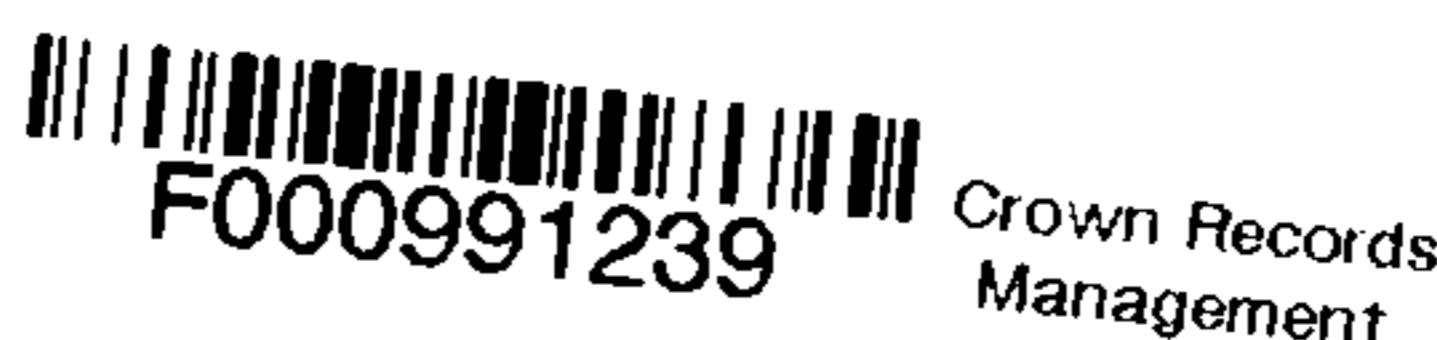


Physiological, biochemical and molecular studies of asparagus postharvest deterioration



A report prepared for
New Zealand Asparagus Council

G King, P Hurst, D Irving, K Davies &
J Seelye
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1 SUMMARY

We are investigating changes in harvested asparagus spears to identify factors influencing deterioration. Identification of key changes may offer the opportunity to delay deterioration, extend storage life, and open the possibility of sea freight of spears to Northern hemisphere markets. Our main findings are:

- * There is a rapid change (away from sugars) in the type of substrates respired by asparagus spears after harvest.
- * Sucrose is the major sugar in tips of spears. The rate of sucrose loss, or size of the available sucrose pool, may be more important than absolute sucrose levels in deterioration.
- * A change in a particular gene is correlated with sugar loss.

These findings suggest sugar loss is a critical feature in the postharvest deterioration of asparagus.

2 INTRODUCTION

Fresh asparagus is airfreighted from New Zealand to markets in Japan and USA. Seafreight would reduce costs, give greater marketing flexibility, and allow industry expansion through export of increased volumes. Seafreight is currently not possible because of tissue deterioration during the ambient shelf-life or marketing period following low temperature storage. Greater understanding of the physiological, biochemical and molecular processes accompanying deterioration of harvested asparagus is essential to provide opportunities for modifying the postharvest performance of the crop.

Our earlier research indicated that several changes accompanied postharvest deterioration of asparagus.

These included:

a rapid decline in respiration rate, altered expression of specific genes, sugar and protein loss, accumulation of amino acids and ammonia, and ultimately loss of tissue integrity.

Two interrelated findings are exciting. We have shown both a rapid loss of sucrose and turning-on of a gene encoding asparagine synthetase (AS; the enzyme responsible for asparagine synthesis in plants) within 3 hours of harvest. These changes parallel respiratory decline, and collectively provide physiological and molecular evidence for rapid altered metabolism by the harvested spear.

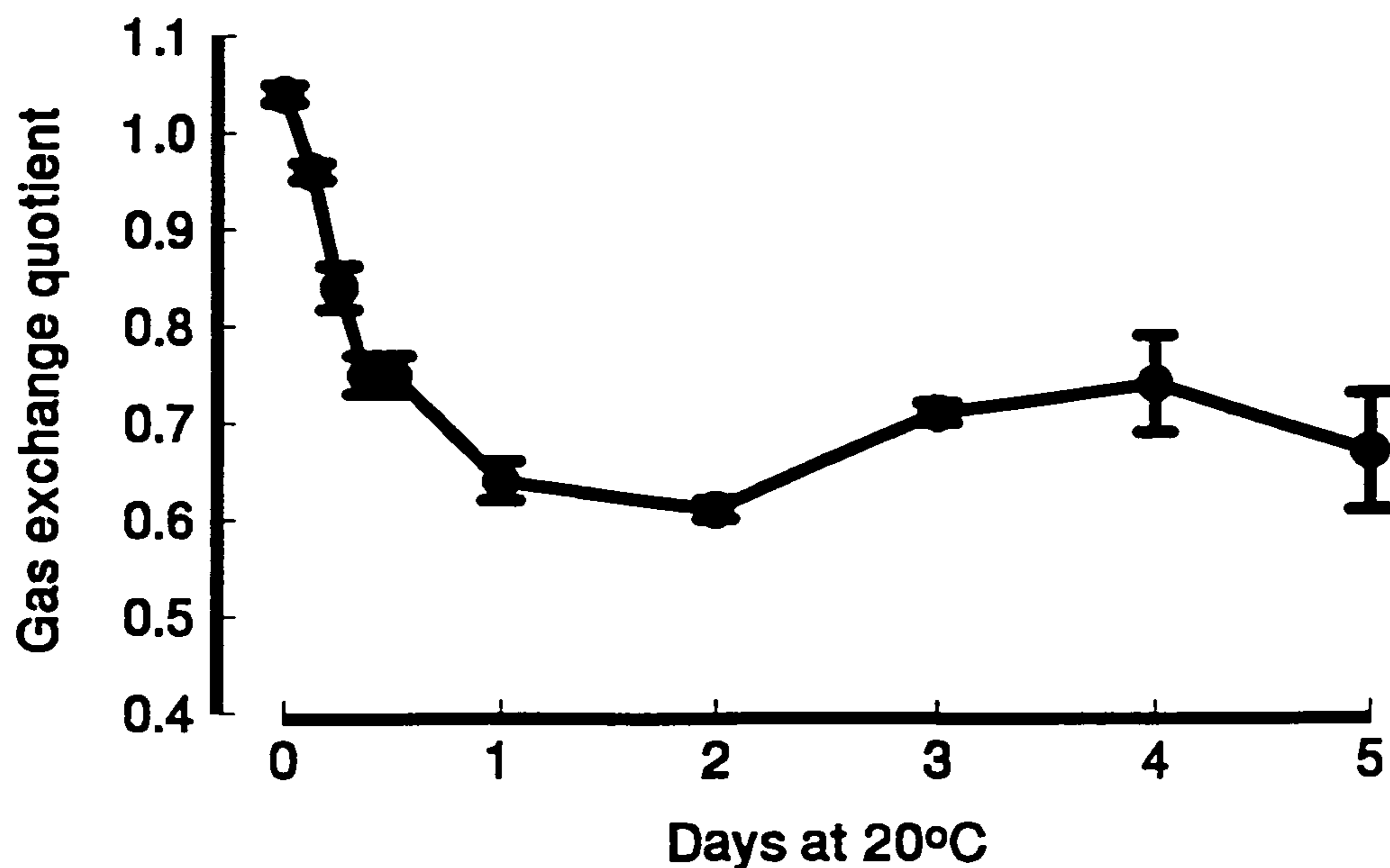
There are published links between sucrose depletion and asparagine accumulation in other plants. A major thrust of our current work is to investigate sucrose decline and AS induction in harvested asparagus spears to further validate this linkage.

2.1 Respiratory metabolism

Respiration declines quickly and sucrose is lost in tips of harvested asparagus spears. Investigations of changes in the respiratory gas exchange quotient (GEQ; ratio of carbon dioxide produced to oxygen consumed by tissue) can give valuable information on the substrates used for respiration in plant systems. Gas exchange quotients are around 1.0 when sugars such as sucrose, glucose or fructose are being consumed. When free amino acids (or amino acids derived from protein breakdown) or fat reserves are used, the GEQ will be lower than 1.0.

There is a substantial fall in the GEQ of tip tissues within 3 hours of spear harvest (Fig. 1), even though sugars are still present in the tissue. This suggests a very rapid shift in the type of substrates being used by spear tissues immediately after harvest. Within 1-2 days at 20°C, the low GEQ indicates that lipids and/or proteins are likely being consumed to support respiration. Breakdown of cellular membranes is a potential source of both lipids and proteins. The GEQ data supports our earlier electron microscopy studies showing that cellular membranes are beginning to deteriorate after 48 hours at 20°C. Further, changes in the GEQ provide additional evidence that processes accompanying deterioration of asparagus begin very soon after the harvester's knife has separated the spear from the crown.

Figure 1: Changes in the gas exchange quotient in tips of harvested asparagus spears held at 20°C.



2.2 Spear sugar changes and nitrogen metabolism

A link between rapid loss of sucrose and induction of an asparagine synthetase (AS) gene in tips of harvested asparagus spears was established in our earlier work. Total soluble sugar (sum of sucrose, glucose, and fructose) content of spear tips declines as the spears grow in the field i.e. tips from short spears tend to have more sugar than tips from tall spears. However, the individual sugar composition in tips of different height spears has not previously been well established. Demonstration that sucrose is the predominant sugar in tips irrespective of spear height, and that tip sucrose levels decline rapidly after harvest, may give a useful experimental system to test further our hypothesis that loss of sucrose induces the AS gene. For example, if the sucrose levels in tips of different height spears decline at different rates, we might expect differences in the timing of AS gene induction and asparagine accumulation.

Sucrose was the predominant sugar in spear tips at harvest irrespective of spear height (Table 1). However, whilst glucose and fructose levels were highest in tips from 40 mm spears, the levels of these sugars were similar in tips from 110, 180 and 250 mm spears. This shows that differences in the total soluble sugar content of tips from different height spears can be mainly attributed to differences in sucrose content.

Table 1: Soluble sugar content (mg/g dry weight) at harvest of tips of different height asparagus spears.

Sugar	Spear height (mm)			
	40	110	180	250
Sucrose	57.3	48.8	50.9	39.8
Glucose	40.0	29.3	30.7	30.4
Fructose	47.5	37.4	39.5	37.5

Sucrose content of tips declined rapidly after harvest, irrespective of spear height (Fig. 2). Although the sucrose levels of the tips from 40 mm spears tended to stay above those from the other height spears, there was no conclusive evidence for differences in the rates of sucrose decline among different height spears.

The characteristic accumulation of asparagine in tips of harvested asparagus spears was unaffected by spear height (Fig. 3). There was no evidence for delayed accumulation of asparagine in tips from 40 mm spears even though these tips tended to have higher sucrose levels than the others. These results suggest that either the

rate of sucrose decline, or size of the readily available sucrose pool, may be more important than absolute sucrose levels in initiating asparagine formation.

Figure 2: Changes in sucrose levels in tips of different height asparagus spears held at 20°C.

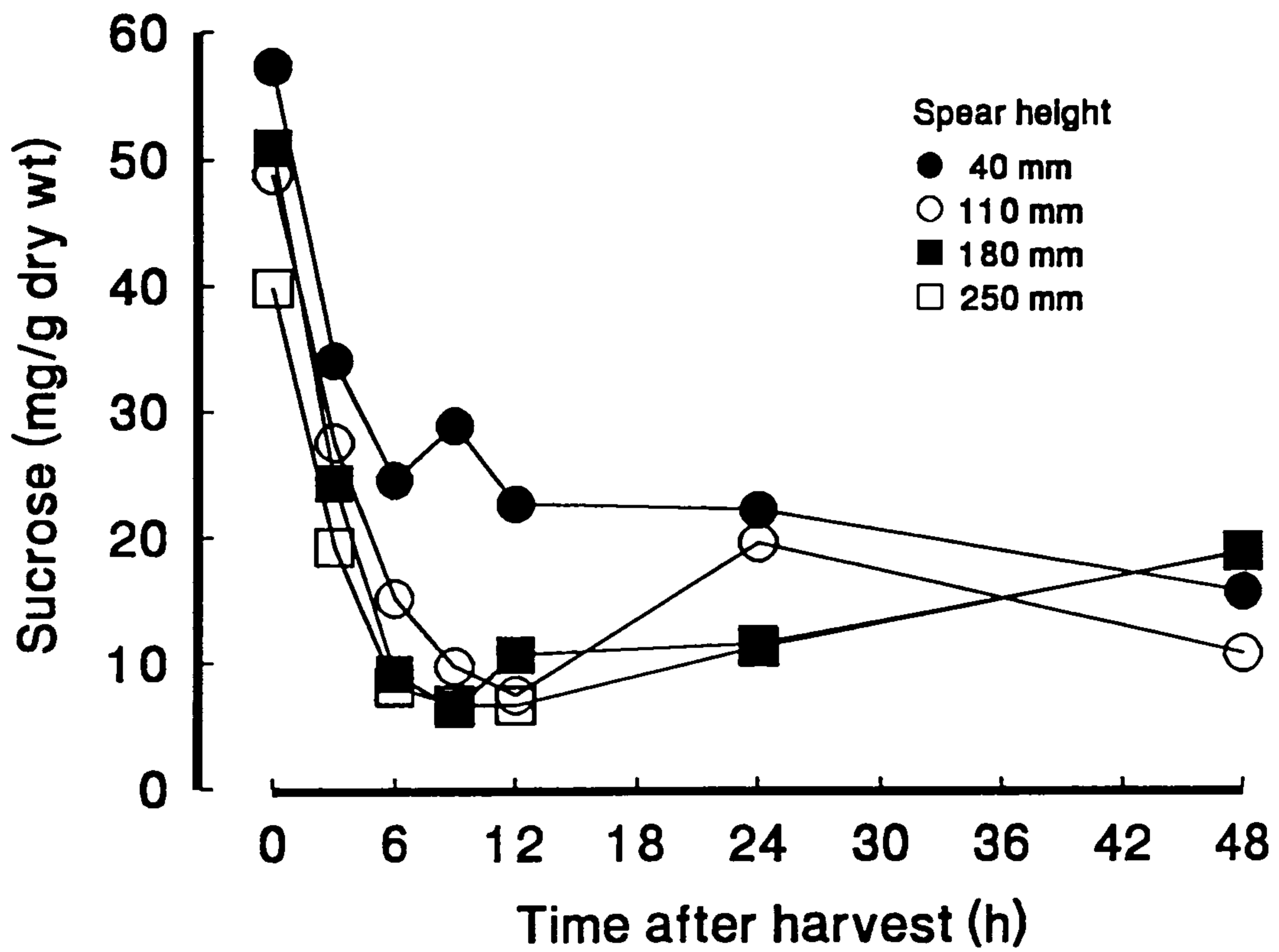
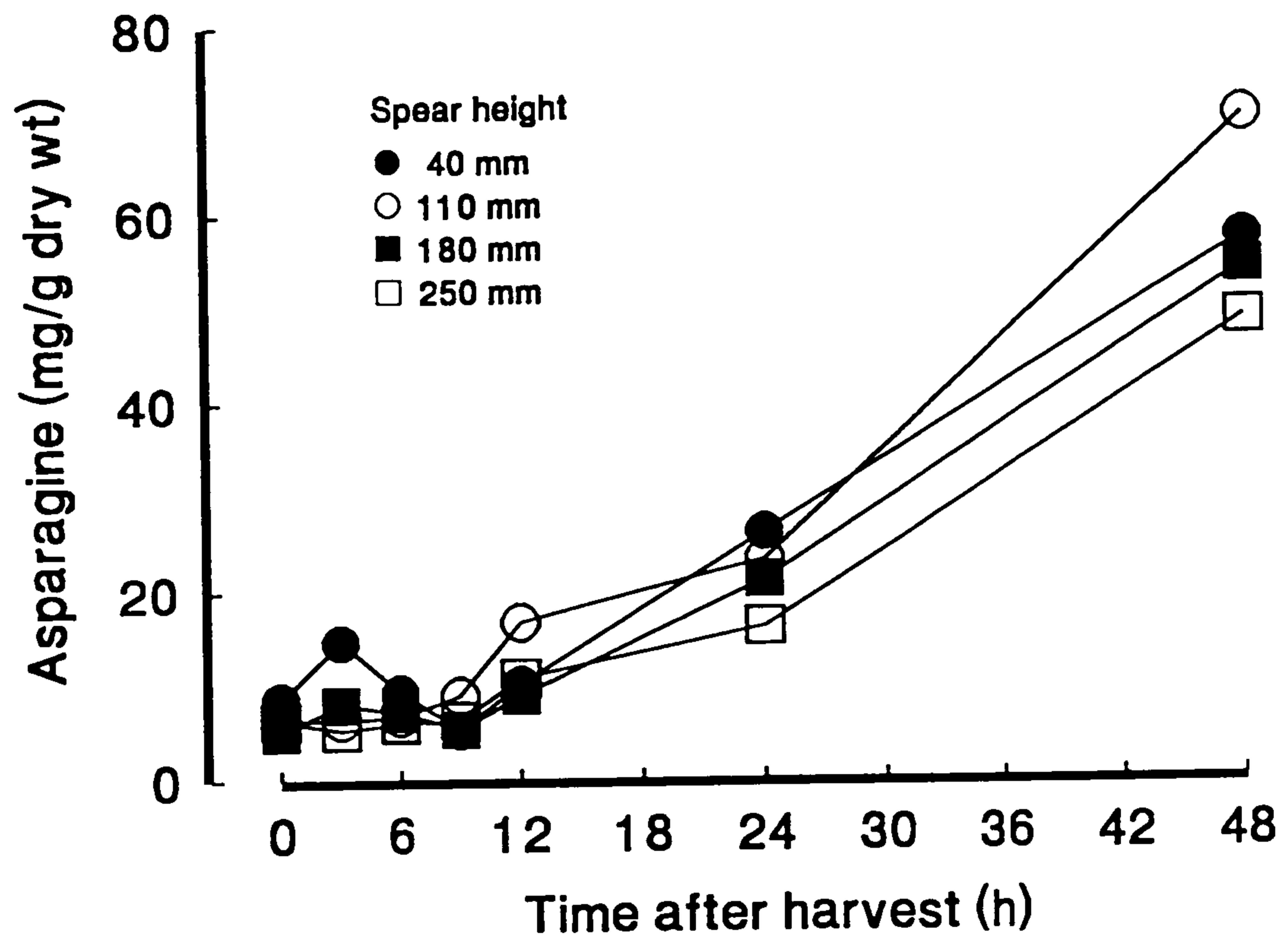


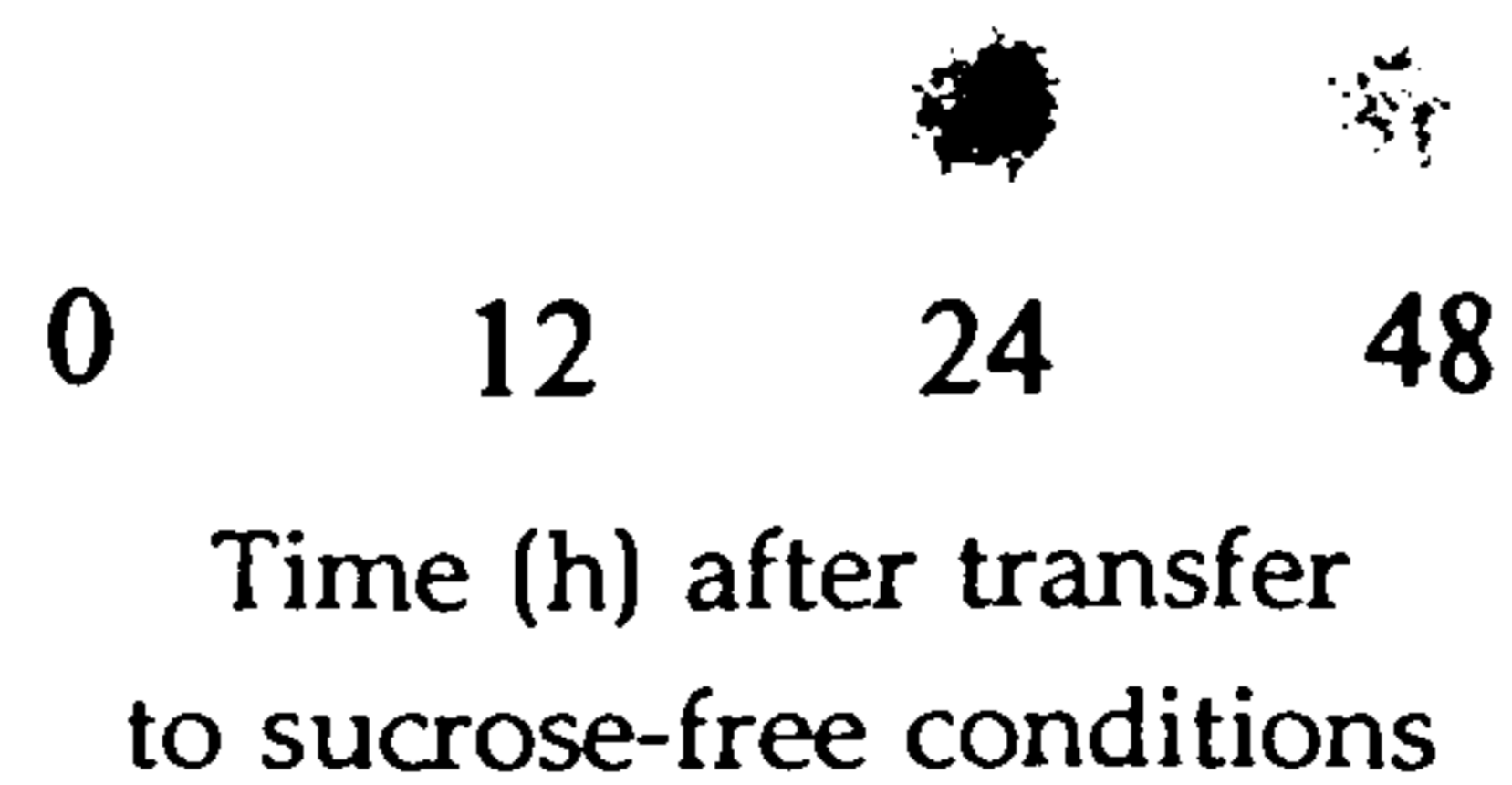
Figure 3: Changes in asparagine levels in tips of different height asparagus spears held at 20°C.



2.3 Cell culture experiments

An alternative approach to investigating the relationship between sucrose decline and AS gene induction was explored using asparagus cell cultures. Cultures grown in sucrose-containing media were transferred to sucrose-free media, and cells sampled at regular intervals. AS gene induction was detected 12 h after transfer, with strong expression evident by 24 h (Fig. 4). This preliminary finding gives direct evidence that sugars are involved in regulating expression of the AS gene.

Figure 4: AS gene expression in asparagus cell cultures transferred to sugar-free media. The darker the dot the greater the gene expression.



2.4 AS gene promoter

A promoter is the piece of DNA controlling a particular gene. Identification and isolation of promoters that can be turned off or on after harvest (enabling controlled expression of genes in the right tissues at the right time) will be key components in future attempts to genetically modify the postharvest performance of horticultural crops.

We have recently shown that the AS gene is turned on after harvest in asparagus spears. This finding has provided the opportunity to isolate a harvest-induced promoter. In collaboration with Dr. Kevin Farnden (Biochemistry Dept., Otago University), we are co-supervising a PhD project aimed at isolating the AS promoter from asparagus. This will allow definitive experiments on factors regulating AS to be undertaken. The AS promoter should also provide a useful molecular tool allowing us to regulate other genes which we may wish to attach to the promoter and turn on in asparagus after harvest.

Preliminary work on isolation of the AS promoter began this year and progress has been excellent to date with potential candidates already isolated. This work is scheduled to continue over the next three years.

3 CONCLUSIONS AND GENERAL SUMMARY

1. A rapid shift in the type of substrates consumed to support respiration is an early response to spear harvest.
2. Sucrose is the major sugar in tips of spears of different heights. However, rate of sucrose loss, or size of the available sucrose pool, may be more important than absolute sucrose levels in initiating asparagine formation.
3. AS gene expression is correlated with transfer of asparagus cell cultures to sucrose-free media.
4. Work on isolating the AS promoter has commenced and is progressing extremely well.

We now have several pieces of evidence demonstrating altered metabolism within 2 - 3 hours of harvesting asparagus spears. These include respiratory decline, changes in the gas exchange quotient, and induction of asparagine synthetase gene expression. These changes are all initiated before spears would be chilled in the normal commercial handling situation. Identification of a stimulus (e.g. sugar depletion) initiating processes leading to deterioration would provide valuable information for plant breeders, enabling them to select for elite plants with improved postharvest performance.

Links between respiratory decline, sugar metabolism, and AS gene expression are becoming better established through our research. In addition to providing information for plant breeders, our research may also assist in providing the opportunity to modify asparagus by molecular means to extend postharvest life. Our collaborative research to isolate the AS promoter for asparagus advances both these goals.