaves of cane are killed by temperatures of -3.5°C, and entire above-ground stalks are killed at -5.5°C (Irvine, 1989). When all the above-ground tissues are killed, sugar concentration declines rapidly. Under these circumstances, the cane must be harvested quickly, before it deteriorates. If the CCS is less than 7, as it often is in the cooler areas where two-year crops are grown, mills are reluctant to process the cane, unless it is mixed with other cane with a higher CCS. Thus, high early sugar content is a way of over-coming losses due to frost, because frosted one year cane with a CCS >7 can be harvested. Although low temperature during winter promotes sugar accumulation, because growth and respiration are reduced more than is photosynthesis, chilling temperatures (-2.0 to 15°C) will reduce sugar accumulation, because photosynthesis is progressively inhibited as temperature falls. For example, sub-zero temperature that does not induce freezing can completely inhibit photosynthesis in the following day. In the absence of similar temperatures, photosynthesis gradually recovers after several days (K.R. Weaich *et al*, unpublished data). The impact of frost and chilling temperatures on photosynthesis of cane is being quantified by an SDRC-funded project, CSC3S (Ludlow, Weaich, Neilsen and Hughes).

Sugar accumulation will probably not be favoured by high temperature, because growth and respiration appear to increase more than photosynthesis (Figure 3). Unfortunately, there are no data on the comparative response of growth, respiration, and photosynthesis at supra-optimal temperatures. Such studies will be facilitated by the new CSIRO, Controlled Environment Facility at St Lucia.

Water stress

Water stress is also an important environmental stress that promotes sugar accumulation in rainfed sugar cane areas of Australia. In irrigated cane production in most parts of the world, water stress is induced by withholding water. Again, the sugar accumulation probably results from the greater sensitivity of the consumption, than the production of fixed carbon. Unfortunately, there are no data available for sugar cane. Data for sugar cane from South Africa (Inman-Bamber and de Jager, 1986) suggest that stem elongation rate is very sensitive to declining leaf water potential, such that it is zero about 1 MPa (Figure 4). However, these field data should be interpreted with great caution, because they are probably confounded by differences in temperature and root signals. Notwithstanding these limitations, it appears that stem, and presumably leaf, elongation is more sensitive to declining leaf water potential, than is leaf photosynthesis of sugar cane and tropical grasses (Figure 4; Ludlow *et al*, 1985; Ludlow *et al.*, 1991). Unfortunately, there are no good data for sugar cane.

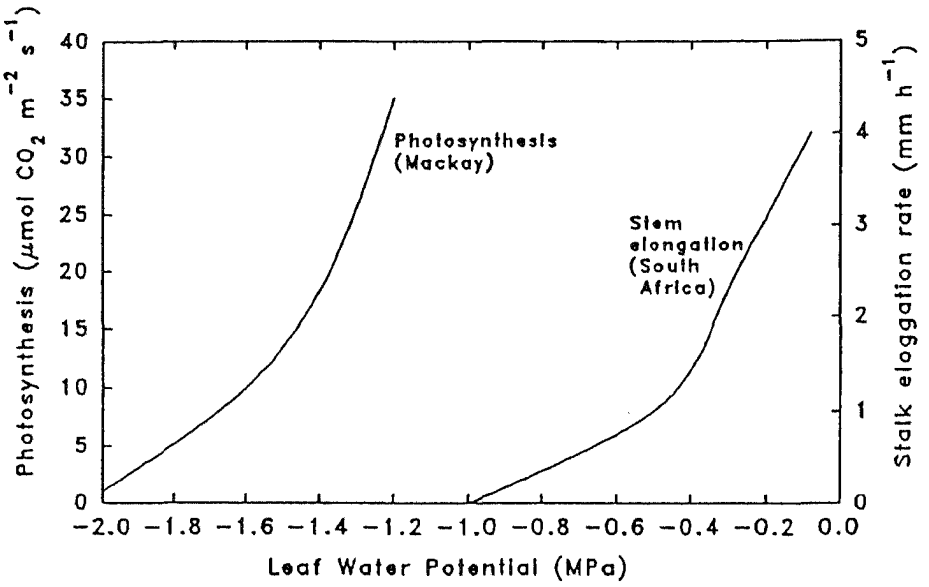


Figure 4. Influence of leaf water potential on leaf net photosynthetic rate (Ludlow *et al*, 1991) measured at Mackay, and stem elongation rate measured in South Africa (Inman-Bamber and de Jager, 1986).

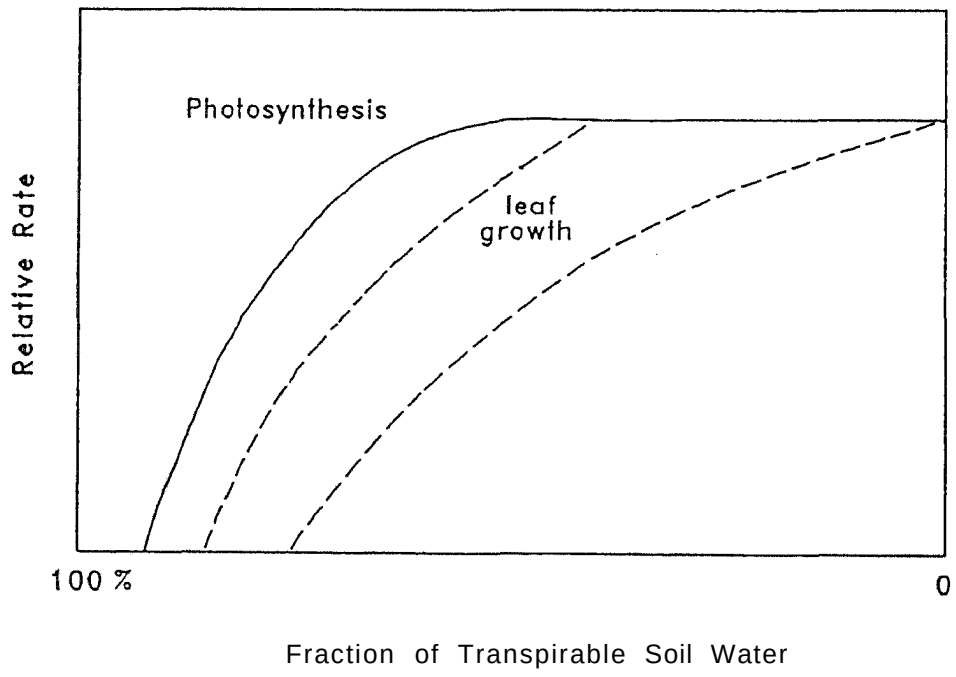


Figure 5 Hypothetical relationships between the fraction of transpirable soil water and photosynthesis and two possible scenarios for leaf growth in sugar cane.

Simulation models for maize and sorghum (Hammer and Muchow, 1991; Muchow and Sinclair, 1991) use relationships between available soil water and both photosynthesis and leaf expansion, which explicitly have leaf elongation rate more sensitive to soil water deficits than is photosynthesis (Figure 5). These models are able to simulate the growth and yield of these two tropical grasses. Therefore, by analogy, the relationships are probably similar in sugar cane. However, such relationships are not available for sugar cane.

Nitrogen stress

The relationship between nitrogen stress and sucrose accumulation has much the same physiological basis as the induction of water stress. There is much evidence (Alexander, 1973; Clements, 1980; Bowen and Anderson, 1992) to suggest that: (i) if too little nitrogen is applied, the cane becomes stressed too soon and cane yield and sugar accumulation suffer; (ii) if too much is applied, vegetative growth continues with little sugar accumulation in the stalks, and additionally in lodged cane many stalks will break and rot, and profuse suckering will smother the primary and secondary stalks that have lodged; and (iii) sufficient nitrogen should be applied for maximum growth early in the season, but the supply should run-out before the end of the growing season to ensure adequate sugar accumulation. In Hawaii, no nitrogen fertiliser is applied to the 24-month crop after it reaches 14 months of age, the theory being that the available nitrogen supplies in the soil will have become exhausted by harvest and the cane will be nitrogen stressed. The consumption of carbon by tropical C4 grasses is more sensitive to leaf nitrogen concentration than is the production of carbon (Wilson, 1975b). Although there are no comparative data for sugar cane, it appears that leaf expansion (assuming sugar cane behaves like a dicotyledon, *Solidago;* Hirose and Werger, 1987) is more sensitive to declining leaf nitrogen concentration than is leaf photosynthetic rate (Ludlow *et al*, 1991),

Anderson and Bowen (1990) have collated data on critical and optimum leaf nitrogen concentrations during early sugar cane growth, but no quantitative data are available on the relationship between leaf nitrogen and crop growth (radiation-use efficiency), nor on the desirable pattern of decline of leaf nitrogen to optimise both cane yield and sucrose accumulation. Dry matter production of C4 grasses is sensitive to leaf nitrogen . Muchow and Davis (1988) and Muchow and Sinclair (1993) have developed quantitative relationships between canopy radiation-use efficiency and specific leaf nitrogen for maize and sorghum. Data are being collected in SRDC Project CSC4S to allow similar relationships to be developed for both biomass and sucrose accumulation in sugarcane.

In contrast to water stress, it is much more difficult to develop management strategies to optimise leaf nitrogen as well as induce nitrogen stress when required to promote sugar accumulation. Crop nitrogen uptake is influenced by residual soil N, fertiliser applied, nitrogen losses associated with rainfall and temperature, and climatic factors influencing crop growth. There is a clear need to integrate both soil and crop processes into a simulation model, and to use the model to optimise nitrogen management for cane yield and

sugar accumulation. Data are being collected in *SKDC* Projects CSC4S and CSC7S to develop some of response functions for this approach, but more field-based research is required to obtain a better quantitative understanding of the interaction of climate and soil factors on sugar accumulation.

Conclusions

Some empirical management practices, which utilise the fact that stress enhances sugar accumulation, have been developed, and they have been successful. However, they are sometimes restricted in their application. New management practices will be required for new varieties, husbandry, and climates. In order to develop these practices in the most efficient and effective manner, we should harness the power of modem research technology, which combines simulation modelling and highly-focussed field experimentation. To utilise this new technology, we need to develop quantitative relationships between stresses and sugar accumulation, so that they can be incorporated in the models. This can be done with an integrated research program, which uses the comparative strengths of controlled environment and field experimentation.

References

Alexander, A.G. (1973). "Sugarcane Physiology". *(Elsevier:* Amsterdam, The Netherlands).

Anderson, D.L. and Bowen, J.E. (1990). Sugarcane nutrition, pp.24-25. (Potash and Phosphate Institute: Atlanta, USA).

Bowen, J.E. and Anderson, D.L. (1992). Sugar-cane cropping systems. *In:* "Ecosystems of the World: Field Crop Ecosystems". (Ed. C.J. Pearson) pp. 143-165. *(Elsevier:* Amsterdam, The Netherlands).

Bull, T.A. (1980). Limits to Sugar Cane Production: A Summary Review of Physiological Research at David North Plant Research Centre, from 1961 to 1977. Unpublished Paper, BSES, QLD, Australia.

Bull, T.A. and Glasziou, K.T. (1975). Sugar cane *In:* "Crop Physiology". (Ed. L.T. Evans) pp. 51-72. (Cambridge Univ Press: Cambridge, U.K.).

Clements, H.F. (1980). "Sugarcane Crop Logging and Crop Control: Principles and Practices". (University Press of Hawaii: Honolulu, USA).

Gosnell, J.M. (1970). Optimum irrigation levels for cane under burnt or trashed conditions. *Proc. South African Sugar Technol Assn.* 44: 121-130.

Hammer, G.L. and Muchow, R.C. (1991). Quantifying climatic risk to sorghum in Australia's semi-arid tropics and subtropics: model development and simulation. *In:* "Climatic Risk in Crop Production: Models and Management for the Semi-arid Tropics and Subtropics. (Eds. R C Muchow and J A Bellamy) pp. 205-232. (CAB International: Wallingford, U.K.).

Hirose, T. and Werger, MJ.A. (1987). Maximising daily canopy photosynthesis with respect to the leaf nitrogen allocation pattern in the canopy. *Oecologia* 72: 520-526.

Inman-Bamber, N.G. and de Jager, J.M. (1986). Effect of water stress on the growth, leaf resistance and canopy temperature in field-grown sugarcane. *Proc. South African Sugar TechnoL Assn.* pp. 156-161.

Irvine, J.E. (1989). Sugar cane anatomy, morphology and physiology. *In:* "Diseases of Sugar Cane, Major Diseases". (Eds. C. Ricaud, B.T. Egan, Ag Gillespie, and C.G. Hughes) pp. 1-17. *(Elsevier:* Amsterdam, The Netherlands).

Jones, C.A., Wegener, M.K., Russell, J.S., McLeod, I.M. and Williams, J.R. (1989). AUSCANE - Simulation of Australian Sugarcane with EPIC. CSIRO, Australia, Division of Tropical Crops and Pastures, Techn. Paper No. 29.

Kingston, G. (1972). An experiment in irrigation scheduling - further comments. *Proc. 37th Conf. Queens. Soc. Sugar Cane Technol.,* pp. 143-151.

Leverington, K.C, Kingston, G. and Skinner, S.O. (1970). An experiment in irrigation scheduling. *Proc. 37th Conf. Queens. Soc. Sugar Cane TechnoL,* pp. 51-56.

Liu, D-L and Kingston, G (1992). Sugar Accumulation in Sugarcane. Unpublished Review, BSES, Bundaberg.

Ludlow, M.M., Ferraris, R. and Chapman, L.S. (1991). Interaction between nitrogen and water supply on the photosynthetic rate of sugar cane leaves. *Proc. 1991 Conf. Aust. Soc. Sugar Cane TechnoL,* pp. 105-110.

Ludlow, M.M., Fischer, M.J. and Wilson, J.R. (1985). Stomatal adjustment to water deficits in three tropical grasses and a tropical legume grown controlled conditions and in the field. *Aust. J. Plant Physiol.* 12; 131-149.

Muchow, R.C. and Davis, R. (1988). Effect of nitrogen supply on the comparative productivity of maize and sorghum in a semi-arid tropical environment. II. Radiation interception and biomass accumulation. *Field Crops Res.* 18: 17-30.

Muchow, R.C. and Sinclair, T.R. (1991). Water deficit effects on maize yields modelled under current and "greenhouse" climates. *Agron. J.* 83: 1052-1059.

Muchow, R.C. and Sinclair, T.R. (1993). Response of leaf photosynthesis and canopy radiation use efficiency to leaf nitrogen in maize and sorghum. *Crop ScL* (in preparation).

Robinson, F.E., Campbell, R.B. and Chang, J.H. (1963). Assessing the utility of pan evaporation for controlling irrigation of sugarcane in Hawaii. *Agron. J,* 55: 444-446.

Wegener, M.K., Jones, C.A. and Russell, J.S. (1988). Simulating cane growth under Australian conditions. *Proc. 1988 Conf Aust. Soc. Sugar Cane TechnoL,* pp. 99-106.

Wilson, J.R. (1975a). Influence of temperature and nitrogen on growth, photosynthesis and accumulation of non-structural carbohydrate in a tropical grass, *Panicurn maximum* var. *trichoglume. Neth. J. Agric. Res.* 23: 48-61.

Wilson, J.R. (1975b). Comparative response to nitrogen deficiency of a tropical and temperate grass in the interrelation between photosynthesis, growth, and the accumulation of non-structural carbohydrate. *Neth. J. Agric. Res.* 23: 104-112.

Yates, R.A. (1983). Influence of pre-harvest temperature and rainfall on cane quality. *Proc. XVIII Cong. Inter. Soc. Sugar Cane TechnoL,* pp 291-308.

Discussion summary
(by J.R. Wilson)

Graham Kingston confirmed that data on the quantitative relationship between nitrogen nutrition and CCS were not available and there were indications from the discussion that there is no conscious N management for maximum CCS.

Mal Wegener said that the AUSCANE model used crude quantitative relationships for environmental effects on CCS, and that there was a need to refine these relationships to obtain better production estimates.

Graham Kingston expressed his view that providing these environmental and nutritional relationships would improve the capability of our current growth models, especially for prediction of improved management procedures in relation to water and nitrogen use.

Bob Mullins indicated that in this research the extension workers should be involved at an early stage.

Chemical Ripening of Sugar cane

G. KINGSTON
BSES
P.*O. Box 651*
Bundaberg, Qld. 4670

Introduction

Low CCS at the commencement, and sometimes at the end of the milling season, has an adverse effect on cane price and farm economics.

The favoured approach to the problem of low early CCS under Queensland conditions has been to rely on breeders to provide varieties with higher early CCS. Many other factors can improve early CCS, these include harvest of standover cane, sensible use of nitrogen fertiliser, good growth during December to March, followed by a cool dry autumn and selection of drier blocks.

Most of the above have a large degree of associated and uncontrollable risk as management factors because of interaction with climatic variables. Wet weather late in spring, which causes extension of the harvest into late November and December, will depress sucrose.

Chemical ripeners have been adopted as management tools to enhance ripening of sugarcane when conditions for natural ripening are poor.

Development of Chemical Ripeners

The first report of chemically induced ripening in sugarcane is attributed to Beauchamp (1949) who used 2,4-D, A major period of research activity from the early 1960s to 1976 culminated in commercial registration of Polaris N, N-bis (phosphono-methyl) glycine - in Hawaii in 1975 (Buren, 1975) and Ethrel - 2, chloroethyl phosphonic acid - in South Africa (Anon, 1976). Polaris is no longer used as a chemical ripener as it was superseded by a series of more active glyphosate products. Roundup - isopropylamine salt of glyphosate - is the major ripening chemical in Hawaii and Columbia and also has commercial registration in South Africa along with Fusilade Super (fluazifopbutyl).

No products are registered in Australia, although a wide range of chemicals including Ethrel and several glyphosates were tested in the period 1976-1977 (Kingston *et aL,* 1978). Ethrel was re-examined in the period 1987-1990 and should be registered for the 1993 milling season.

Development and adoption of chemical ripeners in the Australian sugar industry has lagged behind other canegrowing countries because of a shorter milling season, and generally favourable conditions for natural ripening which result in relatively high sucrose levels (Blume, 1983). Variable responses and low sugar prices led to the termination of ripener research in the 1970s.

Responses to Chemical Ripeners

Hilton *et al* (1980) reported an average response of 2.3 units pol % cane in the upper 13 internodes of seven varieties, in 2 year cane, treated with the glyphosate Mon 8000. Nickell (1988) concluded that commercial use of glyphosate in Hawaii had resulted in an average gain of 1.2 tonnes sugar/ha over un sprayed cane.

Anon (1976) showed an average yield increase of 0.7 tonnes sugar/ha, in 21 field strip experiments, from use of Ethrel in Swaziland, while Sweet *et aL* (1987) showed that a response of 0.53 units pol % cane to Ethrel can be increased by an additional 0.67 units by subsequent treatment with Fusilade Super.

Kingston (1988) showed that yield response from Ethrel in the variety H56-752 at Nambour declined from 1.9 to 0.4 tonnes sugar/ha from early July to Late September; the average response was 1.1 tonnes sugar/ha. In a summary of 21 replicated field experiments, with 8 varieties conducted throughout the Queensland sugar belt from 1987 to 1990, Kingston *et al,* (1991) concluded that Ethrel did not affect cane yield, and 70% of harvests resulted in an increase of at least 0.5 units of CCS.

Anon (1986) recommended that the economics of ripener use in southern Africa be based on a yield response of 0.8 tonnes sugar/ha. Kingston and Hurney (1988) showed that a yield response of only 0.24 tonnes sugar/ha is required to cover the chemical and application costs of Ethrel in Queensland, when the sugar price was $A275 per tonne.

Mode of Action of Chemical Ripeners

Ethrel - 2, chloroethyl phosphonic acid - releases ethylene during its decomposition. Ethylene is regarded as a plant growth regulator (Galston *et al.,* 1980). Ethrel reduces the size and weight of young developing leaves and the 1-2 internodes which were in the rapid elongation phase at the time of spraying, but elongation of subsequent internodes is often increased (Rostron, 1974; Donaldson and van Staden, 1989; Kingston *et al.,* 1991). The daily $C0_2$ uptake in July, of whole plants treated with Ethrel in April, suggested that there had been a marked reduction in photosynthesis compared with untreated cane (Rostron, 1974) The effect which resembled

that of moisture stress on $C0_2$ uptake, was partly due to a reduction in leaf area per plant after treatment with Ethrel, Ethrel does not suppress fresh weight cane yield and had little effect on cane dry matter yield because of an increased proportion of sugar in dry matter (Rostron, 1984). Treatment of relatively mature cane in the autumn, and spring results in stimulation of growth and lowering CCS (observations in semi-commercial field tests, 1989: Rostron, 1977; Kingston *et al*, 1978).

Glyphosate when applied to sugarcane at non-herbicidal rates promotes sucrose storage by inhibiting growth of the meristem (Maretzki *et al*, 1976; Maretzki and Thorn, 1978). Glyphosate is translocated throughout the stalk and stubble to regions of high metabolic activity (Nomura *et al*, 1974 and 1986). C^{14} studies showed that rate of fixation of $C0_2$, and rates of sugar translocation, were unaffected in leaves of plants 11 days after treatment with glyphosate (Maretzki and Thorn, 1978), however less of the fixed carbon appeared in cell walls and reducing sugars while more was directed to sucrose storage. Su *et al*, (1991) have reported that the glyphosate significantly reduced the activity of acid invertase, but not that of other enzymes; addition of auxin restored enzyme activity. Hence the ripening activity of glyphosate.

Ripening activity of Ethrel is restricted to the upper less mature sections of the stalk (Clowes, 1978), whereas glyphosate has the capability to ripen immature internodes and "load" sucrose into the lower stalk (Maretzki and Thorn, 1978; Clowes, 1980; Tianco and Gonzales, 1980).

Limitations to Use of Chemical Ripeners

Varietal reactions

The conclusion that adverse response, or lack of response, to Ethrel was more a reflection of the physiological state of the variety, rather than a varietal effect per *se* (Rostron, 1977) was generally supported by Kingston *et al* (1991) who showed that probability of response was inversely related to the propensity of a variety to produce higher levels of sugar in autumn and early winter. However a current South African variety N14 appears to be non-responsive to Ethrel, but provides good responses to Fusilade Super (R.A. Donaldson and H. Rostron, pers. comm.).

A number of important varieties in Hawaii did not respond to Polaris (Hilton *et al*, 1980), but the higher activity of glyphosate seems to have overcome this problem.

Effects on cane yield

Ethrel has no adverse effect on fresh weight yield of cane (Rostron, 1977; Donaldson and van Standen, 1989; Kingston *et al*, 1991) and treated fields are usually harvested 6 to 12 weeks after spraying. Delaying harvest beyond the Ethrel response window has no negative implications for cane yield. Treatment with Ethrel, however, does promote advanced suckering in varieties such as CP44-101 and Q124 (Kingston *et al*, 1991). Delaying

harvest of such fields beyond mid-August will have a negative impact on CCS because of the diluting effect of suckers.

Cane treated with glyphosate should be scheduled for harvest 3 to 9 weeks after spraying to avoid yield loss compared to unsprayed cane where there was no reduction in stalk mass because of inhibited apical growth.

Fusilade Super kills the apical meristem 3 to 4 weeks after spraying and must be harvested within 4-10 weeks of spraying to avoid loss of yield, relative to unsprayed cane (Rostron *et al.*, 1986; Donaldson and van Staden, 1989).

The glyphosate Mon 8000 induced severe stalk splitting and canopy desiccation in trials with Q90 in north Queensland (Kingston *et al*, 1978).

Carryover effects

No adverse carryover effects into the next ratoon crop have been reported for Ethrel or Fusilade Super. Concern at suppressed shoot population and shoot length in ratoons from crops previously sprayed with glyphosate have been expressed by (Kingston *et al*, 1978; Rice *et al*, 1984 and Sweet *et al*, 1987). However Donaldson and Inman-Bamber (1982) showed that significant carryover effects were likely only where the previous crop had been subjected to water stress between spraying and harvest.

Prediction of responses

The probability of response to Ethrel declines rapidly with increasing juice purity at spraying. For reliable responses pre-spraying juice purity should be less than 75% and preferably less than 70% (Rostron, 1975; Anon, 1986; Kingston *et al*, 1991). In areas such as Swaziland and parts of South Africa where Ethrel is used each year, purity testing and measurement of commercial responses have been abandoned because of the perceived reliability of response.

There are large seasonal and inter-district variations in the prevalence of good early season ripening conditions in the Australian sugar belt; pre-spraying purity testing is likely to be an important criterion until there is sufficient understanding of environmental control of the ripening processes to issue chemical ripening forecasts.

Variability in occurrence of stress conditions after spraying also presents some limitations to the Australian industry, *e.g.* there were very poor responses and a number of negative responses to Ethrel in 1989 associated with cloudy and prolonged wet weather in the 4-6 weeks after spraying. Severe vapour pressure deficits were presumed to have precluded responses to Ethrel in 1991 in an experiment at Bundaberg which was grown under irrigated conditions.

Length of the milling season

Extension of the milling season in Australia would increase the prospects for more widespread use of chemical ripeners.

Responses to crop ripeners in Australia are rarely obtained in cane harvested beyond late July to early August. Up to 30% of the crop could be cut in this period; the potential market would therefore be approximately 100 000 ha. Removal of non-responsive areas and lack of adoption because of small holdings is likely to limit adoption to less than 25 000 ha.

Application techniques

Unfavourable conditions for natural ripening and the large-scale of cane areas are strongly supported by plantation agriculture as factors which have influenced successful adoption of chemical ripening in Hawaii and Swaziland. On Australian cane farms, individual field management and harvest units can be as small as 0.5 to 1 ha, and are commonly 3 to 5 ha. These small areas are difficult to spray successfully by air and would require ground-based application techniques. Aerial spraying has a low level of public acceptance in the more closely settled farming areas.

Research Opportunities

1. An improved understanding and quantification of the sucrose accumulation process in relation to environmental variables will improve the ability to target crops suitable for chemical ripening. Temperature and moisture regimes are likely to be the most significant controlling parameters under Australian conditions.

   Responses to ripeners were no greater when cane yield was boosted by high applications of nitrogen, regardless of whether the soil has a high or low N mineralising capacity (Clowes and Inman-Bamber, 1980; Donaldson, 1986).

2. Useful semi-commercial responses to Ethrel were obtained in the Nambour area in 1989 and 1990 and in a large field strip trial at Rocky Point in 1988. The generally lower early season CCS in these districts and in New South Wales suggests that short-term targeting of these areas may have a high probability of success. Chances of success in NSW will be further enhanced by trends to 12 month-old cane, and the fact that crushing often commences in June.

3. Glyphosate and Fusilade Super should be evaluated for ripening activity in areas where there is a low probability of post-spraying water stress, or for use on younger early harvested cane which is destined for ploughout. These products could also be applied in October in years when a large crop indicates conclusion of the season later than November, and when the seasonal forecast is for above average rainfall.

4. Evaluate new products which show potential as chemical ripeners for sugarcane.

References

Anon, (1976). A chemical ripener for sugarcane. *South. Afr. Sugar J.* 60: 205-207

Anon, (1986). Ripeners. Information Booklet No. 4. South. Afr, Sugar Assn. Expt. Sta., Mt. Edgecombe, January 1986.

Beauchamp, C.E. (1949). A new method for increasing the sugar content of sugarcane. *Proc. 23rd, Ann. Conf. Assn. Tech. Azucar de Cuba,* p. 55.

Blume, H. (1983). Environment and cane sugar yield. *Proc, XVIII Cong, Int. Soc. Sugar Cane Technol.,* pp. 277-288.

Buren, L. (1975). Chemical ripener research succeeds. *Ann. Rep. Hawaiian Sugar Planters' Assn.,* p. 30.

Clowes, M.St. (1978). Early and late season ripening of sugarcane. *Proc, South. Afr. Sugar Technol. Assn.,* 52: 160-165.

Clowes, M.St. (1980). Ripening activity of the glyphosate salts Mon 8000 and Roundup. *Proc. XVII Cong. Int. Soc. Sugar Cane Technol.,* pp. 676-693.

Clowes, M.St., and Inman-Bamber, N. G. (1980). Effects of moisture regime, amount of nitrogen applied and variety on the ripening response of sugarcane to glyphosates. *Proc. South. Afr. Sugar Technol., Assn.,* 54: 127-133.

Donaldson, R. A. (1986). The effects of post-treatment moisture stress and varying amounts of applied nitrogen on the ripening responses of sugarcane to glyphosate and Ethrel. *Proc. South. Afr. Sugar Technol. Assn.* 60, 223-227.

Donaldson. R.A. and Inman-Bamber, N.G. (1982). Residual effect of glyphosate as a ripener on sugarcane. *Proc. South. Afr. Sugar Technol,, Assn.,* 56: 122-124.

Donaldson, R.A. and van Staden, J. (1989). A review of chemicals used as ripeners of sugarcane in South Africa. *Proc. XX Cong. Int. Soc. Sugar Cane Technol.,* pp. 647-654.

Galston, A.W., Davies, PJ. and Satter, RX. (1980). Hormonal control of dormancy senescence and stress. *In:* "The Life of the Green Plant" 3rd Ed. pp. 257-268 (Prentice-Hall, Inc.: Englewood Cliffs, N.J.

Hilton, H.W., Osgood, R.V. and Maretzki, A. (1980). Some aspects of Mon 8000 as a sugarcane ripener to replace Polaris. *Proc. XVII Cong. Int. Soc. Sugar Cane Technol.,* pp. 652-661.

Kingston, G. (1988). Chemical ripening of sugarcane - Time for reassessment in Australia. *Proc. 1988 Conf Aust. Soc. Sugar Cane Technol,,* pp. 157-162.

Kingston, G., Chapman, L.S. and Hurney, A. P. (1978). Chemical ripening of sugarcane - BSES experiments during 1977. *Proc. 45th Conf Qld. Soc. Sugar Cane Technol.,* pp. 37-43.

Kingston, G. and Hurney, A.P. (1988). Use of the growth regulator Ethrel in experiments to ripen sugarcane in 1988. Unpublished BSES Report, November 1988.

Kingston, G., Hurney, A.P. and Kwint, P. (1991). Chemical ripening of sugarcane to improve early season CCS. *Proc. 1991 Conf Aust. Soc. Sugar Cane Technol.,* pp. 1110-1115.

Maretzki, A., Thorn, M. and Moore, P.H. (1976). Growth patterns and carbohydrate distribution in sugarcane plants treated with an amine salt of glyphosate. *Hawaiian Planters' Record* 59: 21-32.

Maretzki, A. and Thorn, M. (1978). Effect of Mon 8000 on photosynthate distribution. *Ann. Rep. Hawaiian Sugar Planters' Assn.,* pp. 30-32.

Nickell, L.G. (1988). Plant growth regulator use in cane and sugar production - Update. *Sugar J.* 50: 7-11.

Nomura, N., Kameda, S., Yauger, W., Ching, S. and Hilton, W. (1974). Progress report on glyphosate. *Ann. Rep. Hawaiian Sugar Planters' Assn.,* pp. 37-38.

Nomura, N., Hayamichi, Y. and Hilton, W. (1986). Some physiological effects from glyphosate applied to sugarcane foliage. *Proc. XIX Cong. Int. Soc. Sugar Cane Technol,* pp. 284-297.

Rice, E.R., Holder, D.G. and Little, T.D. (1984). Recent tests on chemical ripening and regrowth of two sugar cane varieties in Florida. *Sugar J.* 47: 9-11.

Rostron, H. (1974). Chemical ripening of sugar cane with Ethrel and Polaris. *Proc. XIV Cong. Int. Soc. Sugar Cane Technol,* pp. 953-965.

Rostron, H. (1975). An assessment of chemical ripening of sugarcane in South Africa. *Proc. South. Afr. Sugar Technol Assn.,* 49: 160-163.

Rostron, H. (1977). A review of chemical ripening of sugarcane with Ethrel in southern Africa. *Proc. XVI Cong. Int. Soc. Sugar Cane Technol,* pp. 1605-1616.

Rostron, H., Barnes, D.A., Jenkins, CM., Marsh, RX, Park, R.I. and van Coller, A J. (1986). Recent sugar cane ripening experiments with Fusilade Super. *Proc. XIX Cong. Int. Soc. Sugar Cane Technol,* pp. 252-257.

Su, L.Y., Dela Cruz, A. and Moore, P.H. (1991). The relationship of glyphosate treatment to sugar metabolism in sugarcane : New physiological insights. *Ann. Rep. Hawaiian Sugar Planters' Assn.,* pp. 18-19.

Sweet, C.P.M., White, P.W. and Dodsworth, G.H. (1987). Commercial experience with chemical sugarcane ripeners at Simunye Sugar Estate in Swaziland. *South. Afr. Sugar J.* 71: 413-419.

Tianco, A.P. and Gonzales, M.Y. (1980). Effect of glyphosate ripener on growth response and sugar yield of sugarcane. *Proc. XVII Cong. Int. Soc. Sugar Cane Technol,* pp. 694-709.

Discussion summary
(by A. Maretzki)

There appears to be agreement that a chemical ripening program has a place in the Australian sugar industry.

Some concern was expressed that chemicals be chosen which do not destroy the photosynthetic apparatus of the plant and preferably cause limited physical damage to the apical tissue. A specific primary, inhibitory target reaction is preferred.

A question was raised regarding the effect on fibre content by the application of ethryl. The precise value is not known but it is probable that at least a 5% difference in CCS is needed under field conditions to detect a change. In Hawaii, increases in sucrose are considerably greater than changes in fibre content. It was pointed out that each 0.1 point increase in CCS raises the value of the crop by $2.70 per ton cane.

The danger of damage to neighbouring crops in the application of ripeners to sugarcane fields was discussed. The chemicals used as ripeners could be very damaging to such crops as mango or papaya, for instance.

A marked varietal difference in response to ripener was mentioned in the talk. A Canal Point variety was found to have a very low ripening response, while a Hawaiian variety had the highest response. This raised the question of whether there is a difference in response between temperate and tropical zone varieties in Australia. No such difference has been detected. Paul Moore pointed out that in other areas of the world temperate zone varieties tend to be less responsive to ripeners than tropical zone varieties.

General Overview of Sugar Metabolism and Storage

J.S. HAWKER
Department of Plant Science
University of Adelaide
Glen Osmond, SA. 5064

Introduction

A brief description of the topic has been presented recently, (Hawker *et al*, 1991) and should be consulted in conjunction with the following updated comments. Further recent and/or relevant information can be found in Hawker (1985), Preiss (1991), Slone and Buckhout (1991), and Gallet *et al*, (1992).

Hawker *et al*, (1991), referred to above, described pathways and control of pathways of sucrose and starch synthesis in leaves and storage organs and diagramatically illustrated the processes. More recent work has attempted to determine control coefficients for the partitioning of photosynthate between sugars and starch in leaves using mutants of higher plants in which the activity of one enzyme at a time is reduced (Neuhaus and Stitt, 1990). Although the approach has its problems and critics, it is at least a beginning in our understanding of steps which could be limiting synthesis of particular compounds of interest. In some plants mutants are not available, e.g. wheat, but it is possible to use heat treatment to get some insight into limiting steps in pathways. Although the classical approaches of Newsholme and Start (1973) and Kacser and Burns (1973) are not always strictly applicable because more than one enzyme is often affected it is or should be possible to determine which enzymes to manipulate for the improvement of crop yield or quality. Since the final proof of the importance of a control point will be in the production of a transformed plant, protracted studies of control theory will be time wasting. The proof of the pudding must be in the eating. In heated wheat ears, soluble starch synthase is the most affected enzyme, it has relatively low activity, there is a correlation between its activity and starch synthesis and studies of

metabolite levels all point to it limiting starch storage in the treated ears (C.R Jenner and J.S. Hawker, unpublished). Further work in wheat is concentrating on this enzyme.

Sucrose Metabolism

In a general treatment of sugar metabolism in plants it should be remembered that we are seeking control steps in sucrose accumulation in sugarcane from $C0_2$ and light entrapment by leaves all the way through the plant to maintenance of sucrose concentrations in the sugarcane stalks. Since Australia provides a ripening climate for sugarcane i.e. cool sunny winters in which growth stops while photosynthesis continues, it is likely that changes in stalk metabolism should be aimed for to increase the sucrose percentage of the cane. Sucrose is transported via the phloem from the leaves to the stalks. There is some evidence that sucrose synthase is involved in phloem loading and unloading (Tomlinson *et al,* 1991) but sucrose proton cotransport is also likely (Slone and Buckhout, 1991). Sugarcane stalks have a typical monocot anatomy with vascular bundles scattered in parenchyma. There is evidence, which is not universally accepted, that sucrose concentrations in the free space of the parenchyma approach those in the vacuoles (Hawker, 1985). It is important to resolve the whole question of the pathway and mechanism of transport of sucrose from leaves to stalk vacuoles to determine limiting steps. Other speakers will cover these processes in more detail but it is relevant to note here that there is likely to be a controversy over the role of sucrose synthase in sinks as an indicator of sink strength as supported by Claussen *et al,* (1986) and the schools of Black (Sun *et al,* 1992) and Davies (Ross and Davies, 1992) and recently denied by Geigenberger and Stitt (1992). Whether sucrose synthase is limiting in sinks or whether it is present in sufficient amounts to supply enough substrates for sinks (sucrose to UDP glucose and fructose) and whether there are species differences should be evident after further work. Of course, the presence and roles of invertases are further complications (Ranwala *et aL,* 1992). Studies of mutants and/or transgenic plants should provide answers of a general nature but specific plants may need to be investigated for specific answers.

Accumulation of Sucrose in Stem Parenchyma

The rate of sucrose accumulation in the vacuoles of sugarcane stalks needed to maintain the high concentrations present depends on the gradient of sucrose between the free space and the vacuole and this may or may not be large depending on the concentration of sucrose in the free space. Since xylem concentrations of sucrose are not high there cannot be high concentrations of sucrose throughout the stem free space. There is an ill-defined endodermis present and it is tempting to speculate that it may have a role in sucrose transport and separation of the free space into parenchyma and stele. The pathways and control point(s) of sucrose movement from phloem to storage vacuoles need to be determined in sugarcane stems and a possible role of sucrose synthase should not be ignored because of its suggested involvement in translocation and sinks in many plants.

Vacuolar sucrose carriers have been described as either energy dependent (redbeet, sugar beet, Japanese artichoke) or energy independent (facilitated diffusion; barley, sugarcane) (see Keller, 1992). The proposed group translocator at the tonoplast involving sucrose phosphate has fallen into disrepute but cannot be ignored (see Hawker, 1985; Hawker *et al*, 1991). Some enzymes in sugarcane stem which might warrant manipulation are sucrose synthase, invertases, sucrose phosphate synthase and/or sugar carriers to increase sucrose storage. It is of interest to note that the equilibrium constant for sucrose phosphate synthase is close to that of sucrose synthase (Barber, 1985) which could change future approaches to research into sucrose storage.

Inorganic Pyrophosphate Role

The importance of PPi (inorganic pyrophosphate) in plant metabolism was highlighted by Hawker *et al*, (1991). Recent papers include these by Wong *et al*, (1990), Claassen *et al*, (1991) and Viola and Davies (1991). The relationships between PPi, PPi phosphofructokinase, fructose 2, 6-P and other metabolites and enzymes are possible control sites in sugarcane stalks if work with leaves is any guide. The regulatory properties of fructose 2, 6-P in micromolar concentrations makes it worth considering in any carbohydrate pathway. In Hawker *et al*, (1991) glucose 1-P was illustrated as the carbohydrate transported across the amyloplast envelope. Recent work has suggested that glucose 6-P may be the transported sugar (Kiss and Sack, 1989; Hill and Smith, 1991). ADPglucose has been shown to be transported across isolated chloroplast and amyloplast envelopes but no transport has been demonstrated *in vivo* (Pozueta-Romero *et al*, 1991).

Potential Modifications of Sucrose Metabolism

The prodigious work of Stitt and his colleagues referred to by Hawker *et al*, (1991) has continued and includes work on flux-control coefficients in transgenic tobacco plants expressing yeast invertase (Stitt, Schaewen and Willmitzer, 1990), and inhibition of photosynthesis by supplying glucose to detached leaves of spinach (Krapp *et al*, 1991). Presumably experiments and manipulation of sugarcane by techniques evolving (Lorz *et al*, 1988; Franks and Birch, 1992) will allow modification of carbohydrate metabolism along the lines done by Stitt.

Trends in current research in carbohydrate metabolism can be seen in the abstracts of the Society of Experimental Biology, Lancaster Meeting, 1992 on pages 10-16. Phloem transport of sucrose, sucrose-degrading enzymes, sucrose synthase, sucrose phosphate synthase, the phosphorylation of sucrose phosphate synthase, turgor regulation of sucrose synthesis and sucrose as a novel plant growth regulator are some of the topics. Further recent reports of work on sugar metabolism which illustrate the importance of sucrose phosphate synthase, invertases and hexose transporters in sugar synthesis and accumulation in plants have recently appeared (Rausch, 1991; Castrillo *et al*, 1992; Dali and Yelle, 1992; Ranwala *et al*, 1992.

Future research targets could include modification of plants to increase rates of sucrose translocation, rates of sucrose movement into storage vacuoles and/or free space, maintenance of higher concentrations of sucrose in vacuoles and/or free space, and different partitioning of carbohydrate between sucrose and cell wall material. Identification of control steps in sucrose storage and cell wall synthesis might allow modification of enzyme activity or efficiency to achieve higher levels of sucrose or faster filling of stalks.

References

Barber, G.A. (1985). The equilibrium of the reaction catalyzed by sucrose phosphate synthase. *Plant Physiol.* 79: 1127-8.

Castrillo, M., Kruger, N.J. and Whatley, F.R. (1992). Sucrose metabolism in mango fruit during ripening. *Plant Sci.* 84: 45-51.

Claassen, P.A.M., Budde, M.A.W., de Ruyter, H.J., van Calker, M.H. and van Es, A. (1991). Potential role of pyrophosphate : fructose 6 - phosphate phosphotransferase in carbohydrate metabolism of cold stored tubers of *Solanum tuberosum* cv Bintje, *Plant Physiol* 95: 1243-9.

Claussen, W., Loveys, B.R. and Hawker, J.S. (1986). Influence of sucrose and hormones on the activity of sucrose synthase and invertase in detached leaves and leaf sections of eggplants *(Solanum melongena), J. Plant Physiol.* 124: 345-57.

Dali and Yelle. (1992). Suc-P synthase in Tomato sugar accumulation. *Plant Physiol* 99: 434-438.

Franks, T. and Birch, R.G. (1992). Gene transfer into intact sugarcane cells using microprojectile bombardment. *Aust. J.Plant Physiol* 18:471-80.

Gallet, O., Lemoine, R., Gaillard, C, Larrson, C. and Delrot, S. (1992). Selective inhibition of active uptake of sucrose into plasma membrane vesicles by polyclonal sera directed against a 42 Kilodalton plasma membrane polypeptide, *Plant Physiol* 98: 17-23.

Geigenberger, P. and Stitt, M. (1992). Sucrose synthase degrades sucrose by a reversible reaction within the phloem and in cells receiving sucrose via symplastic unloading. Soc. Exp. Biol. Abs. Lancaster Meeting, pp.13.

Hawker, J.S. (1985) Sucrose. *In:* "Biochemistry of Storage Carbohydrates in Green Plants". (Eds P.M. Dey and R.A. Dixon), pp. 1-51, (Academic Press: New York).

Hawker, J.S., Jenner, C.F. and Niemietz, CM. (1991). Sugar metabolism and compartmentation. *Aust.* J. *Plant Physiol* 18: 227-37.

Hill, L.M. and Smith, A.M. (1991). Evidence that glucose 6-phosphate is imported as the substrate for starch synthesis by the plastids of developing pea embryos. *Planta* 185, 91-96.

Kacser, H. and Burns, J.A. (1973). The control of flux. *Symp. Soc. Exp. Biol* 27: 65-107.

Keller, F. (1992). Transport of stachyose and sucrose by vacuoles of Japanese artichoke *(Stachys sieboldii)* tubers. *Plant Physiol* 98: 442-5.

Kiss, J.Z. and Sack, F.D. (1989). Reduced gravitropic sensitivity in roots of a starch deficient mutant of *Nicotiana sylvestris. Planta* 180: 123-30.

Krapp, A., Quick, W.P. and Stitt, M. (1991). Ribulose-1, 5-bisphosphate carboxylase-oxygenase, other Calvin-cycle enzymes, and chlorophyll decrease when glucose is supplied to mature spinach leaves via the transpiration stream. *Planta* 186: 58-69.

Lorz, H., Gobel, E. and Brown, P. (1988). Advances in tissue culture and progress towards genetic transformation of cereals. *Plant Breeding* 100: 1-25.

Neuhaus, H.E. and Stitt, M. (1990). Control analysis of photosynthate partitioning. Impact of reduced activity of ADP-glucose pyrophosphorylase or plastid phosphogluco-mutase on the fluxes to starch and sucrose in *Arabidopsls thaliana* (L.) Heynh. *Planta* 182; 445-54.

Newsholme, E.A. and Start, C. (1973). "Regulation in Metabolism" (Wiley, Chichester).

Pozueta-Romero, J., Ardila, F. and Akazawa, T. (1991). ADP-glucose transport by the chloroplast adenylate translocator is linked to starch biosynthesis. *Plant Physiol* 97; 1565-72.

Preiss, J. (1991). Biology and molecular biology of starch synthesis and its regulation. *Oxford Surveys Plant Molec. Cell Biol.* 7: 59-114.

Ranwala, A.P., Suematsu, C. and Masuda, H. (1992) Soluble and wall-bound invertases in strawberry fruit. *Plant Sci.* 84: 59-64.

Rausch, T. (1991). The hexose transporters at the plasma membrane and the tonoplast of higher plants. *Physiol. Planta.* 82: 134-42.

Ross, H.A. and Davies, H.V. (1992). Sucrose metabolism in tubers of potato *{Solanum tuberosum* L.). Effects of sink removal and sucrose flux an sucrose-degrading enzymes, *Plant Physiol.* 98; 287-93.

Slone, J.S. and Buckhout, T.J. (1991) Sucrose-dependent H^+ transport in plasma-membrane vesicles isolated from sugarbeet leaves *(Beta vulgaris* L.). Evidence in support of the H^+-symport model for sucrose transport. *Planta.* 183; 584-9.

Stitt, M., von Schaewen, A. and Willmitzer, L. (1990). "Sink" regulation of photosynthetic metabolism in transgenic tobacco plants expressing yeast invertase in their cell wall involves a decrease of the Calvin-cycle enzymes and an increase of glycolytic enzymes. *Planta.* 183: 40-50.

Stitt, M., Quick, W.P., Schurr, U., Schulze, E.D., Rodermel, S.R. and Bogorad, L. (1991). Decreased ribulose-1, 5-bisphosphate carboxylase-oxygenase in transgenic tobacco transormed with "antisense" rbc S. II Flux-control coefficients for photosynthesis in varying light, $C0_2$, and air humidity. *Planta.* 183: 555-66.

Sun, $J._r$ Loboda, T., Sung, S.S. and Black, C.C. (1992). Sucrose synthase in wild tomato, *Lycopersicon chmielewskii,* and tomato fruit strength. *Plant PhysioL* 98: 1163-9.

Tomlinson, P.T., Duke, E.R., Nolte, K.D. and Koch, K.E. (1991). Sucrose synthase and invertase in isolated vascular bundles. *Plant PhysioL* 97: 1249-52.

Viola, R. and Davies, H.V. (1991). Fluoride-induced inhibition of starch biosynthesis in developing potato, *Solanum tuberosum* L., tubers is associated with pyrophosphate accumulation. *Plant PhysioL* 97: 638-43.

Wong, J.H., Kiss, F., Wu, M, and Buchanan, B.B. (1990). Pyrophosphate fructose-6-P 1-phosphotransferase from tomato fruit. Evidence for change during ripening. *Plant PhysioL* 94: 499 506.

Discussion summary
(by Paul Moore)

Sucrose in the apoplasrn may be involved in apoplastic transport or in retrieval mechanisms as it is leaked from the cytosol.

Controls in the leaf determine the partitioning between sucrose and starch through:-

(a) a feed forward mechanism by which triose-P and 3PGA, exported from the chloroplast, inhibit synthesis of Fructose 2, 6-P, which in turn increases Fl, 6-P hydrolysis and allows synthesis of sucrose to proceed.

(b) a feed back mechanism such that when sucrose synthesis exceeds the rate of withdrawal of sucrose from the cytosol, build up of sucrose feeds back to inhibit both the synthesis and hydrolysis of sucrose phosphate resulting in build up of F6-P. F6-P inhibits breakdown of F2,6-P which slows sucrose synthesis, accumulates 3PGA and leads to starch synthesis.

In the sink, sucrose is metabolized by either one of three invertases (cell wall acid, cytosolic neutral, or vacuolar acid invertase) or by sucrose synthase to produce 2F1,6-P.

It is the balance of these leaf and sink reactions that control sucrose accumulation. Learning how to control (manipulate) the leaf and sink regulatory steps might lead to increased sucrose storage in sugar cane.

Metabolic Regulation and Genetic Engineering of Sucrose and Starch Synthesis in C_4 Leaves

R.T. FURBANK
CSIRO Division of Plant Industry
P.O. Box 1600
Canberra ACT 2601

Introduction

In this presentation I will briefly review the mechanism and regulation of sucrose and starch synthesis in leaves of C4 plants. Opportunities for manipulating these processes using genetic engineering and transformation of sugarcane will be discussed.

While our understanding of the regulation of sucrose and starch synthesis has advanced considerably only in the last decade, the pathway of starch and sucrose synthesis and the location of the enzymes involved has been known since the 1970's (for a comprehensive review see Stitt *et al*, 1987; Stitt and Quick, 1989). Despite the recent concentrated effort in this area, very little information is available on the enzymology and regulation of sucrose and starch synthesis in sugar-cane or most other C4 leaves. Work with maize (Furbank and Foyer, 1988) suggests that a compartmentation of starch and sucrose synthesis occurs where, under normal growth conditions, starch is synthesised almost exclusively in the bundle sheath cells, and sucrose in the mesophyll cells (Figure 1). Whether this compartmentation can be extrapolated to other C4 species is doubtful, however the localisation of starch in the bundle sheath chloroplasts of sugarcane (Alexander, 1973) suggests that the hypothesis may hold for this species at least.

The key regulatory enzymes in leaf sucrose synthesis are believed to be cytosolic fructose 1,6-bisphosphatase (FBPase) and sucrose phosphate synthase (SPS). In this discussion these enzymes will form the basis for a strategy to genetically manipulate end-product synthesis in C4 plants. The extractable activities of these two enzymes are only just sufficient to account for rates of foliar sucrose synthesis and they appear to be closely regulated in vivo (Stitt and Quick, 1989). Under most conditions, the activities of

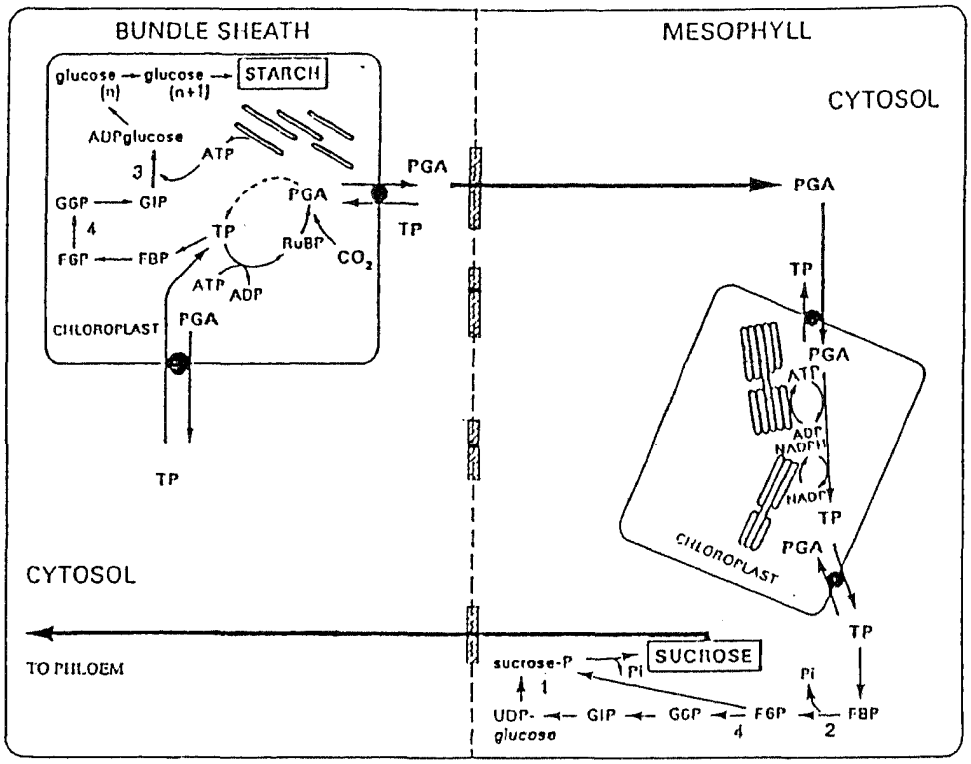


Figure 1. The compartmentation of sucrose and starch synthesis in C4 leaves. Cycling of the 3-C compounds phosphoglycerate (PGA) and triose phosphate (TP) between bundle sheath and mesophyll is also shown. Key enzymes mentioned in the text are: 1. sucrose phosphate synthase; 2. fructose 1,6-bisphosphatase; 3. ADP-glucose pyrophosphorylase; 4. phosphoglucose isomerase.

these enzymes track the photosynthetic rate but this is not always the case as it is thought that SPS and FBPase are also control points for partitioning of photosynthate between starch and sucrose (Stitt *et al*, 1987). FBPase activity is controlled mainly by the regulatory metabolite fructose 2,6-bisphosphate (F2,6P2), a potent allosteric inhibitor of FBPase. Cellular levels of this metabolite change in response to the photosynthetic rate and environmental conditions, providing both a feed-back and feed-forward control of this step which is very sensitve to the availability of photosynthate, light and C02. SPS is regulated by a complex combination of covalent modification by protein phosphorylation, allosteric regulation by metabolites and diurnal changes in the amount of SPS protein. There is a large amount of variation between species in the types of regulation present and little information is available for C4 plants other than maize.

Does Capacity for Leaf Sucrose Synthesis Limit Sucrose Accumulation?

A first step in addressing the problem of improving sugar accumulation in sugarcane via metabolic engineering must be to identify rate limiting steps in the process as a whole. The process of sugar accumulation may be limited (or co-limited) at a number of steps, some of which are listed below:

1. Leaf reactions, e.g. photosynthetic rate, enzymes of sucrose synthesis, carbon partitioning within the leaf.

2. Rate of phloem loading and transport to the ripening stalk.

3. Rate of sucrose transport into the storage parenchyma and vacuoles.

4. Genetic/developmental constraints of a variety, e.g. duration of maturation and "filling" capacity of the stalk tissue.

Any potential for improvement of sugar yield by manipulation of leaf reactions is based on the premise that these processes at the very least co-limit sugar accumulation. Once again, information bearing on this point is scarce. A study of photosynthetic rate (on a leaf area basis) suggests that high yielding cultivars of sugarcane have lower maximum rates of photosynthesis than their progenitors (Irvine, 1967). Most of the productivity gains in C3 cereals seem to be correlated with changes in leaf morphology (more leaf area; longer, narrower leaves (Wardlaw, 1990). However, studies on C4 plants (e.g. sorghum) show close correlations between maximum photosynthetic capacity and both grain yield and biomass production (Peng *et al*, 1991). Since the cold ripening phenomenon in Australia relies on continued high rates of photosynthesis while stalk growth is reduced, it may be that the integrated capacity for sucrose synthesis of all foliage over the ripening period is an important limiting factor in determining stalk sugar content.

Molecular "Tools" for Metabolic Engineering of Sucrose Synthesis

The following molecular techniques have been used to study rate limitations in metabolic pathways and to control flux through pathways in transgenic C3 plants and micro-organisms.

1. Over-expression of a native gene coding for a "rate - limiting" enzyme to relieve a potential "bottle-kneck" in a pathway.

2. Expression of a "foreign" gene coding for an enzyme from another species or organism. This technique can be used to divert flux into another product or eliminate metabolic control of a step if the foreign enzyme is not subject to the same regulatory mechanisms as the native enzyme.

3. Gene suppression (antisense and ribozyme technology) can be used to reduce the amount of a key enzyme to examine its role in determining flux or to divert flux from one pathway to another where there is competition for common substrates.

The application of these techniques to stalk localised enzymes of sucrose synthesis and breakdown will be dealt with elsewhere in this workshop. There is considerable scope for the use of transgenic sugarcane in elucidating rate limiting steps in sucrose accumulation and possibly for improvement of sugar production. Ways in which these techniques could be applied to the leaf reactions are set out below.

Over-expression of SPS and cytosolic FBPase in sugarcane would be a good starting point in a program of this nature. SPS from maize has recently been cloned and a cDNA sequenced (Worrell *et al*, 1991). This cDNA could be expressed in sugarcane under the control of a strong leaf specific promoter to relieve a bottle-kneck at this point. Maize SPS has recently been expressed in tomato (Worrell *et al.*, 1991) causing a diversion of photosynthate from starch toward sucrose. Also, evidence from this work suggests that maize SPS expressed in tomato is no longer strictly regulated. A program aimed at expressing maize SPS in the C_4 plant *Flaveria bidentis* is currently underway in CSIRO, Division of Plant Industry.

Cytosolic FBPase is also amenable to immediate application of the over-expression strategy as a cDNA for this protein has been cloned from C3 species (Hur *et al.*, 1992). Transformation of sugarcane with the C3 form of this enzyme might alter rates of sucrose synthesis as the C3 enzyme has a higher affinity for its substrate than the C_4 form (M. Stitt, pers commun).

One way in which the over-expression and gene suppression strategies might be used to promote sucrose synthesis in sugarcane is to attempt to divert carbon away from starch toward sucrose. Although modern cane varieties have low starch levels in the stem, leaf starch may form up to 4% of leaf dry matter at the end of the photoperiod (Alexander, 1973). As discussed above, over-expression of enzymes in sucrose synthesis may shift carbon partitioning although evidence from C_3 mutants deficient in the starch synthesis enzyme phosphoglucose isomerase (PGI) suggests that restriction of starch synthesis may inhibit photosynthesis rather than divert carbon toward sucrose (Kruckeberg *et al.*, 1989). In contrast, potatoes in which expression of the starch synthesis enzyme ADP-glucose pyrophosphorylase has been suppressed by antisense (Muller-Rober *et al*, 1992) showed increased sugar content of both leaves and tubers, suggesting that a constitutive reduction in enzyme activity can affect the carbohydrate content of both source and sink tissue. Although these genes would have to be cloned in sugarcane to generate effective antisense constructs, these initial studies show great promise.

Lastly, if the rate of sucrose synthesis in the leaf can limit sugar accumulation in the stalk and if, ultimately, all photosynthate is converted efficiently to stalk sucrose, we must aim to improve the rate of photosynthesis. Traditional plant breeding could be used to increase total photosynthate production over the life of the cane foliage, particularly during ripening. C_4 photosynthesis is substantially inhibited by low temperatures. The site of this inhibition has not been elucidated. Work currently underway on *Flaveria* using molecular approaches to determine the

rate limiting steps in C4 photosynthesis should help direct attempts to improve photosynthetic capacity in other C4 species.

References

Alexander, A.G. (1973). "Sugarcane Physiology" *(Elsevier:* Amsterdam).

Furbank, R.T. and Foyer, C.H. (1988). C_4 plants as valuable model experimental systems for the study of photosynthesis. *New Phytol.* 109: 265-277.

Hur, Y., Unger, E.A. and Vasconcelos, A.C. (1992). Isolation and characterisation of a cDNA encoding cytosolic fructose 1,6-bisphosphatase from spinach. *Plant Mol. Biol.* 18: 799-802.

Irvine, J.E. (1967). Photosynthesis in sugarcane varieties under field conditions. *Crop Sci.* 7: 297-300

Kruckeberg, AX., Neuhaus, H.E., Feil, R., Gottleib, L.D. and Stitt, M. (1989). Reduced activity mutants of phosphoglucose isomerase in the cytosol and chloroplast of Clarkia xantiana. *Biochem. J.* 261: 457-467.

Muller-Rober, B., Sonnewald, U. and Willmitzer, L. (1992). Inhibition of ADP-glucose pyrophosphorylase in transgenic potatoes leads to sugar storing tubers and influences tuber formation and expression of tuber storage protein genes. *EMBO J.* 11: 1229-1238.

Peng, S. Krieg, D.R. and Girma, F.S, (1991), Leaf photosynthetic rate is correlated with biomass and grain production in grain sorghum lines. *Photosyn. Res.* 28: 1-7.

Stitt, M., Huber, S. and Kerr, P. (1987). Control of photosynthetic sucrose formation. *In:* "The Biochemistry of Plants", Vol 10, (Academic Press: NY.).

Stitt, M. and Quick, P. (1989). Photosynthetic carbon partitioning. Its regulation and possibilities for manipulation. *Physiol. Planta. 11:* 633-641.

Wardlaw, I.F. (1990). The control of carbon partitioning in plants. *New PhytoL* 116:341-381.

Worrell, A.C, Bruneau, J-M., Summerfelt, K. and Boersig, M. (1991). Expression of a maize sucrose phosphate synthase in tomato alters leaf carbohydrate partitioning. *The Plant Cell* 3: 1121-1130.

Discussion summary
(by R. Henry)

Andi Maretzki pointed out that the tomato sucrose phosphate synthase had been overexpressed in leaves of tomatoes, and shifted synthesis from starch to sucrose.

John Patrick indicated that the role of photosynthesis in sugar accumulation did not need to be defended. There was then discussion that physiological experiments were needed to determine whether sugar accumulation in the leaf during the day caused feedback inhibition of photosynthesis in sugar cane. Merv Ludlow emphasised the importance of the photosynthetic rate per unit leaf area. This was followed by a general discussion of photosynthesis. George Stewart introduced the topic of leaf angle and area to the discussion.

Robert Furbank (when pressed by Robert Henry) agreed that the overexpression of sucrose phosphate synthase in sugarcane leaves was likely to be successful in achieving higher levels of sugar accumulation in the leaves. The question of transport to the stalk and resulting accumulation in the stalk was not so easy to predict. Robert Furbank indicated that maize SPS clones were available for testing in sugar cane.

Sugar Transport to Stalk

J.W. PATRICK
Department of Biological Sciences,
University of Newcastle, NSW 2308

Introduction

Photosynthate flows from source leaves through the phloem path to the various sink organs. Whole leaf photosynthetic rate and partitioning of reduced carbon to the transport pool within the leaf determines the instantaneous amount of photosynthate available for transport. Path and sink transport characteristics determine the pattern of partitioning of exported photosynthate between the competing sink regions. This presentation focuses on potential regulation of photosynthate flow by the path and sink components of the transport catena.

The current consensus is that phloem translocation exhibits characteristics consistent with mass flow, driven by the turgor potential gradient established between the source and sink ends of the phloem pathway (Minchin and Thorpe, 1987a). Thus, determinants of flow along the phloem path may include cross-sectional area (i.e. number of sieve tubes) and limiting path radius (i.e. sieve pores) as well as viscosity and concentration of the translocated solution. In contrast, to directly influence translocation rate, all sink functions must act to alter the turgor potential at the sink end of the transport path.

Path Versus **Sink Control of Assimilate Transport**

The saturation kinetic relationship of sink metabolism/compartmentation with the size of the sink photosynthate pool can be used to deduce the influence of path and sink on phloem transport. For sink-limited import (i.e. photosynthate saturation of metabolism/ compartmentation), increases in the maximal rate of sink metabolism/compartmentation can be shown to be realised fully by feedback mass action adjustments in the rate of phloem import without any need for change in phloem transport properties (Patrick, 1988; 1991). In contrast, for source-limited conditions (i.e. photosynthate supply limiting metabolism/compartmentation), mass action induced decline in sink pool size can be demonstrated to lead to an attenuated realisation of the sink's potential for photosynthate metabolism/compartmentation (Patrick,

1988; 1991). Under these conditions, a coordinated increase in the transport properties of the phloem path has the potential to ensure that the sink pool size remains unaltered and as a result, the sink potential would be realised fully.

The above analysis demonstrates that path control only could be of significance under source-limited conditions. These conditions pertain to sinks undergoing rapid cell division (Patrick, 1988) and to plants subjected to various forms of environmental stress (Wardlaw, 1990). It is speculated that the hydraulic properties and numbers of functional sieve elements of a differentiating phloem pathway could impose limitations on photosynthate import by meristems (Patrick, 1988). Since the remaining phloem path, including that of the intercalary meristems (Patrick, 1972; Wood, 1992), has spare transport capacity (Wardlaw, 1990), it is unlikely that photosynthate transport to established stem storage sinks would be controlled by sieve element transport properties. However, the hydraulic properties of specific vascular interconnections at the nodal regions may exert some influence on partitioning patterns between aero- and basipetal flows of photosynthate (Patrick and Wardlaw, 1984). Longitudinal flow rate is also influenced by the ability of the phloem path to buffer the concentration of transported photosynthate through exchange with the stem apoplast (Minchin *et al.*, 1984) and ultimately the stem symplast (Wardlaw, 1990). An osmoregulatory mechanism probably accounts for the former phenomenon in which sugars and potassium are used interchangeably (Smith and Milburn, 1980). Indeed, lateral exchange of potassium might act to regulate phloem turgor and hence flow rates to particular sink regions (Lang, 1983).

For sink-limited conditions, sucrose accumulation from the phloem path ultimately must be determined by the capacity of the stem sink tissues to metabolically interconvert or compartment sucrose.

Cellular Pathway of Radial Transfer from the Phloem

The rate of and extent to which alterations in potential sink accumulation capacity are realised may be influenced significantly by the characteristics of radial transfer from the sieve elements to the sites of utilisation in the stem. This claim is explored beginning with a consideration of the possible cellular pathways for radial transfer.

The mechanism and hence control of photosynthate transfer from the sieve elements to the sink cells, at least to some extent, will be determined by the cellular pathway followed. Therefore, before any sensible progress can be made regarding the mechanism and control of radial transfer, the cellular pathway needs to be be elucidated. This alters during organ development. In the case of both root and shoot apical meristems, flow from the differentiating phloem path is through the symplast (Patrick, 1991). A similar conclusion has been drawn for the hook region of etiolated pea shoots (Gougler Schmalstig and Cosgrove, 1990). For these tissues, solute unloading from the phloem displays characteristics consistent with a mass flow mechanism regulated by wall extensibility of the recipient sink cells (Gougler Schmalstig and Cosgrove, 1990). In addition, Meshcheryakov

et al., (1992) demonstrated that water and solute supply, together with their partitioning between apoplast and symplast compartments, can influence turgor gradients and growth rates. Whether similar unloading pathways and mechanisms are operative within the intercalary meristems of monocotyledenous stems remains to be determined. With progression of stem development to a phase dominated by cell extension, radial photosynthate transfer appears to include an apoplastic component (Glasziou and Gayler, 1972; Minchin *et al.,* 1984; Gougler Schmalstig and Cosgrove, 1990; Wood, 1992). Correlates of stem growth rates with extracellular invertase activities have implicated this enzyme as a key regulant of photosynthate unloading in elongating sugarcane stems (Glasziou and Gayler, 1972). These experiments could be repeated to include measurements of the transmembrane sucrose concentration gradient from the unloading cells to the apoplast talcing into account water supply effects on apoplast solute content (Meschcheryakov *et aL,* 1992). The integration of invertase activity with growth demand could be mediated through apoplast pH regulated by plasma membrane H+-ATPases (Eschrick, 1980).

Glasziou and Gayler (1972) speculated that transfer from the sieve elements in mature sugarcane stems could follow a symplastic pathway but on balance favoured an apoplastic route. Similar conclusions have been drawn for stems of Vicia faba infected by the vascular parasite, Cuscuta, the haustoria of which encapsulate the host sieve elements but do not invade the host cytoplasm (Wolswinkel, 1986). Responses of the apoplast sugar pool size to alterations in the rate of phloem transport through *Phaseolus* stems were found to be quantitatively consistent with radial transport of photosynthates that includes an apoplastic step (Patrick and Turvey, 1981; Hayes and Patrick, 1985). A quantitative appraisal of the plasma membrane surface area of the se-cc complexes indicated that this was adequate to support the observed rates of photosynthate unloading (Hayes *et al.,* 1985). However, these structural studies also demonstrated that there were sufficient plasmodesmata present to permit passage through a potential symplast route at observed rates of radial transfer (Hayes *et al.,* 1985). Plasmolytic disruption of the interconnecting plasmodesmata showed that the cellular pathway of unloading depended upon the sugar status of the stem with symplastic unloading being favoured under high sugar loads (Hayes *et aL,* 1987; Shen and Patrick, 1990). The switching between apo- and symplastic routes may be regulated by plasmodesmatal valving in response to transplasmodesmatal pressure gradients (Patrick, 1990). Since Gayler and Glasziou reviewed the sugarcane literature in 1972, the question of unloading pathway in sugarcane stems surprisingly has remained in limbo until the recent report by Jacobson *et al* (1992). Investigations of cellular pathway are aided by the use of membrane impermeable tracers such as fluorochromes to delineate potential apoplast and symplast routes (Grignon *et al.,* 1989; van Bel and Kempers, 1990; Jacobsen *et aL,* 1992). While tedious, quantitative structural studies provide the framework to refine the findings from tracer studies and to erect defensible hypotheses of possible cellular routes. Finally, experimental manipulation of plasma membrane and plasmodesmatal transport with concurrent monitoring of photosynthate accumulation rates and cellular pool sizes provides indirect corroborative evidence for the operation of a particular unloading route. Opportunities for

more elegant approaches to manipulate plasmodesmatal conductivity will emerge as their biology is elucidated (Robards and Lucas, 1990). The presence of apoplastic barriers in sugarcane stems (Jacobsen *et aL,* 1992), and high radial sucrose fluxes favour movement through the symplast. Since sucrose levels in the apoplast of mature canes are extraordinarily high (Welbaum and Meinzer, 1990), our *Phaseolus* observations suggest the possibility of an entire symplast passage to the storage parenchyma. However, symplast transfer to an anatomical point where sufficient membrane surface area is available to permit membrane exchange to the stem apoplast cannot be excluded on present evidence. Such a cellular pathway operates in developing fruit of tomato during the period of peak sugar storage (Offler and Horder, 1992).

Mechanism and Control of **Radial Transfer**

Until the cellular pathway of radial sucrose transfer is elucidated, any attempt to describe transfer mechanism and control remains speculative. The close anatomical interrelationship between the phloem and ground tissues of stems presents considerable technical difficulties to unambiguously investigate radial photosynthate transfer from the phloem. For instance, the apparent promise of monitoring phloem unloading to the apoplast by wash out of isolated stem segments preloaded with 14C photosynthates (Wolswinkel, 1986; Aloni *et al.,* 1986) is flawed on two grounds. Firstly, the 14C photosynthate pulse rapidly accumulates in the phloem parenchyma (Patrick and Turvey, 1981) so that efflux is likely to be dominated by exchange from cells other than the sieve elements. Moreover, even if care is taken to ensure that the 14C pulse is primarily localised in the sieve elements, normal rates on unloading would result in exhaustion of this relatively small pool of photosynthates within minutes of stem excision (Patrick, 1990). Given these considerations, it would seem essential that studies of radial photosynthate transfer are undertaken with intact stems. Monitoring responses of the apoplast pool size of photosynthates of intact stems, after perturbing plasma membrane transport, were found wanting as some treatments appeared to switch unloading to a symplastic route (Hayes *et aL,* 1987). Real time measurements of 11C entering and exiting a portion of a segment of an intact stem provides the least equivocal assessment of photosynthate unloading from the stem phloem (Minchin and Thorpe, 1987a). Unfortunately the technology to generate 11C is not readily available. The high sucrose concentrations in the apoplast of sugarcane stems (Welbaum and Meinzer, 1990) suggests that the plasma membrane efflux to the stem apoplast might be facilitated and possibly energised as found for unloading from coats of developing legume seed (Patrick, 1990). Furthermore, the relatively low cell turgors of the storage parenchyma (Moore and Cosgrove, 1991) provide appropriate conditions for the operation of a turgor-dependent unloading mechanism which integrates sucrose accumulation from the apoplast with unloading to it (Patrick, 1990; Patrick, 1991). Alternatively, given the appropriate compartmentation of photosynthates (see below) and that sufficient plasmodesmata interconnect the sieve elements with the storage parenchyma, radial photosynthate transfer through the stem symplast could well be the principal path for radial transfer in sugarcane stems. The prerequisite of a symplast sucrose

concentration gradient to drive diffusion from the sieve elements to the cytosol of the storage parenchyma could be generated and maintained by sucrose inversion or membrane transfer to the vacuole in the storage parenchyma. Depletion of cytosolic sucrose levels can be shown to exert limited influence on the radial transfer rates suggesting that significant control may involve integrated changes in plasmodesmatal transport properties (Patrick, 1991). In sugarcane stems, radial photosynthate transfer rates could well exceed those by diffusion alone and principally depend upon bulk flow. This could be in the form of cytoplasmic streaming accompanied by diffusion through the interconnecting plasmodesmata (Bostrom and Walker, 1976) or entirely as a bulk sap flow driven by a hydrostatic pressure gradient established between the sieve elements and storage parenchyma (Murphy, 1989). The hydrostatic pressure gradient could be amplified by or indeed depend upon a barrier separating the vascular and storage parenchyma apoplasts (Jacobsen *et al.*, 1992). Under these conditions, rates of radial transfer would be maintained as sucrose concentrations (and hence osmolality) rose in the storage parenchyma by a turgor homeostat controlling photosynthate exchange to the stem apoplast (Patrick, 1990; 1991). Hydraulic conductance of plasmodesmata could operate a fine control of photosynthate flow through subtle changes in pore radius as predicted by Poiseuille's law. In order to prevent loss of the substantial levels of photosynthates located in the stem apoplast (Welbaum and Meinzer, 1990), water return to the xylem (Glasziou and Gayler, 1972) would need to follow an apoplastic route that passed through a region selectively permeable to water prior to xylem entry. The remobilisation of sugars from stem storage pools is not well understood despite its clear contribution to yield determination in a number of crops including sugarcane (Wardlaw, 1990). Given the presence of an apoplast barrier surrounding the vascular bundles flow would of necessity follow the unloading route and could be driven by a reversed gradient of turgor pressure between the storage cells and the phloem.

References

Aloni, B., Wyse, R. and Griffith, S. (1986). Sucrose transport and phloem unloading in stem of Vicia faba . Possible involvement of a sucrose carrier and osmotic regulation. *Plant Physiol.* 81: 482 - 487.

Bostrom, T.E. and Walker, N.A. (1976). Intercellular transport in plants. *J. Exp. Bot.* 27: 347-357.

Eschrich, W. (1980). Free space invertase, its possible role in phloem unloading. *Ber. d. Deutsch. Bot. Ges.* 93: 363-378.

Glasziou, K.T. and Gayler, K.R. (1972). Storage of sugars in stalks of sugarcane. *Bot. Rev.* 38: 471-490.

Gougler Schmalstig, J. and Cosgrove, DJ. (1990). Coupling of solute transport and cell expansion in pea stems. *Plant Physiol.* 94: 1625-1634.

Gougler Schmalstig, J. and Cosgrove, D. (1992). Differential phloem unloading during gravitropic curvature. *Plant Physiol.* S99:61.

Grignon, N., Touraine, B. and Durand, M. (1989). 6(5) carboxyfluoroscein as a tracer of phloem translocation. *Amer. 7. Bot.* 76: 871-877.

Hayes, P.M., Offler, C.E. and Patrick, J.W. (1985). Cellular structures, plasma membrane surface areas and plasmodesmatal frequences of the stem of *Phaseolus vulgaris* L. in relation to radial photosynthate transfer. *Ann. Bot.* 56: 125-138.

Hayes, P.M. and Patrick, J.W. (1985). Photosynthate transport in stems of *Phaseolus vulgaris* L. treated with gibberellic acid, indole-acetic acid or kinetin. Effects at the site of hormone application. *Planta* 106: 371-379.

Hayes, P.M., Patrick, J.W. and Offler, C.E. (1987). The cellular pathway of radial transfer of photosynthates in stems *of Phaseolus vulgaris* L.: Effect of cellular plasmolysis and p - chloromercuribenzene sulfonic acid. *Ann. Bot,* 59: 635-642.

Jacobsen, K.R., Fisher, D.G., Maretzki, A. and Moore, P.H. (1992). Developmental changes in the anatomy of the sugarcane stem in relation to phloem unloading and sucrose storage. *Bot. Acta* **105:** 70-80.

Lang, A. (1983). Turgor-regulated translocation. *Plant, Cell Envir.* 6: 683-689.

Meschcheryakov, A. Steudle, E. and Komor, E. (1992). Gradients of turgor, osmotic potential and water potential in the cortex of the hypocotyl of growing Ricinus seedlings. Effect of the supply of water from the xylem and of solutes from the phloem. *Plant Physiol.* 98: 840-853.

Minchin, P., Ryan, K. and Thorpe, M. (1984). Further evidence of apoplastic unloading into the stem of bean : Identification of the phloem buffering pool. *J. Exp. Bot,* 35, 1744-1753.

Minchin, P.E.H. and Thorpe, M.R. (1987). Is phloem transport due to a hydrostatic pressure gradient? Supporting evidence from pressure chamber experiments. *Aust. J. Plant Physiol.* 14: 397-402.

Minchin, P.E.H. and Thorpe, M.R. (1987b). Measurement of unloading and reloading of photo - assimilate within the stem of bean. *J. Exp. Bot.* 38, 211-220.

Moore, P.H. and Cosgrove, D.J. (1991). Developmental changes in cell and tissue water relation parameters in storage parenchyma of sugarcane *Plant Physiol* 96: 794-802.

Murphy, R. (1989). Water flow across the sieve tube boundary: estimating turgor and some implications for phloem loading and unloading. II. Phloem in the stem. *Ann. Bot.* 63: 551-559.

Offler, C.E. and Horder, B.A. (1992). The cellular pathway of short-distance transfer of photosynthates in developing tomato fruits. *Plant Physiol.* S99: **41.**

Patrick, J.W. (1972). Vascular system of the stem of the wheat plant. II. Development. *Aust. J. Bot* 20: 65-78.

Patrick, J.W. (1988). Assimilate partitioning in relation to crop productivity. *HortScience* 23: 33-40.

Patrick, J.W. (1990). Sieve element unloading: Cellular pathway, mechanism and control. *Physiol. Planta.* 78: 298-308.

Patrick, J.W. (1991). Control of phloem transport to and short-distance transfer in sink regions. *In* "Recent Advances in Phloem Transport and Assimilate Compartmentation". (Eds. J.L. Bonnemain, S. Delrot, J. Dainty and W. J. Lucass). pp. 167-177 (Ouest Editions).

Patrick, J. W. and Turvey , P. M. (1981). The pathway of radial transfer of photosynthate in decapitated stems of *Phaseolus vulgaris* L. *Ann. Bot.* 47: 611-621.

Patrick, J.W. and Wardlaw, I.F. (1984). Vascular control of photosynthate transfer from the flag leaf to the ear of wheat. *Aust. J. Plant Physiol.* 11: 235-241.

Robards, A.W. and Lucas, W.J. (1990). Plasmodesmata. *Ann. Rev. Plant Physiol. Mol. Biol* 41: 369-419.

Shen, Y.-W. and Patrick, J.W. (1990). Cellular pathway and hormonal promotion of phloem unloading in stems of *Phaseolus vulgaris* L. *Acta Phytophysiol. Sinica* 16: 115-124.

Smith, J.A.C. and Milburn, J.A. (1980). Phloem turgor and the regulation of sucrose loading in *Ricinus communis* L. *Planta* 148: 42-48.

van Bel, A.J.E. and Kempers, R. (1990). Symplastic isolation of the sieve element - companion cell complex in the phloem of *Ricinus communis* and *Salix alba* stems. *Planta* **183:** 69-76.

Wardlaw, I.F. (1990). The control of carbon partitioning in plants. *New Phytol.* 27: 341-381.

Welbaum, G.E. and Meinzer, F.C. (1990). Compartmentation of solutes and water in developing sugarcane stalk tissue. *Plant Physiol.* 93: 1147-1153.

Wolswinkel, P. (1986). Phloem unloading in stems parasitized by Cuscuta. *In:* "Phloem Transport." (Eds. J. Cronshaw, W.J. Lucas, and R.T. Giaquinta). pp. 239 - 246. (Alan R. Liss, Inc.: New York.)

Wood, R.M. (1992). Transfer of photosynthates and potassium in the elongating stem of *Phaseolus vulgaris* L. PhD Thesis. The University of Newcastle. NSW 2308. Australia.

Discussion summary
(by R. Henry)

Robert Furbank asked about options for control of sink strength. John Patrick replied that turgor was the important factor controlling phloem loading in the leaf John Wilson raised the question of a possible control of phloem unloading in the stem through high turgor developed in mature stem parenchyma cells as sucrose accumulation in the vacuole and cytoplasm increased to high levels. If the walls were lignified, and hence rigid and unable to store sucrose to maintain osmotic balance, then cell turgor could increase greatly. Paul Moore indicated that direct measurements of turgor pressure showed that it did not increase greatly in these cells as sucrose accumulated.

George Stewart suggested that starch was a better sink. Andi Maretzki discussed the role of plasmodesmata 'valving' in the control of symplastic/apoplastic transport.

Robert Furbank suggested that transformation to dilate plasmodesmata might be possible, such plasmodesmatal enlargement is seen with certain viral infections.

Inter- and Intra- Cellular Transport and Storage in Stem Parenchyma

A. MARETZKI
Hawaiian Sugar Planters Association
PO Box 1057
Hawaii, USA 96701

Introduction

Evidence for carrier (or binding) proteins inserted into biological membranes dates back to the 50's and 60's (e.g. Stanier *et aL,* 1970, Singer, 1974) when definitive evidence for existence of proteins assisting the transfer of metabolites across membrane barriers was obtained in microorganisms and animals, long before this was possible with higher plants. The chemo-osmotic theory of Mitchell (1966) placed membrane transport into a new, dynamic light from which our present concepts of proton-linked metabolite movement against concentration gradients, including those for sugars, are derived (Komor and Tanner, 1980). In general, evidence for the membrane transport of sugars as well as other metabolites in plants was based for many years largely on indirect experimentation (e.g. Reinhold and Kaplan, 1984). Only more recently have concepts advanced and means been found in plants for recognising and tagging transport related proteins *in situ* (Galet *et aL,* 1989; Ripp *et aL,* 1988), achieving vesicle isolation, solubilisation, and reconstitution (e.g. Martinoia *et aL,* 1991, Thume and Dietz, 1991), raising specific antibodies (e.g. Lemoine *et aL,* 1989), measuring electrical channels (patch clamping) (e.g. Cosgrove and Hedrich, 1991) and generally tying molecular approaches to transport mechanisms. All have contributed to current advances in plants and confirm many of the concepts previously suggested from indirect evidence. Leading the way have been the numerous elegant studies on ATPases fueling the energy requirements for membrane transport to function, both at the plasma membrane (Bennett *et aL,* 1989; Ewing *et al.,* 1990; Serrano, 1990;), as well as at the tonoplast (e.g. Nelson, 1989). The most promising recent advance in terms of plasma membrane sugar transport has been the isolation, cloning, and sequencing of a glucose transporter gene (Sauer and Tanner, 1989), followed now also by similar success with a sucrose carrier gene (Riesmeier and Frommer, 1992)

Membrane Transport

How this new evidence impinges on membrane transport in the stalk sink of the sugarcane plant is still controversial. In our own lab we are sufficiently encouraged by the information from other plant systems that we decided to plunge into molecular aspects, while our colleagues, more esteemed than we in their knowledge of plant physiology, argue about whether membrane transport of sugars is necessary, desirable, or feasible under biophysical conditions existing in the sink tissue. We are quite confident that we will find genes coding for hexose and sucrose transport but their significance in a sugar loading sink will need to be evaluated. One approach which then becomes feasible will be to determine the distribution of expression of the relevant protein in the tissues.

The original premise from which both Glasziou's group and we proceeded was that the plasma membrane of stalk storage parenchyma cells recognised hexoses but not sucrose for transport. The evidence came from several sources, the most persuasive of which was C14 scrambling of asymmetrically labelled sucrose. Recent reexamination by this approach (Lingle, 1989) has thrown some doubt on the original interpretation but it is still not at all clear whether it is the parenchyma storage cells or cells belonging to the vascular bundle that accept sucrose for transport (Thorn and Maretzki, 1992). In addition, there is strong evidence that uphill loading of the sucrose storage cells may not be necessary since the apoplast contains high concentrations of this sugar (Welbaum and Meinzer, 1990). The evidence for the existence of glucose/fructose-proton cotransport sites on the plasma membrane is strong, at least in cells cultured from parenchyma cells capable of storing about 0.2M sucrose (Komor *et aL,* 1981). Molecular probes for an Arabidopsis and Chlorella plasma membrane glucose carrier isolated and cloned by Norbert Sauer are being used to probe cDNA libraries from sugarcane. Since these probes appear to have a wide spectrum of recognition of other carriers, it is likely that we will find other carriers as well. Indeed, there has been hybridisation to numerous plaques which are presently being sequenced to ascertain their homology with reported carrier genes.

Intracellular Transport

Our more recent attention has been focused primarily on the intracellular transport of sucrose. The question of compartmental concentrations has become one begging a definitive answer. A need for uphill transport of sugars from cytoplasm to vacuole had always been assumed but now appears to be questionable. There is no doubt that the vacuole occupies over 90% of the space of storage parenchyma cells and the cytoplasm only a fraction of the remainder. However, concentrations of sucrose appear to be equally as high in the cytoplasmic compartment as in the vacuole. This would obviate the need for an active transport mechanism to assist uphill movement of sugars across the tonoplast. The existence of a proton-generating ATPase on the tonoplast has been shown (Williams *et aL,* 1990). Also, there is some evidence that sucrose uptake is at least ATP-assisted (Getz *et aL,* 1991). The isolation of clean vacuoles in large

numbers has not proven as feasible from sugarcane stalk tissue as it has from beet. Isolation of tonoplast vesicles has, however, been successful and has yielded much data (Getz *et al.,* 1991; Williams *et al,* 1990). A monoclonal antibody raised against tonoplast vesicles has been an important aid for the isolation of a tonoplast protein with a sucrose carrier function (Thorn *et al,* 1992). This protein has been inserted into an artificial membrane and the resulting proteoliposomes have been found to take up sucrose. Again, ATP appears to stabilise the vesicles for uptake of the sugar but there was no evidence for a proton/sucrose antiport mechanism. Since the protein is very low in its abundance in the solubilised vesicles from the young stalk tissue, it is not feasible to isolate a sufficiently large amount of this carrier-associated protein via the present method. Other methods of isolation suggested by its properties toward inhibitors are being investigated. While the monoclonal antibody has been useful as mentioned above, it has not proven possible to identify a corresponding gene from DNA libraries at high stringencies. The problem is likely to be the low abundance of the messenger RNA in the tissue.

Concluding Remarks

Our present thrusts for investigations of transport-related mechanisms are all oriented toward the underlying molecular genetics. As already indicated, we need to move away from phenomenology and look at the most fundamental aspects of sugar transport before we attempt to relate them to the physiology of the system. Fortunately, the tools for such approaches are now fully available and the future looks much more promising than the past. Whether or not it will be either feasible or desirable to manipulate sugar transport per se remains a fascinating question. At the moment, sugar transport, at least in the stalk sink, does not appear to be a critical regulatory point in the overall scheme of sugar accumulation. Perhaps this will turn out to be an advantage in trying to modify one or more transport sites for achieving higher sucrose yields.

References

Bennett, A.B., Ewing, N.N., Wimmers, L.E. and Meyer, DJ. (1989). In: "Plant Membrane Transport: the Current Position" (Eds. J. Dainty, M.I. DeMichelis, E. Marre, F. Frasi-Caldogno) pp. 449-454. *(Elsevier:* London).

Cosgrove, D.J. and Hedrich, R. (1991). Stretch-activated chloride, potassium, and calcium channels coexisting in plasma membranes of guard cells in *Vicia faba* L. *Planta* 186, 143-153.

Ewing, N.E., Wimmers, L.E., Meyer, D.J., Chetelat, R.L and Bennett, A.B. (1990) Molecular cloning of tomato plasma membrane H^+-ATPase. *Plant Physiol.* 94, 1874-1881.

Galet, O., Lemoine, R., Larsson, C. and Delrot, S. (1989) The sucrose carrier of the plant plasma membrane. I.. Differential affinity labelling. *Biochem Biophys Acta* 978, 56-64.

Getz, H.P., Thorn, M. and Maretzki, A. (1991). Proton and sucrose transport in isolated tonoplast vesicles from sugarcane stalk tissue. *Physiol. Plant.* 83, 404-410.

Komor, E. and Tanner, W. (1980). Cotransport of sugars in plants. In: "Plant Membrane Transport: Current Conceptual Issues" (Eds. R.M. Spanwick, WJ. Lucas and J. Dainty) pp. 247-257 *(Elsevier:* London).

Komor, E., Thorn, M. and Maretzki, A. (1981) The mechanism of sugar uptake by sugarcane suspension cells, *Planta* 153, 181-192.

Lemoine, R., Delrot, S., Gallet, 0. and Larsson, C. (1989). The sucrose carrier of the plant plasmamembrane. II. Immunological characterisation. *Biochem Biophys Acta* 978,65-71.

Lingle, S. (1989). Evidence for the uptake of sucrose intact into sugarcane internodes. *Plant Physiol* 90, 6-8.

Martinoia, E., Vogt, E., Rentsch, D. and Amrheim, N. (1991) Functional reconstitution of the malate carrier of barley mesophyll vacuoles in liposomes. *Biochem Biophys Acta* 1062,271-278.

Mitchell, P. (1966). Chemiosmotic coupling in oxidative and photosynthetic phosphorylation. *Biol. Rev. Comb, Philos Soc.* 41, 445-502.

Nelson, N. (1989). Structure, molecular genetics and evolution of vacuolar H^+-ATPases. *J. Bioenerg. & Biomembranes* 21, 553-571.

Reinhold, L. and Kaplan, A. (1984). Membrane transport of sugars and amino acids. *Am. Rev. Plant Physiol.* 35, 45-83.

Riesmeier, J.W., Willmitzer, L. and Frommer, W.B. (1992). Isolation and characterization of a sucrose carrier cDNA from spinach by functional expression in yeast. *EMBO J.* 11 (in press).

Ripp, K.G., Viitanen, P.V., Hitz, W.D. and Franceschi, V.R. (1988) Identification of a membrane protein associated with sucrose transport into cells of developing soybean cotyledons. *Plant Physiol.* 88, 1435-1445.

Sauer, N. and Tanner, W. (1989). The hexose carrier from Chlorella c-DNA cloning of a eucaryotic H^+ cotransporter. *FEB Lett.* 259, 43-46.

Serrano, R. (1990). Recent molecular approaches to the physiology of the plasma membrane proton pump, *Bot. Acta* 103, 230-234.

Singer, S.J. (1974). The Molecular organisation of membranes. *Ann. Rev. Biochem.* 43, 805-833.

Stanier, R.Y., Douderoff, M. and Adelberg, E.A. (1970). "General Microbiology" *(MacMillan:* London).

Thorn, M., Getz, H.P. and Maretzki, A. (1992). Purification of a tonoplast polypeptide with sucrose transport properties. *Physiol. Plant,* in press.

Thom, M. and Maretzki, A. (1992) Evidence for direct uptake of sucrose by sugarcane stalk tissue. *J. Plant Physiol.* 139, 555-559.

Thume, M. and Dietz, K.J. (1991). Reconstitution of the tonoplast amino acid carrier into liposomes. Evidence for an ATP-regulated carrier in different species. *Planta* 185, 569-575.

Welbaum, G.E. and Meinzer, F.C. (1990). Compartmentation of solutes and water in sugarcane stalks. *Plant Physiol* 93, 1147-1153.

Williams, L., Thom, M. and Maretzki, A. (1990). Characterisation of a proton translocating ATPase and sucrose uptake in a tonoplast-enriched vesicle fraction from sugarcane. *Physiol Plant.* 80, 169-176.

Discussion summary
(by J. Patrick)

On questioning, Andi Maretzki re-affirmed that current evidence was consistent with ATPase-dependent (i.e. proton coupled transport) across the plasma membrane and that there was qualified support for facilitated diffusion across the tonoplast.

Rob Birch floated the proposition that vaculolar accumulation of sucrose might therefore be enhanced by engineering the plasma membrane porter system into the tonoplast. John Hawker pointed out that the prospects for obtaining the desired outcome were limited as the tonoplast proton gradient would only drive an antiport system and that it would seem unlikely that altering the plasma membrane symporter to an antiport configuration would be possible.

Andi Maretzki acknowledged that Briskin 's work with tonoplast vesicles of sugar beet demonstrating a proton/sucrose antiport was sound. He suggested that the absence of this porter system from the sugar cane tonoplast could result from the use of cell wall degrading enzymes to obtain membrane vesicles compared to the "cleaner" mechanical isolation used for sugar beet

Merv Ludlow enquired whether membrane transport would dampen the effect of overexpression of key sugar metabolising enzymes on sucrose accumulation. Andi Maretzki considered that membrane transport was not sufficiently well understood to offer a sound prediction.

Robert Furbank posed the question of the relative sucrose concentrations in the cytosol and vacuole to resolve whether tonoplast transport was energised. Current data available were considered not sufficiently reliable to apply a vigorous test but Heldt was developing techniques for maize which might be able to be used for sugar cane to provide these estimates. Pursuing this issue further, Robert Furbank suggested that, if the tonoplast porter system occurred in relatively larger quantities, technologies are now available to generate and isolate monoclonal antibodies with relative ease. This would pave the way to obtaining clones in which the tonoplast porter protein was overexpressed. Andi Maretzki pointed out that porter proteins, including the tonoplast sugar porter, are not abundant. At this juncture John Hawker reminded all assembled that sucrose phosphatase could be the key to tonoplast transport as part of a group translocator system.

John Wilson enquired whether all the transport studies described were based on work with cell cultures which are an undifferentiated cell population. Andi Maretzki indicated some studies had been carried out with tissue slices but more could be done by investigating transport properties of cells at different stages of development.

Biophysical and Structural Changes in Sugarcane Stalk Storage Tissue During Development: A Role in Regulating Sucrose Storage?

P.H. MOORE[1,2] AND K.R. JACOBSEN[2]
[1] *ARS, USDA*
[2] *Expt Stn Hawaiian Sugar Planters' Association*
P.O. Box 1057
Aiea, Hawaii, USA 96701

Introduction

The sugarcane *(Saccharum officinarum* L.) stem stores sucrose exceeding 62% of the dry weight or 25% of the fresh weight (Bull and Glasziou, 1963) which approaches molar concentrations. On the other hand, some of the wild relatives of sugarcane *(Saccharum spontaneum)* store less that 2% of the fresh weight as sucrose. These striking differences in sink activity are not paralleled by differences in source activity; the photosynthetic rates of S. spontaneum are nearly twice that of *S. officinarum* and 30% greater than that of hybrid cultivars (Irvine, 1975). Thus, differences in sucrose storage appears to be regulated at the level of the sink or within the translocation system between the source and sink. Knowledge of where and how sucrose storage is regulated is so critical for developing strategies for agronomic or genetic regulation, that a more complete understanding of the processes at the structural, physiological, biochemical and molecular levels is needed.

Information needed for understanding sucrose storage includes details on both phloem sieve element (SE) unloading and sink parenchyma cell loading. Considerable progress has been made working on the accessible junctures in developing fruit between maternal SE and embryo parenchyma tissue. Comparable information has not been obtained on vegetative organs such as roots, tubers or stems because of the inaccessibility of component SE within the sink tissues (Oparka, 1990). Clues about regulation of sucrose storage in vegetative tissues might be obtained by analyzing biophysical and anatomical properties involved in water and solute conductance.

Water Relations of Storage Tissues

Wolswinkel (1985) showed that the rate of assimilate transport Into developing seeds of legumes is controlled by the osmotic environment of seed coat tissues. Patrick, *et al.* (1986) showed turgor-sensitive unloading of assimilate into seed coats. Wyse *et al* (1986) showed clearly that, in sugar beet parenchyma, the lowering of all turgor enhances active sucrose uptake at the plasmalemma; this work indicated that high turgor may be an important factor in limiting sink strength. Oparka and Wright (1988) showed that turgor regulated not only the uptake of sucrose into potato tubers but also controlled the portion partitioned into starch. Perry, *et al.* (1987) reported that sugar beets not only accumulated sucrose at low turgor but also exported sucrose at high turgor to maintain a relatively constant turgor under varying osmotic pressure. Thus there is ample evidence that turgor and possibly osmotic pressure of sink organs regulates assimilate unloading and storage. While the effect of turgor might involve a strictly physical modification of membranes to effect transport properties, it more likely involves complex biological processes such as would be mediated through changes in gene transcription (Guerrero, *et* ai., 1990).

In the sugar-storing members of *Saccharum*, the stalk parenchyma cells may accumulate sucrose having osmotic potential of more than 20 bars pressure. Thus developing stalk parenchyma cells must experience a wide range of turgors, or have an ability to regulate it. Recent thermodynamic analyses of water relations (Welbaum and Meinzer, 1990) and more direct pressure probe measurements (Moore and Cosgrove, 1991) indicate that turgor of sugarcane parenchyma during development is in the same range as non-sucrose storing tissues of otter plants, ie. 2 to 8 bars (Husken *et al*, 1978). Turgor in sugarcane stem parenchyma was maintained relatively low and constant by partitioning a fraction of the cellular solutes between the symplast and apoplast (Welbaum and Meinzer, 1990). When sugarcane parenchyma tissue was rinsed in water to remove the apoplastic solutes, turgor increased to 7 to 15 bars (Moore and Cosgrove, 1991). Thus, it appears that sugarcane parenchyma osmotically adjusts to the developmental accumulation of sucrose, even though it can not adjust rapidly to dilution of the apoplastic solution.

Regardless of whether sugarcane parenchyma cells actually experience increasing turgors during development, or somehow regulate it, the wide range of osmotic pressures that occurs must be accommodated physiologically. During maturation of intemodes from TVD+5 to TVD+I5 there was a three-fold decrease in membrane hydraulic conductivity and an eight fold increase in cell wall elasticity (Moore and Cosgrove, 1991). These changes were apparently associated with a cellular switch from expansive growth to maturation with sucrose accumulation. How these changes axe mediated are not known, but one might expect the parenchyma cells to develop membrane and cell wall structural changes to facilitate changes in the biophysical characteristics of the sucrose storage tissue during maturation.

Anatomy of Stem Storage Tissue

The sugarcane stem is composed of storage parenchyma tissue within which are numerous vascular bundles. The vascular bundles are surrounded by a sheath of two or more layers of thick-walled, lignified sclerenchyma cells; the storage parenchyma cells become lignified at a later stage of development (Artschwager, 1925). The pattern of increasing lignification and suberization in maturing intemodes more or less parallels the increase of sucrose in stem tissue. In mature internodes having a high sucrose concentration, the vascular tissue is surrounded by thick-walled, lignified and suberized sclerenchyma cells; most of the storage parenchyma, except for portions of walls of isolated cells, was likewise lignified and suberized (Jacobsen, *et al.*, 1992). The role of the isolated cells having only primary cell walls is unknown.

Water is thought to freely penetrate the matrix of cell walls but not the crystalline regions of the microfibrils. The amount of water in walls varies from cell to cell and depends on the degree of wall lignification; increased lignification results in less water, and presumedly reduced capacity for water and solute movement. In sugarcane, lignification of the bundle sheath and parenchyma cells may be important to processes involved in phloem unloading and sucrose storage. In addition, cell wall suberization provides layers strongly impervious to the passage of water so that the presence of suberin is sign of a second possible constraint to movement of water and solutes between apoplastic and symplastic compartments. These wall barriers to plasmamembrane transport can be penetrated by direct cell to cell communication through plasmodesmata connecting the plasmamembranes and cytoplasm of adjacent cells (Robards and Lucas, 1990). The development and distribution of plasmodesmata in sugarcane storage tissue is the subject of current studies.

Implicit in many sucrose transport studies in the sugarcane stem is the assumption that the sucrose unloaded from the phloem freely traverses the apoplastic cell wall spaces before being taken up by the parenchyma cells. Difficulties with this assumption include the expected very slow in vivo tissue conductance of solutes through unstirred cell wall spaces in the absence of mass flow of water, and the high amount of cell wall lignification, especially of the sclerenchyma cells surrounding the vascular tissues. Several pathways exist for assimilate unloading in the various sink tissues of different plant species (Giaquinta, *et al*, 1983). In many reproductive sinks, assimilates are unloaded into the apoplast of maternal tissue surrounding the developing embryo and then taken up from the free space prior to their utilization by the embryo or endosperm (Patrick, 1990). However, in vegetative sinks, such as root tips of maize, there is evidence that unloading in vegetative tissues is via the symplast (Giaquinta, *et al*, 1983).

Concluding Remarks

The considerable data supporting the model of phloem unloading sucrose into the apoplastic space where it is inverted before uptake, over the

alternatives of direct sucrose transfer through either the apoplast or symplast, has been critically reviewed (Glasziou and Gaylor, 1972). However, direct uptake of sucrose by sugarcane tissue slices (Lingle, 1989; Thorn and Maretzki, 1992) indicates that it is not necessary for sucrose to be inverted before uptake by parenchyma cells. In addition, reports of high concentrations of sucrose and low concentrations of hexoses in the sugarcane stem apoplastic space (Hawker, 1965; Welbaum and Meinzer, 1990; Moore and Cosgrove, 1991) and water relations studies showing the isolation of xylem water from that of the stalk storage tissues (Welbaum, *et al*, 1992) further weaken parts of this model. Anatomical development studies and data using tracer dyes (Jacobsen, *et al*, 1992; Welbaum, *et al*, 1992) provide additional evidence against apoplastic transport.

Collectively, data indicates the presence of two separated apoplasts, one within the vascular bundle and one surrounding the storage parenchyma cells. How phloem is unloaded into the apoplastic compartment of the storage parenchyma is still unanswered. Current research integrating studies at the structural, physiological, biochemical and molecular levels should provide us data for developing a better understanding of the controls of sucrose accumulation in the sugarcane stem.

References

Artschwager, E. (1925). Anatomy of the vegetative organs of sugar cane. 7. *Agr. Res.* 30: 197-221.

Bull, T.A. and Glasziou, K.T. (1963). The evolutionary significance of sugar accumulation in *Saccharum*. *Aust. J. Biol. Sci.* 16: 737-42.

Giaquinta, R.T., Lin, W. Sadler, N.L. and Franceschi, V.R. (1983). Pathway of phloem unloading of sucrose in corn roots. *Plant Physiol.* 72: 362-67.

Glasziou, K.T. and Gayler, K.R. (1972) Storage of sugars in stalks of sugar cane. *Bot. Rev*. 38: 471-90.

Guerrero, F.D., Jones, J.T. and Mullet, J,E. (1990). Turgor-responsive gene transcription and RNA levels increase rapidly when pea shoots are wilted. Sequence and expression of three inducible genes. *Plant Mol. Biol.* 15: 11-26.

Hawker, J.S. (1965). The sugar content of cell walls and intercellular spaces in sugar-cane stems and its relation to sugar transport. *Aust. J. Biol. Sci.* 18: 959-69.

Hiisken, D., Steudle, E. and Zimmermann, U. (1978). Pressure probe techniques for measuring water relations of cells in higher plants. *Plant Physiol.* 61: 158-63.

Irvine, J.E. (1975). Relations of photosynthetic rates and leaf and canopy characters to sugarcane yield. *Crop Sci.* 15: 671-76.

Jacobsen, K.R., Fisher, D.G., Maretzki, A. and Moore, P.H. (1992) Developmental changes in the anatomy of the sugarcane stem in relation to phloem unloading and sucrose storage. *Bot. Acta* 105, 70-80.

Lingle, S.E. (1988). Evidence for the uptake of sucrose intact into sugarcane internodes. *Plant Physiol.* 90: 6-8.

Moore, P.H. and Cosgrove, DJ. (1991). Developmental changes in cell and tissue water relations parameters in storage parenchyma of sugarcane. *Plant Physiol.* 96: 794-801.

Oparka, K.J. (1990). What is phloem unloading? *Plant Physiol.* 94: 393-396.

Oparka, K.J. and Wright, K.M. (1988). Influence of cell turgor on sucrose partitioning in potato tuber storage tissues. *Planta* 175: 520-26.

Patrick; J.W. (1990). Sieve element unloading: cellular pathway, mechanism and control. *Physiol Plant* 78: 298-308.

Patrick, J., Jacobs, E., Offler, C.E. and Cram, W.J. (1986). PhotosynthAte unloading from seed coats of *Phaseolus vulgaris* L. Nature and cellular location of turgor-sensitive unloading. *J. Exp. Bot.* 37: 1006-1019.

Perry, C.A., Leigh, R.A., Deri Tomos, A., Wyse, R.E. and Hall, J.L. (1987). The regulation of turgor pressure during sucrose mobilisation and salt accumulation by excised storage-root tissue of red beet. *Planta* 170: 353-61.

Robards, A.W. and Lucas, W.J. (1990). Plasmodesmata. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 41: 369-419.

Thorn, M. and Maretzki, A. (1992). Evidence for direct uptake of sucrose by sugarcane stalk tissue. *J. Plant Physiol.* 139:555-59.

Welbaum, G.E. and Meinzer, F.C. (1990). Compartmentation of solutes and water in developing sugarcane stalk tissue. *Plant Physiol.* 93: 1147-53.

Welbaum, G.E., Meinzer, F.C, Grayson, R.L. and Thornham, K.T. (1992). Evidence for and consequences of a barrier to solute diffusion between the apoplast and vascular bundles in sugarcane stalk tissue. *Aust. J. Plant Physiol.* 19 (in press).

Wolswinkel, P. (1985). Phloem unloading and turgor-sensitive transport: factors involved in sink control of assimilate partitioning. *Physiol. Plant* 65: 331-39.

Wyse, R.E., Zamski, E. and Deri Tomos, A. (1986). Turgor regulation of sucrose transport in sugar beet taproot tissue. *Plant Physiol.* 81: 478-81.

Discussion summary
(by M.M. Ludlow)

George Stewart pointed out that the concentrations of sucrose in the vacuole and the cytoplasm do not match-up to achieve osmotic balance. Paul Moore answered that there is a discrepancy, but that the question I addressed was to look at the changes in cell turgor with internode maturity, [solutes other than sucrose will also accumulate in the cytoplasm to create osmotic balance, ed.J.

Robert Furbank asked if there are plasmadesmata between the storage cells of cane stems? Paul Moore replied that there were numerous plasmodesmatal connections between the cells.

John Wilson pointed out that Paul Moore[r]s slides concentrated on cells near the rind in showing by stain response the extent of lignification and suberisation, and also that parts of parenchyma cell walls showed no stain reaction. What about the main storage cells in the centre of the pith that occupy most of the stem volume (e.g. 80% of the cross-sectional area area of stem in sorghum) ? Paul Moore indicated that the cells in the pith did give the same patchy stain response.

John Hawker mentioned that the earlier work ofGlasziou and Hawker also showed that there was a high concentration of sucrose in the apoplast, agreeing with the current information from Paul Moore. This seems to conflict with the view that suberised and lignified cell walls should not be able to store sucrose. How do we know suberised and lignified cells are not permeable to sucrose ? Studies with dye of the same molecular size as sucrose indicate that it could not penetrate such walls.

In view of the apparently adverse effects of lignification on cell expansion, and the ability of sucrose to permeate and be stored in the stem parenchyma walls, Merv Ludlow asked if there was any direct evidence that lignification limits the maximum storage capacity of stem cells for sucrose. Paul Moore said there was no evidence to support this.

Molecular and Biochemical Aproaches to an Understanding of Sucrose Metabolism

A.MARETZKI
Hawaiian Sugar Planters Association
P.O. Box 1057
Aiea, Hawaii USA 96701

Introduction

Sucrose is a readily reversible end product of carbohydrate metabolism. In leaf tissue of sugarcane, sucrose-starch light-dark cycles are comparable to those in other plants, but in the stalk storage tissue starch formation has been relegated to a minor role, presumably by breeding selection. Sucrose thus dominates as an end product in stalk storage parenchyma where sugar accumulation occurs, and a plastid function may be largely bypassed. Cytoplasmic and vacuolar enzymes most directly responsible for sucrose metabolism, i.e., sucrose synthase (SS), sucrose phosphate synthase (SPS), and acid invertase, may steer but not necessarily control sucrose deposition. Recognising the limitations, these enzymes, their distribution, their localised tissue expression, isomeric forms, genes coding for them, and their biochemical characteristics have been receiving our attention.

Sucrose Phosphate Synthase

SPS has been the subject of many investigations, most in leaf tissue but some also in storage sinks other than sink leaves (e.g., Fieuw and Willenbrink, 1987; Kalt-Torres *et al*, 1987; Koehler *et al.*, 1988; Siegland Stitt, 1990; Hubbard *et al*, 1991; Salerno *et al*, 1991). SPS in stalk tissue of sugarcane can be purified by a series of steps, including PEG, Superose 6, and Mono-Q fractionation. A final gel electrophoretic isolation permits sufficient polypeptide recovery to make N-terminal amino acid analyses and polyclonal antibody formation, possible. SPS in stalk tissue differs from the leaf enzyme in terms of active subunit size and some regulatory characteristics; other fine control regulation by metabolites like glucose-6-phosphate and Pi turns out to be remarkably similar between the leaf vs. the stalk enzyme. It is probable that a single SPS gene exists in sugarcane. The cDNA cloning of this gene, using a polyclonal antibody, is in progress.

Sucrose Synthase

SS in sugarcane exists in two isomeric forms, as it does in other monocot species (e.g. Echt and Chourey, 1985; Chourey *et al,* 1986; Chan *et al.,* 1990; Marana *et al.* 1990). The genes have been isolated and characterised and show close homology with SS corn genes in the coding region, but also a 20% difference between the two sugarcane genes in the promoter region. The promoter of only one of them contains the sequences for an ARS element and it is the transcription of only this form which is sensitive to anaerobic conditions. The two SS protein forms of stalk tissue have been separated on a Mono-Q FPLC column, followed in the case of SS2 by a final purification step on an immunoaffinity column. In the case of the SSI protein this procedure will not work and it is necessary to do the isolation from old internodes where Western blots show the expression of SSI only. These preparations are being used to do exhaustive kinetic characterisation. The hypothesis that one form of SS can function in the direction of sucrose synthesis under some physiological conditions has yet to be proven. Also still outstanding is definitive information about extra- and intra- cellular distribution of these two enzymes. In general, it is believed that SS is localised in the vascular bundles. It is possible but unproven that it is also located in the cytoplasm of storage tissue parenchyma cells where it would be in direct competition with SPS if its function is only degradative. Also in progress is an approach to transform plants with coding regions cloned in the reverse order to block expression of the SS proteins. These experiments have reached a stage where a number of transformed plants have been confirmed by Southern blots. As yet, they are too young to have permitted biochemical analyses.

Invertases

Attempts are being made to synthesise acid invertase clones via PCR from suitable DNA primers but beyond this effort, fitting invertase into the present spectrum of investigative possibilities is lagging far behind in sugarcane. Thanks to the ever increasing number of reports on molecular aspects of plant invertases (e.g. Karuppiah *et al,* 1989; Sturm and Chrispeels, 1990; Unger *et al*$_t$ 1992) it is now finally possible to look forward to obtaining antibodies suitable for probing DNA libraries of sugarcane for these genes, rather than attempting to isolate the relevant polypeptides from sugarcane itself. From the patterns of distribution of acid invertase in youngest to fully mature internodes, it is still quite possible that one or more invertase genes hold the key to many of the critical questions with respect to sucrose accumulation. Assuming that acid invertase must be localised in the vacuole of storage parenchyma cells, its rapid disappearance with increasing age of the tissue remains a fascinating and vital link to our understanding of sucrose accumulation.

Usefulness of the Antisense Approach

The three above categories of enzymes must be placed into a broader context to evaluate their impact on equilibria of carbohydrate partitioning in stalk tissue. The antisense approach with SS is one way to do so.

Research in other laboratories illustrates clearly that this method can be used to implant powerful metabolic blocks which enable us to learn about the radiating effects of a specific block in the glycolytic pathway (von Schaewen *et al,* 1990; Sonnewald *et al.,* 1991; Mueller-Roeber *et al,* 1992). There are, of course, downsides to this approach as well. It is not likely to pinpoint mechanisms that identify the exact switch from one metabolic mode to another. To do so, either a signal must be introduced that allows turning transcription/translation on or off at will at the site of desired intervention, or the time point at which such a signal is tripped in normal metabolism must be known. A case for the use of cell cultures in an antisense approach could be made.

Concluding Remarks

From a practical point of view, no industry is going to be interested in ways to divert greater amounts of carbohydrate toward product (i.e., sucrose) formation if it is at the expense of growth vigor, disease susceptibility, or elasticity of the plant, to name a few possible trade-offs. At the present time, the only overproduction or antisense approaches that I know of for industrial applications in crop plants are being used to modify post-harvest physiology, and the genes involved turn out not to be important during the development of the plant. The full biochemical/physiological impact of similar, seemingly appropriate intrusions into the genome of the sugarcane plant will have to be carefully evaluated before one embarks on an approach involving genetic transformation. The analytical efforts needed at the agronomic level, once so embarked, are not trivial. Perhaps site-specific mutations to modify the active site of an existing gene will prove to be an important approach, but it too will require a full understanding of the enzyme coded for by the gene of choice for the intervention, as well as kinetics of field tests to verify success. There are exciting and promising possibilities for sucrose accumulation increases in sugarcane in sight via the use of molecular genetics but they will require much thought and effort.

References

Chan, H.Y., Ling, T.Y., Juang, R.H., Ting, I.N., Sung, H.Y., Su, J.C. (1990). Sucrose synthase in rice plants. *Plant Physiol.* 94, 1456-1461.

Chourey, P.S., Latham, M.D., Still, P.E. (1986). Expression of two sucrose synthase genes in endosperm and seedling cells of maize: Evidence of tissue-specific polymerization of protomers. *Mol. Gen. Genet.* 203, 251-255.

Echt, C.S., Chourey, P.S. (1985). A comparison of two sucrose synthase isomers from normal and shrunken maize. *Plant Physiol.* 79, 530-536.

Fieuw, S., Willenbrink, J. (1987). Sucrose synthase and sucrose-phosphate synthase in sugar beet plants, *(Beta vulgaris* L. ssp. *altissima). J. Plant Physiol.* 131, 153-162.

Kalt-Torres, W., Kerr, P.S., Huber, S.C. (1987). Isolation and characterisation of multiple forms of maize leaf sucrose-phosphate synthase. *Physiol. Plant* 70, 653-658.

Karrupiah, N., Vadlamudi, B., Kaufman, P.B. (1989). Purification and characterisation of soluble (cytosolic) and bound (cell wall) isoforms of invertases in barley. *Plant Physiol.* 91, 993-998

Koehler, J., Komor, E., Thorn, M., Maretzki, A. (1988). Activity of sucrose-phosphate synthase in sugarcane leaves. *Phytochem.* 27, 1605-1608.

Marana, C., Garcia-Ohneda, F., Carbonero, P. (1990). Differential expression of two types of sucrose synthase-encoding genes in wheat in response to anaerobiosis, cold shock and light. *Gene* 88, 167-172.

Mueller-Roeber, B., Sonnewald, U., Willmitzer, L. (1992). Inhibition of ADP-glucose pyrophosphatase in transgenic potatoes leads to sugar-storing tubers and influences tuber formation and expression of tuber storage proteins. *EMBO J.* 11, 1229-1238

Salerno, G.L., Crespi, M.D., Zabaleta, E.J., Pontis, H.G. (1991). Sucrose-phosphate synthase from wheat characterisation of peptides by immunoblotting analysis. *Physiol. Plant.* 81,541-547.

Siegl, G., Stitt, M. (1990). Partial purification of two forms of spinach leaf sucrose-phosphate synthase which differ in their kinetic properties. *Plant Sci.* 66,205-210.

Sonnewald, U., Brauer, M., van Schaewan, A., Stitt, M., Willmitzer, L. (1991). Transgenic tobacco plants expressing yeast-derived invertase in either the cytosol, vacuole, or apoplast: a powerful tool for studying sucrose metabolism and sink-source interactions. *The Plant J.* 1, 95-106.

Sturm, A., Chrispeels, M.J. (1990). cDNA cloning of carrot extracellular B-fructosidase and its expression in response to wounding and bacterial infection. *The Plant Cell* 2, 1107-1119.

Unger, C, Hofsteenge, J., Sturm, A. (1992). Purification and characterisation of a soluble B-fructofuranosidase from Caucus-carota. *Eur. J. Biochem.* 204, 915-921.

VonSchaewen, A., Stitt, M., Schmidt, R., Sonnewald, U., Willmitzer, L. (1990). Expression of a yeast-derived invertase in the cell wall of tobacco and *Arabidopsis* plants leads to accumulation of carbohydrate and inhibition of photosynthesis and strongly influences growth and phenotype of transgenic tobacco plants. *EMBO J.* 9, 3033-3044.

Discussion summary
(by R. Birch)

The development of a gene transfer system makes it possible to manipulate genes for enzymes involved in sucrose metabolism, with the aim of increasing sucrose levels in the sugarcane stalk. The discussion of this presentation commenced with some points of technical clarification on the innovative research described by Dr. Maretzki, whose research team are clearly the world leaders in exploration of sucrose metabolism in sugarcane. The discussion then moved to a consideration of (i) the gaps in our knowledge which need to be filled in order to select the best candidate genes for genetic manipulation, and (ii) the best experimental approaches to fill these gaps.

(i) Gaps in knowledge:

The major enzymes involved in sucrose metabolism are known. The HSPA group have made good progress in isolating and comparing sugarcane leaf and stalk sucrose phosphate synthase, and are attempting to clone the gene. Work is also well advanced on two sugarcane sucrose synthases, with different expression patterns in stalks. Work with acid invertase is at a much earlier stage.

Important gaps in knowledge include the distribution of these key enzymes at the cellular level, the extent to which they naturally limit sucrose accumulation, and the metabolic control mechanisms that fine tune their activities or switch cells between metabolic modes.

(ii) Experimental approaches:

Further biochemical characterisation of these enzymes will help to indicate what molecular modifications should be attempted for altered enzymatic properties affecting sucrose accumulation.

In situ enzyme localisation studies will help resolve questions concerning both sucrose transport and metabolism, and may indicate the most promising enzymes for overexpression or modification.

However, most participants agreed that in the short term the approach most likely to provide a quick practical benefit (while also increasing basic understanding) is to go ahead withgenetic manipulation of sugarcane to deliberately increase or decrease the levels of each suspected key enzyme; or "try it and see\ This is already being attempted for several enzymes at HSPA, and complementary work is also proposed by CSIRO. In the longer term fine tuning will probably require results from the biochemical and enzyme localisation studies.

Other Physiological Approaches to Increasing Sugar Accumulation

J.R. WILSON
CSIRO, Division of Tropical Crops and Pastures
306 Carmody Road
St Lucia, Brisbane, Qld. 4067

Introduction

This paper covers some other metabolic approaches to raising the capacity of the sugarcane plant for higher sucrose accumulation. These approaches are largely associated with my background experience and expertise with forage species. The CSR David North group, in Brisbane, in the 1970's estimated maximum potential CCS in cane of 22-27%, so there is perhaps good reason to believe that 1-2 units increase over current levels of c. 11-16% is achievable.

To help focus our thoughts a simplistic empirical growth model (Charles-Edwards, 1982) can be adapted to think about factors influencing net growth in stored sucrose (G_5. The derivation of G_5 can be on a daily or seasonal basis.

$$G_s = n_1 n_2 \; E J - V \qquad (1)$$

$$E = (P - R) / J \qquad (2)$$

Where n_1 and n_2 are partitioning coefficients; e.g.
n_1 = proportion sucrose distributed to storage stem as against leaf or root
n_2 = proportion fixed photosynthetic carbon distributed to stored sucrose as against cell wall fibre, Iignin, starch or other C compounds.
P = gross photosynthetic C input, R = respiration losses, J = photosynthetically active radiation intercepted per unit of time, and V = losses of previously stored C (e.g remobilisation of stored sucrose from stem parenchyma cells).

It is perhaps useful to reflect a little on where the extra C units for higher sucrose accumulation and higher sucrose yield will come from. Obviously,

increasing P per unit ground area over a substantial growth period will provide additional C input. Field physiology studies in Queensland are looking at tillering, leaf area, and overall light use efficiency, and environmental effects have been considered earlier in this workshop. The biochemistry of photosynthesis is being investigated in many laboratories, but so far in crops there has been little evidence of yield gains from selecting for higher photosynthetic rates per unit leaf area. Moore (1989) reviewed at length the photosynthetic and growth efficiency of cane using a more elaborate discussion of the model above. The items discussed below are more in line with increasing harvest index, which has been most successful for yield improvement in other field crops.

Partitioning (n)

Prospects for altering partitioning of C to more stored sucrose in stem at the expense of less in leaves or less carbohydrate reserves stored as starch will be covered by Robert Furbank in an earlier discussion.

We could consider here though the potential for reducing the partitioning of fixed C to fibre (cell wall polysaccharides and lignin). A certain level of fibre is necessary to give stems the strength to resist the compressive forces imposed upon them and to prevent lodging. However, much of the strength comes from the architectural arrangement of the tissue types within the stem, with the narrow layer (rind) of thick-walled sclerenchyma and vascular cells as near to the periphery of the stem as possible, following well-established engineering principles (Wilson, 1990). This is supported by the literature on lodging resistance of stems, especially cereals, which often reveals a poor relationship between resistance and individual stem parameters such as cross-sectional area of these cell types, or stem cell wall or lignin content. Consequently, there may be an opportunity to reduce the quantity of fixed carbon locked up in fibre without sacrificing stem strength. My own work with sorghum shows that whilst stem rind may occupy only 14-16% of the stem volume it contains 66-68% of the stem cell wall material and 82% of the lignin. Stem fibre shows much genotypic variation in most grasses and sugarcane is no exception with *S. spontaneum* at c. 34% and *S. officinarum* c. 10% (on fresh weight basis). So reducing the volume of rind could be a possibility , thereby conserving glucose units (c. 1:1, see Table 1) for conversion to sucrose and adding to the volume of storage parenchyma cells in the stem.

Reducing general lignification of the stem, without reducing wall thickness of the rind cells could be a more promising option. This is especially so based on the conversion efficiences shown in Table 1, because one unit reduction in lignin would conserve 2.3 units of glucose for potential conversion to sucrose. Lignin reductions of 30-50% (without other modifications of either cell wall content or anatomical structure) have been found in stems of brownrib mutants of maize, sorghum and millet. Programs to reduce lignin by antisense RNA or ribozyme techniques to

interfere with enzyme activity are now in progress in a number of laboratories. The CSIRO DTCP group have recently produced transgenic tobacco plants in their program.

Table 1 Glucose costs for synthesis of various compounds within the plant [based on biochemical pathway analysis]

Compound	Glucose cost (g glucose/g compound)
Lipids	3.03
Nitrogenous compounds	2.48
Carbohydrates (cell wall polysaccharides)	1.09
Organic acids	0.91
Lignin	2.32

Adapted from Penning de Vries *et al.*, 1974; Lambers and Rychter, 1989

Another possibility in lignin reduction is to specifically target the storage pith parenchyma cells. This would be aimed not at increasing conversion efficiency of fixed C to sucrose but at possibly increasing the maximum sucrose storage capacity of these cells. The pith cells become lignified and perhaps suberised with age (Jacobsen *et al.*, 1992), although less so than the other cells in the stem, e.g. in cane, acetyl bromide lignin was 13% (pith), 19% (vascular bundles) and 22% (sclerenchyma) (He and Terashima, 1990), and for comparison, comparable values in sorghum using acid detergent lignin were 1.5% (pith), 4.5% (vascular bundles) and 4.7% (sclerenchyma) (J.R. Wilson, unpublished data). Nevertheless thelignin present would stop further expansion of the parenchyma pith cells and result in the walls becoming hydrophobic and impervious to sucrose diffusion (Brett and Waldron, 1990). Thus the apoplastic transfer pathway of sugars to storage within these cells would be blocked and a high turgor pressure would be expected to develop. However, recent information from pressure probe analysis suggests that turgor pressure does not rise in mature storage cells (Moore and Jacobsen, 1992) which is perhaps a matter requiring further examination.

Cell-specific promoters for gene modification of pith cells only will undoubtedly become available. Also, the lignin composition of pith cells is different from that of the rind cell types in sugarcane (He and Terashima, 1990) and in sorghum (J.R. Wilson, unpublished data). Thus perhaps specific enzyme targets could be defined and their action modified to affect pith cells only. Would little or no lignin in these storage cells increase their maximum capacity for sucrose accumulation? Experiments could be designed to test this using the various metabolic inhibitors of lignin synthesis currently available.

Respiration Losses (R)

Sugarcane, as for other grasses, loses 20-50% of gross photosynthate in respiration (Glover, 1973). The proportional loss increases as the cane crop matures (Fig. 1). Can some of this loss be avoided to increase the net C gain for storage as sucrose?

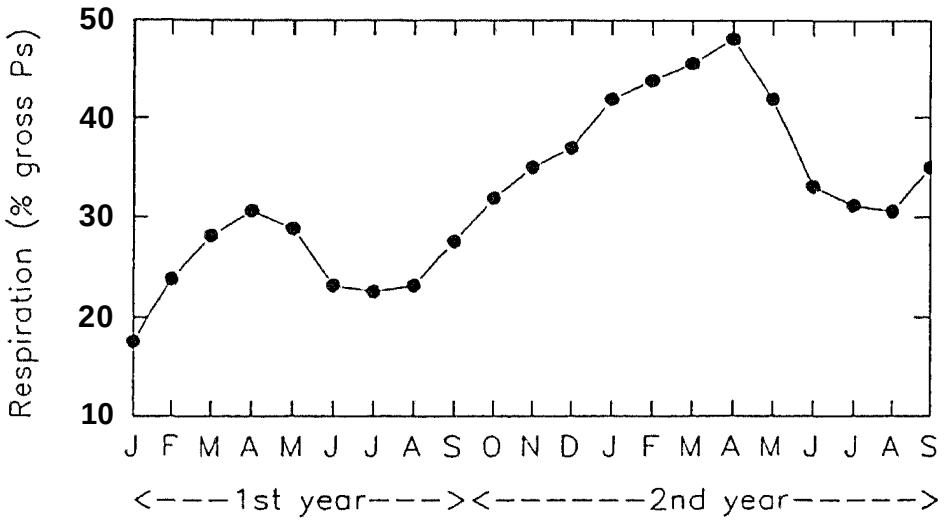


Figure 1 Whole plant respiration as a percentage of gross photosynthesis in sugarcane [adapted from Glover 1973].

A negative relation between level of dark respiration and yield has been demonstrated in several grass species (e.g. Fig. 2), and selection and testing of "low" dark respiration genotypes of perennial ryegrass is well documented.

Kraus *et al.*, (1989) have summarised these studies which show an annual yield advantage in the field of 13% for "low" respiration ryegrass selections. The differences in dark respiration are only apparent for mature tissue, they have a cumulative effect which increases (1) as the proportion of mature tissue in the crop increases, (2) as the length of time between harvests increases, (3) with high nitrogen additions, and (4) with higher temperature and light growth conditions. During summer the yield gain for N-fertilised ryegrass increased to 22% (Pilbeam and Robson 1992). These conditions for maximum effect describe a sugarcane crop admirably, so if low respiration lines could be selected in cane then we might expect a considerable advantage for greater biomass production and, hopefully, higher levels of sucrose accumulation. Another, possible advantage of low respiration lines could be shown in ratooning, because in ryegrass these lines permit more substrate, remobilised from stubble after harvest, to be

employed in new leaf production to re-establish light interception (Kraus *et al.,* 1989). So far as I am aware, selection for low respiration lines has not been investigated in cane. The biochemical basis for the low dark respiration is unclear but no disadvantages have yet been identified (Kraus *et al,* 1989). The work on ryegrass suggests that low respiration rate is only controlled by one or two genes.

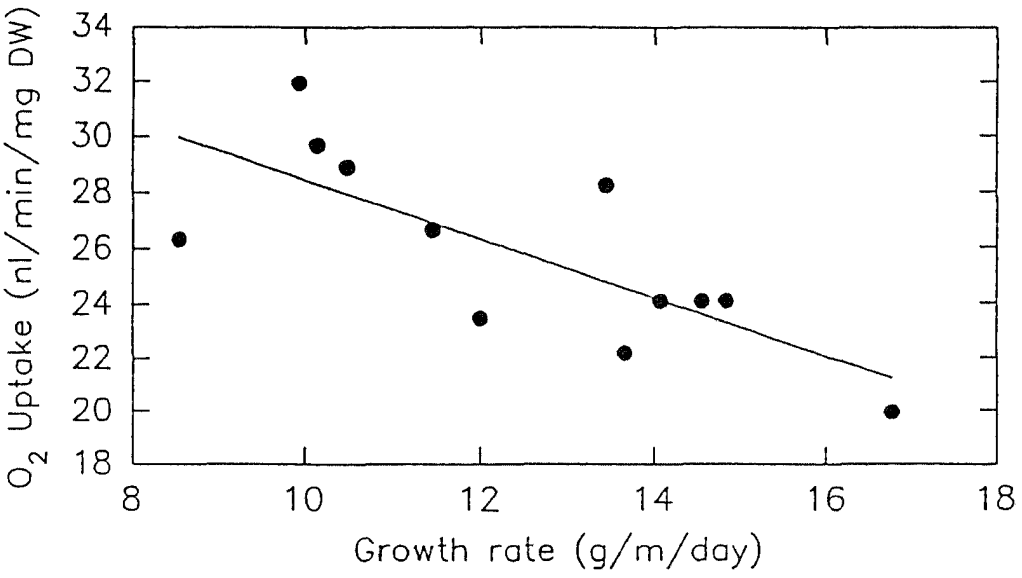


Figure 2 Negative correlation between mature leaf dark respiration and growth of vegetative swards of genotypes of *Lolium perenne* S23 [from Kraus *et al.,* 1989].

Maintenance respiration (MR) can run as high as 1-5% of total living plant biomass/day, and thus can be substantial in a crop with large biomass such as sugar cane. Variation in MR can result in significant alterations in plant productivity (Amthor 1984), simulations in sugar beet support this (Hunt and Loomis (1979). Most respiration is channeled through the cytochrome pathway which yields 3 ATP for each O atom consumed (Hirose *et al.,* 1989). Inhibition of this pathway by cyanide has recently shown the existence of an alternative non-phosphorylating electron transport respiration pathway which is less efficient and yields only 1 ATP/ O atom (Lambers and Rychter 1989). Hirose *et al.* (1989) claim that there is much evidence that plants can change the relative proportion of respiration via the two pathways, and that if the alternative pathway ("cyanide-insensitive") was inactivated then growth would increase by 10%. The pathway is engaged when the level of reserve carbohydrates in tissues is high (Lambers and Rychter 1989). So possibly the enzymes of this pathway could be a target

for gene inactivation; the gene for alternate oxidase has recently been cloned. The alternate pathway is not involved in the selection for low respiration lines described earlier (Kraus *et al.,* 1989).

Losses of Stored Sucrose (V)

In older internodes, as cane ripens, there can be a significant loss of stored sucrose (Liu and Kingston, 1992). Also, towards the end of the harvest season if growing conditions alter with early rain to favour new vegetative growth, then stored sucrose is remobilised from the stem to support new growth and CCS will drop. It would be an advantage if such losses of stored sucrose could be controlled/avoided, perhaps by chemical sprays that inhibit enzymes (associated with hydrolysis of sucrose) which are specific to pith cells. Alternatively, perhaps metabolic inhibitors to be used as sprays could be found to temporarily interfere with N transfer to, or protein metabolism at, the meristem to hold back leaf growth until the cane can be harvested. Should work along these lines be considered?

References

Amthor, J.S. (1984). The role of maintenance respiration in plant growth. *Plant, Cell and Envir.* 7: 561-9.

Brett, C. and Waldron, K. (1990). "Physiology and Biochemistry of Plant Cell Walls". (Unwin Hyman: London).

Charles-Edwards, D.A. (1982). "Physiological Determinants of Plant Growth". (Academic Press: Sydney).

Glover, J. (1973). The dark respiration of sugar cane and the loss of photosynthate during the growth of a crop. *Ann. Bot.* 37: 842-52.

He, L. and Terashima, N. (1990). Formation and structure of lignins in monocotyledons. III. Heterogeneity of sugarcane *{Saccharum officinarum* L.) lignin with respect to the composition of structural units in different morphological regions. J. *Wood Chem. & Technol* 10: 435-59.

Hirose, T., Lambers, H., Konings, H. and Van der Werf, A. (1989). Modelling of respiration: effect of variation in respiration on plant growth in two *Carex* species. *Fund. Ecol* 3, 655-65.

Hunt, W.F. and Loomis, R.S. (1979). Respiration modelling and hypothesis testing with a dynamic model of sugar beet growth. *Ann. Bot.* 44: 5-17.

Jacobsen, K.R., Fisher, D.G., Maretzki, A. and Moore, P.H. (1992). Developmental changes in the anatomy of the sugarcane stem in relation to phloem unloading and sucrose storage. *Bot. Acta.* 105: 70-80.

Kraus, E., Wilson, D., Robson, MJ. and Pilbeam, CJ. (1989). Respiration: Correlation with growth rate and its quantitative significance for the net assimilation rate and biomass production. *In:* "Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants". (Eds. H. Lambers *et a*/.), pp. 187-98. (SPB Academic Publishing: The Hague, Netherlands).

Lambers, H. and Rychter, A.M. (1989). The biochemical background of variation in respiration rate: respiratory pathways and chemical composition. *In:* "Causes and Consequences of Variation in Growth Rate and Productivity of Higher Plants". (Eds H. Lambers *et al.),* pp. 199-225. (SPB Academic Publishing: The Hague, Netherlands).

Liu, D.L. and Kingston, G. (1992). Sucrose accumulation in sugarcane. [in press].

Moore, P.H. (1989). Physiological basis for varietal improvement in sugarcane. *In:* "Sugarcane varietal improvement". (Eds. K.M. Naidu *et al.)* pp. 19-55 (Sugarcane Breeding Inst.: Coimbatore).

Moore, P.H. and Jacobsen, K.R. (1992). Biophysical and structural changes in sugar cane stalk storage tissue during development: A role in regulating sucrose storage. *In:* "Improvement of Yield in Sugar Cane through Increased Sucrose Accumulation - Workshop Report (Ed. J.R. Wilson), pp. 67-72. (CSIRO/SRDC: Brisbane, Australia)

Penning de Vries, F.W.T., Brunsting, A.H.M. and Van Laar, H.H. (1974). Products, requirements and efficiencies of biosynthetic processes: a quantitative approach. J. *Theor. Biol.* 45: 339-77.

Pilbeam, C.J. and Robson, MJ. (1992). Response of populations of *Lolium perenne* cv S23 with contrasting rates of dark respiration to nitrogen supply and defoliation regime. I. Grown as monocultures. *Ann. Bot.* 69: 69-77.

Wilson, J.R. (1990). Influence of plant anatomy on digestion and fibre breakdown. *In: "* Microbial and Plant Opportunities to Improve the Utilization of Lignocellulose by Ruminants". (Eds. D.E. Akin *et a*/.), pp. 99-117. *(Elsevier* Sci. Publ.: New York).

Discussion summary
(by G,R. Stewart)

Discussion centred on the biochemical and physiological constraints to sucrose accumulation in stem parenchyma cells and how molecular genetics might be applied to the enhancement of sucrose accumulation. The roles and subcellular localisation of various sugar metabolising enzymes were highlighted as key areas in which precise quantitative data were lacking. This is essential if we are to genetically manipulate levels of biosynthetic or catabolic enzymes in order to alter the equilibrium of carbohydrate partitioning in stalk tissue. The role of sucrose synthase is somewhat of an enigma and in the discussion little consensus emerged as to wether or not it can function in the direction of sucrose synthesis. Information is lacking as to the specific roles of the two isoforms of sucrose synthase. Although the potential of molecular genetics was realised enthusiasm for this approach was tempered by our lack of physiological and biochemical information that would provide the necessary framework for the molecular approach.

It was recognised that there is still scope for the use of classical physiological approaches in the manipulation of sucrose concentrations. Various possibilities were considered in the context of altering the partitioning of photosynthate between different pools within the sugarcane plant. Reducing the lignin content of sugarcane, particularly that of pith cells was suggested as a possible approach to releasing carbohydrate to the sucrose pool There was discussion of the progress being made by other groups who have targeted reduction in lignin as strategy for increasing forage digestibility. Another approach to reducing competition for carbohydrate within the plant was to select for varieties with reduced rates of respiration. In other Gramineae a negative correlation has been found between dark respiration and yield. Some surprise was expressed during the discussion that plants had what appeared to be "surplus" respiratory activity. A third possibility that was considered was the use of inhibitors to block the utilisation of stored sucrose during the later stages of cane ripening. Whilst this approach might have some merit there seemed to be a general view that the use of more chemicals was undesirable. However the suggestion was made that genetically altered plants might be constructed that suppressed sucrose mobilisation.

PART 3

COLLATION

SESSIONS

Collation session: Genetic and Environmental control of sugar accumulation.

Collator: R.C. Muchow

1. Desired outcome:

The desired outcome is increased sugar accumulation per hectare from conventional breeding programs, and through better management procedures to maximise favourable environmental and nutritional affects on sucrose accumulation. Whilst the primary focus of the workshop is on sugar content (CCS), it is important to consider the interaction of both cane biomass and sugar content when considering the impact of genetic and environmental manipulations.

2. Current knowledge/background:

Genetic variability in CCS has been observed with the variation being greater for early-season CCS than for late-season CCS. The genotype x environment interaction for CCS is relatively low. Substantial progress is being made by current breeding programs in selection for early-season CCS. Further genetic improvement in CCS through cultivar development is more likely early in the season than later in the crushing season, and more so in southern sugar-growing locations. There is a need to understand the genetic basis for the interaction between cane yield and CCS among varieties.

There are differing sensitivities of photosynthesis, structural growth and hence sucrose accumulation to environmental factors. This results in CCS varying within and across seasons and across locations. Low temperature (except frost and chilling), water stress and nitrogen stress decrease structural growth more than photosynthesis resulting in increased sucrose accumulation. Most of this knowledge is qualitative, and quantitative response functions are not available to allow dynamic simulation of sucrose accumulation. There is a clear need to develop the capability to explore options for maximising sucrose accumulation by manipulating these environmental factors. Then appropriate improvements to crop management can be investigated.

The role of chemical ripeners to promote sucrose accumulation is very much dependent on climate. Variable responses dependent on location, season and chemical type have been observed. There is a need to better understand the interaction of ripeners with climatic factors so that their effectiveness can be improved, before further field testing is conducted to achieve an outcome for the industry. Chemical ripeners are seen as a tool

clarification of their role and usefulness in the light of public environmental concerns about agricultural chemicals. Also, because results are so variable there is a real risk of litigation against suppliers or extension advisors who make recommendations on their use.

3. Research strategies and priorities:

1. Ongoing research with breeding programs to select for early-season CCS is a high priority and the probability of success in the next 3-5 years is high. Selection for mid- to late-season CCS has a lower probability of success with a 15-20 year time-frame.

2. Research to develop the capability to exploit environmental and management manipulation to increase sucrose accumulation, using dynamic simulation is a high priority with a high probability of success in 5-7 years. Quantitative response functions relating cane yield and sucrose accumulation to environmental factors are required for incorporation into crop simulation models. The response to temperature can best be quantified in controlled environments with some field validation, whereas the response to water and nitrogen stress can best be obtained in field experiments to allow integration of soil and crop processes. The basic yield physiology of varietal differences in yield accumulation and the trade-off between cane yield and sucrose content also need to be quantified.

   Once the response functions have been incorporated into simulation models and optimal management strategies have been identified for different locations and varieties using simulation, further field testing of the identified strategies would be required for 3-5 years to deliver an outcome to the industry.

3. Further general field testing of new and existing chemical ripeners in the short-term is low priority. Rather, priority should be given to quantifying the interaction between current recommended ripeners and environmental factors so that their effectiveness can be improved. There is a need to utilise opportunistic research in this area (i.e. within existing projects rather than setting up projects dedicated to ripener research).

4. Research collaboration:

Current collaboration between BSES, CSR, NSWDA, NSWSMA and CSIRO will continue to be fostered. Further collaboration between CSIRO and BSES will be developed if research is pursued on the interaction between climatic factors and ripener efficiency or the interaction between climatic and edaphic factors and sucrose accumulation.

5. Strategic capacity building:

A database on sugar accumulation is required for integration with the databases currently being developed for soils, climate and biomass. These

data could be obtained by collaboration with existing SRDC projects CSC4S and CSC7S, and together with controlled environment studies to provide quantitative relationships between sugar accumulation and environmental factors, will allow the use of a crop growth simulation model to explore planting, harvesting, watering and nutrition management options to increase sugar accumulation. A researcher with whole plant and crop physiology skills is required to implement this work.

The development of this strategic research to establish a database for sugar accumulation will require concurrent building of capacity for analysis of sugars in cane, using newer technologies of HPLC and rapid enzyme-based assays for routine analytical requirements.

Genetic improvement in sugar accumulation would be enhanced by increased quarantine facilities for an expansion of cane introductions, particularly to expand the *S. officinarum* germplasm.

Collation Session: Molecular Modification for Increased Sucrose Accumulation

Collator; R.G. Birch

1. Desired outcome:

The desired outcome is genetically transformed cultivars of sugar cane with greater efficiency of sucrose metabolism and/or storage, resulting in higher sugar yield per hectare.

2. Current knowledge/background:

Molecular modification of sugar cane has only become a practical possibility within the last year, following the development of techniques for the production of transgenic plants. This new technical capability provides unique new opportunities for sugar cane improvement, including new experimental approaches to achieve increased sucrose accumulation.

At present, genetic transformation technology is limited to introduction or modification of only one or a few genes at a time. Because specific genes are transferred, or specific alterations are made to genes, the technology is ideally suited to testing specific hypotheses about the genetic, biochemical or physiological factors affecting sucrose accumulation. This is a major breakthrough because it allows the first direct testing of some long standing ideas about the role of known enzymes and transport proteins in sucrose metabolism, transport, storage and remobilisation.

3. Research strategies:

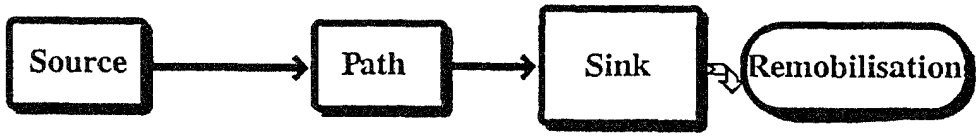
Experimental approaches to increasing sucrose accumulation can be considered in two broad categories:

(1) Optimising the existing process

(2) Redesigning sugar cane as a sucrose accumulator.

3.1 Optimising the existing process of sucrose accumulation

The process of sucrose accumulation involves several stages, shown diagrammatically below:



Photosynthesis	Transport	Stalk Storage	Losses
•Light Capture	•via Apoplast	+Fibre	•Growth
•Sucrose Synthesis (vs starch etc.)	(Walls , spaces)	+Protein etc.	•Microbes
	>via Symplast (cytosol, membr.)		
•Sucrose Breakdown			

Approaches to optimise this existing process include:

(a) Breeding and selection, which continues to provide improved cultivars based on favourable combinations of genes following planned cross-pollination.

(b) Agronomy and extension, which provide growers with information necessary to achieve the potential of specific cultivars for sucrose accumulation, for example by correct application of fertilisers, irrigation or chemical ripeners.

(c) Crop physiology and modelling, which generate new information about responses of sugar cane, often a valuable starting point for (b) or (d).

(d) Molecular modification, to relieve "bottlenecks" in the process or reduce wasteful reactions that reduce stored sucrose.

In planning to optimise the existing process, a molecular biologist would therefore seek answers to two questions:

> Which enzymes, transport proteins or other gene products are likely to result in increased sucrose storage if they are appropriately modified or increased in sugar cane cells? (Or, more simply, what are the rate-limiting steps?)
>
> What are the most wasteful, unnecessary elements of the process?

In both cases, additional data is needed from continuing biochemical and physiological research, perhaps coupled with modelling of the process. However, it is now possible to make "best guesses" from available data, and use gene transfer technology to test these ideas by over expressing, reducing or modifying the selected target genes as appropriate.

3.2 Redesigning sugar cane as a sucrose accumulator

The gene pool of *Saccharum officinarum* and its wild relatives is the result of an evolutionary process which has selected for genes and gene combinations valuable for survival and reproduction of plants, before

intensive cultivation by humans. Traditional breeding works to obtain the most favourable combinations of the genes in this gene pool, but is constrained by the limits of this wild heritage.

Molecular modification provides a powerful new approach to complete the domestication of sugar cane, because it allows new genes for desired traits to be added to the limited *Saccharum* gene pool, it allows the patterns of expression of existing genes to be changed to favour growth or maturation, and it allows undesired genes to be specifically "switched off, even when they show tight genetic linkage to desired genes.

Novel capabilities that might be added by gene transfer include cell membrane or vacuole membrane sucrose pumps to increase sucrose compartmentalised in storage parenchyma cells, or inducible control of senescence genes to permit ordered ripening of blocks before harvest.

Examples of processes which might divert resources away from sucrose storage, and which could be reduced or specifically blocked by molecular modification include lignification (excess fibre), futile cycles of sucrose metabolism and synthesis, or production of floral structures.

The technical approaches to overexpression, modification or suppression of target genes have been outlined in presentations by Dr. Furbank and Dr. Maretzki.

4. limitations on molecular modification:

At present, decisions on molecular targets most likely to allow increased sucrose accumulation are best guesses based on available data from biochemical and physiological studies. Support is needed for additional research to better define such targets .

Molecular modification of any process requires identification and isolation of the gene to be modified. In some cases the target genes isolated from other organisms can be used in sugar cane, but biological and commercial barriers may interfere. Isolation of desired genes from sugar cane is likely to be slower, and initially more expensive. In the short term, it may be undertaken based on homology with known genes from other species, or based on properties of known gene products. In the longer term, cloning desired genes will be assisted by the work commencing to develop a genomic map for sugar cane, and by other new gene tagging technologies.

In most cases, commercial application will require appropriate patterns of expression of introduced genes. This will be assisted by work underway to isolate appropriate gene regulatory sequences (promoters and enhancers) for suear cane.

The efficiency of the current sugar cane genetic transformation system is sufficient for transfer of known useful genes with the objective of selecting commercially useful transformants. However, larger numbers of transformants are required for experimental work to test hypotheses about

the regulation and effects of expression of specific genes of interest. More work is required to obtain higher sugar cane transformation frequencies, or we are likely to become limited by the logistics of gene transfer once a range of genetic constructs need to be tested.

Finally, it is important to bear in mind that specific changes to a single gene can potentially trigger a series of consequential changes in a transgenic plant. The poorer the available background data, the greater the possibility of unpredicted consequential changes. Thus much early molecular modification will be experimental, with a need for very thorough characterisation of resulting transgenic plants before moving to commercial application.

5. Research priorities:

As indicated in the attached Table, group discussion suggested the following current key priorities based on feasibility and probability of success.

1. Genetic manipulation of sugar cane to alter levels of expression of key enzymes or transporters involved in sucrose metabolism and storage.

2. Isolation of promoters resulting in appropriate tissue-specific expression patterns for the selected enzymes in sugar cane.

3. Increased transformation efficiency to facilitate production of the required number of transformed sugar cane plants for evaluation of effects on sucrose accumulation.

6. Research collaboration:

Collaboration needs and opportunities were covered in discussion and are shown in the Table. Scientists from CSIRO, HSPA and UQ met after the workshop was finished to map out collaborative research efforts on cloning, transfer and expression of genes in sugar cane.

7. Strategic capacity building:

With the initiative by CSIRO, DTCP to set up a molecular biology laboratory dedicated to genetic modification for increased sucrose accumulation, there will be at least three laboratories in Brisbane with a major commitment to sugar cane molecular improvement. Some large equipment items may be beyond the resources of a single laboratory or project, but could be heavily used, and would increase the efficiency of a range of projects if shared. An obvious example is apparatus to automate sequencing of DNA, which will be a frequent task in all genetic manipulation projects. Assistance from SRDC in the purchase or operation of such large equipment items for shared use would be a valuable strategic investment in sugar cane molecular improvement.

Table: Summary of research strategies and priorities:

This table summarises the group discussion on research priorities in molecular modification for increased sucrose accumulation.

Opportunities & Needs		Technical Feasibility	Probability of Success /Benefits	Time Span / Costs	Interested Collaborators	Priorities
↑ or ↓ Expression of:	↕ Sucrose Synthase 1,2	✓	Medium	3-5 years work by one research scientist plus technician per enzyme, to glasshouse testing stage	HSPA	H
	↑ SPS - stem, leaf	✓	High		HSPA in progress CSIRO?	H
	↓ Invertase	✓	Medium		HSPA	H
	↑ FBPase (leaf)	✓	Medium		CSIRO PI?	H
	↓ ADP Glucose PPase	✓	High		CSIRO?	H
	↓ Lignin biosynthesis	✓	See next section		CSIRO/UQ	
Data on:	Compartmentation of-					
	Sucrose	✓	High	Several years each for 1-2 scientists	HSPA in progress	
	Enzymes	✓	High		HSPA in progress	
	Turgor Regulation	✓	Low (at present)			L
	Sucrose Transporters	✓	Low (difficult)			L
	Effects of Novel sinks	✓	Low (at present)			L
	Regulation of Enzyme Activity	✓	Medium	1 biochemist for 1-2 y per isolated enzyme	HSPA?	
Isolation of:	Storage Parenchyma Specific Promoters	✓	High	2-3 years for 1 scientist and technician	CSIRO/HSPA/UQ	H
Other:	↑ Transformation Efficiency	✓	High	1-2 years for a skilled technician	UQ/HSPA/BSES	H

Collation Session: Chemical and Physiological Approaches

Collator: G.R. Stewart

1. Desired outcome:

The desired outcome is the identification of physiological traits that can be either selected for or genetically manipulated so as to increase sucrose content at harvest while maintaining maximum cane biomass yield. Implicit in this is an affirmation that, in this molecular age, basic plant physiology can still make a contribution to crop improvement.

2. Current knowledge/background:

Sugar cane is a well researched species and we have considerable general information on its physiology. Much of this centres on growth studies and its photosynthetic characteristics- There is, however, a clear need to develop a carbon balance model for the crop under various environmental constraints. In particular, we need to quantify carbohydrate partitioning within the plant through its various growth phases.

3. Research strategies and priorities:

The physiological contribution to enhancing sucrose accumulation can be direct or indirect. In the former, we are concerned with identifying and selecting for existing physiological processes that influence sucrose metabolism. In the later, we are developing the theoretical background against which the molecular biologist can manipulate the sucrose accumulating capacity of sugar cane.

3.1 Physiological targets for enhanced sucrose accumulation

Within the sugar cane plant, there are a number of competing carbohydrate pools. In addition to sucrose they include cell protein (in particular the photosynthetic machinery), structural carbon (polysaccharides and and lignins), starch, respiratory substrates and root exudates.

Within these, the magnitude of carbon utilisation in respiration was identified as being a suitable physiological characteristic for further investigation. Dark respiration can account for as much as 50 % of gross photosynthate and there is evidence from work with other *Gramineae* that selection for low rates of dark respiration can enhance biomass yield. Research into the variation in dark respiration rates among sugar cane varieties should be undertaken as a high priority. If sufficient variation exists, there is a high probability that selection for low rates of dark respiration would be successful in increasing yield biomass

and hence sucrose production. The relevant expertise and facilities are available for this type of work.

The remobilisation of stored sucrose to support growth and protein synthesis represents a potential sucrose yield loss. Investigations of the mechanisms controlling the allocation of carbon between sucrose accumulation and protein synthesis and the relationship between carbon status and protein turnover could be of value particularly since several of the enzymes of nitrogen catabolism are subject to catabolite repression, that is, their activity increases in response to sucrose limitation. The mobilisation of protein carbon to sucrose accumulation is a long term target that might be amenable to molecular modification.

3.2 Physiological framework for molecular biology

A high priority area that is an essential prerequisite for genetic engineering of the sucrose accumulating capacity of sugar cane is the development of quantitative models for the partitioning and turnover of carbon within the plant. One area highlighted as a target for genetic engineering is lignin production. The allocation of carbon to lignin biosynthesis needs to be investigated prior to work aimed at reducing the lignin content of selected sugar cane tissues. Precise, quantitative physiological studies into the relationship between lignification and lodging resistance need to be carried out as part of this program.

4. Research collaboration:

Collaboration between CSIRO, DTCP and the Botany Department, UQ is being developed on some aspects of sugar cane physiology. Further collaborative research between UQ, CSIRO and BSES should be fostered in this area in the future.

5. Strategic capacity building:

Maintenance of the existing SRDC post-graduate fellowship scheme, or possibly expanding it, would help the longer term strategic aim of building up information on sugar cane physiology and providing a flow-through of new researchers with special skills and understanding of the cane plant.

PART 4

OVERVIEW OF THE WORKSHOP

Overview of Workshop

M M LUDLOW
CSIRO Division of Tropical Crops and Pastures
306 Carmody Rd,
St Lucia, Brisbane, Qld. 4067

The objective of the workshop was to review the opportunities for increasing sugar yield in cane by increasing sucrose accumulation. The following is a brief summary of the some of the significant points raised at the workshop.

Industry needs

combination of short-term and long-term outcomes
continuing development of new technologies to increase industry efficiency and competiveness
need for research to give an industry outcome
higher CCS is desirable, especially early season CCS
SRDC strategic plan embraces cane productivity, viz. to increase and sustain sugar yield/ha/annum

Conventional breeding

20-30% increase in sugar yield over the years, largely due to higher biomass than higher CCS
good progress in seeking higher early season CCS, variability available, heritability high, low G x E interaction
introduction of wider range of S. *officinarum* is desirable
continued future gains in increasing CCS through breeding will become more difficult
collaboration required between institutes on genetic markers, and on understanding reasons for genotype differences in CCS

Management options

need capacity for more rapid response to assess management changes to suit new cultivars, new practices, environmental laws, climatic change, etc
need growth and sugar simulation models to explore management options, and reduce *ad hoc* experimentation
quantitative relationships between sugar accumulation and environment and nutritional factors essential for model
understanding of climatic interactions with ripener effectiveness is needed

Genetic engineering for sucrose accumulation

theoretically, there is ample scope for increasing CCS above present maximum levels
presently have good general understanding of metabolic pathways
need better definition of limiting processes, control points, and location of sugars and enzymes at the cellular level
techniques, cloned genes available for genetic modification of a number of enzymes in cane
believe it to be time to go ahead and make test under- and over-expression of key enzymes rather than seek further biochemical understanding; the latter will flow from gene modification studies
a number of possible target leaf and stem enzymes were indentified
overexpression of enzymes may not show gains if sugar transport is key limitation

Other structural and physiological aspects

apoplastic storage of sugars allows high sucrose storage in cytosol
high turgor does not develop in stem cells as they accumulate sucrose and is thus not seen as a limiting factor to sugar storage
suberisation and lignification of stem storage parenchyma restrict may sucrose accumulation? Lignin level can be genetically modified
low respiration genotypes could be sought to conserve C units for extra growth or CCS
explore significance of alternative "cyanide-resistant" respiration pathway in sugar cane for inefficient use of fixed C, gene for enzyme involved is cloned
genetically reduce lignin in plant to favour redistribution of fixed C from fibre to sucrose

LIST OF ATTENDEES

Sugar Research and Development Corporation

Dr E F Henze
Chairman
Sugar Research and Development Corporation
PO Box 12050
Brisbane QLD 4002
[Ph: 07 210 0495] [Fax: 07 210 0506]

Mr Eion Wallis
Executive Director
Sugar Research and Development Corporation
PO Box 12050
Brisbane QLD 4002
[Ph: 07 210 0495] [Fax: 07 210 0506]

Dr Vinee E Mungomery
Sugar Research and Development Corporation
PO Box 12050
Brisbane QLD 4002
[Ph: 07 210 0495] [Fax: 07 210 0506]

Hawaiian Sugar Planter's Association

Dr Andrew Maretzki
Hawaiian Sugar Planters Association
PO Box 1057
Aiea
Hawaii USA 96701-1057
[Ph: 808 487 5561] [Fax: 808 486 5020]

Dr Paul H Moore
Hawaiian Sugar Planters Association
PO Box 1057
Aiea
HAWAII USA 96701-1057
[Ph: 808 487 5561] [Fax: 808 486 5020]

University of Adelaide

Dr John S Hawker
Dept of Plant Science
University of Adelaide
Glen Osmond SA 5064
[Ph: 08 372 2327] [Fax: 08 232 3297]

University of Newcastle

Dr John W Patrick
Dept Biological Sciences
University of Newcastle
Newcastle NSW 2308
[Ph: 049 21 5700] [Fax 049 216923]

Bureau Sugar Experiment Stations

Brisbane

Dr R T Muffins
Bureau Sugar Experiment Stations
PO Box 86
Indooroopilly QLD 4068
[Ph: 07 371 6100] [Fax: 07 371 4115]

Dr D M Hogarth
Bureau Sugar Experiment Stations
PO Box 86
Indooroopilly QLD 4068
[Ph: 07 371 6100] [Fax: 07 371 4115]

Dr Grant Smith
Bureau Sugar Experiment Stations
PO Box 86
Indooroopilly QLD 4068
[Ph: 07 371 6100] [Fax: 07 371 4115]

Dr G. Leonard
Bureau Sugar Experiment Stations
PO Box 86
Indooroopilly QLD 4068
[Ph: 07 371 6100] [Fax: 07 371 4115]

Bundaberg

Dr Michael C Cox
Bureau Sugar Experiment Stations
P O Box 651
Bundaberg QLD 4670
[Ph: 071 59 3228] [Fax: 071 59 3383]

Mr G Kingston
Bureau Sugar Experiment Stations
P O Box 651
Bundaberg QLD 4670
[Ph: 071 59 3228] [Fax 071 59 3383]

Dr De-Li Liu
Bureau Sugar Experiment Stations
P O Box 651
Bundaberg QLD 4670
[Ph: 071 59 3228] [Fax 071 59 3383]

Department of Primary Industries

Dr Robert Henry
QLD Agricultural Biotechnology Centre
University of Queensland
St Lucia 4072
[Ph: 07 365 4962] [Fax: 07 377 0466]

CSIRO - Division of Plant Industry

Dr Robert T Furbank
CSIRO Division of Plant Industry
PO Box 1600
Canberra ACT 2601
[Ph: 06 246 5218] [Fax: 06 246 5000]

Sugar Research Institute

Dr O L Crees
Process Research Unit
Sugar Research Institute
P O Box 5611
Mackay Mail Centre QLD 4741
[Ph: 079 52 1511] [Fax: 079 52 1734]

CSIRO - Division of Tropical Crops and Pastures

Dr Merv M Ludlow
CSIRO Div. Tropical Crops and Pastures
306 Carmody Rd
St Lucia QLD 4067
[Ph: 07 377 0322] [Fax: 07 377 0410]

Dr John R Wilson
CSIRO Div. Tropical Crops and Pastures
306 Carmody Rd
St Lucia QLD 4067
[Ph: 07 377 0321] [Fax: 07 377 0410]

Dr Russell C Muchow
CSIRO Div. Tropical Crops and Pastures
306 Carmody Rd
St Lucia QLD 4067
[Ph: 07 377 0253] [Fax: 07 377 3946]

Dr Bob Ferraris
CSIRO Div. Tropical Crops and Pastures
306 Carmody Rd
St Lucia QLD 4067
[Ph: 07 377 0340] [Fax: 07 377 3946]

Dr Phillip A Jackson
CSR
Box 59
PO Macknade QLD 4850
[Ph: 077 77 0203] [Fax: 077 77 7657]

Dr K Lynne McIntyre
CSIRO Div. Tropical Crops and Pastures
306 Carmody Rd
St Lucia QLD 4067
[Ph: 07 365 2815] [Fax: 07 377 3946]

Dr Christopher Grof
CSIRO Div. Tropical Crops and Pastures
306 Carmody Rd
St Lucia QLD 4067
[Ph: 07 377 0321] [Fax: 07 377 0410]

Australian Cane Fanners Association

Mr J N Farley
General Manager
Australian Cane Farmers Assocoiation Ltd
GPO Box 608
Brisbane QLD 4001
[Ph: 07 229 9899] [Fax: 07 229 3130]

Queensland Sugar Corporation

Dr David Rutledge
Chief Executive Officer
Queensland Sugar Corporation
P O Box 981
Brisbane QLD 4001
[Ph: 07 231 0199] [Fax: 07 2212906]

Canegrowers

Mr R Chapman
Canegrowers
GPO Box 1032
Brisbane QLD 4001
[Ph: 07 864 6444] [Fax: 07 864 6429]

Australian Sugar Millers Council

Dr Phillip A Jackson
CSR
Box 59
PO Macknade QLD 4850
[Ph: 077 77 0203] [Fax: 077 77 7657]

University of Queensland

Dr George R Stewart
Botany Department
University of Queensland
St Lucia QLD 4072
[Ph: 365 2727]

Dr Robert G Birch
Dept. of Botany
University of Queensland
St Lucia QLD 4072
[Ph: 07 365 3347] [Fax: 07 365 1699]

Mr Mal K Wegener
Agriculture Department
University of Queensland
St Lucia QLD 4072
[Ph: 07 365 2929]