

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**YIELD AND QUALITY OF ASPARAGUS (*Asparagus officinalis* L.) AS
AFFECTED BY CARBOHYDRATE DISTRIBUTION IN RELATION TO
DAYLENGTH, FRUCTAN LEVELS, AND BUD NUMBER**

A thesis presented in partial fulfillment of the requirements
for the degree of Master of Applied Science
in Plant Science at Massey University
New Zealand

K A R N O

1999

ABSTRACT

Three different aspects of carbohydrate reserves in asparagus were evaluated in this thesis. The first aspect was the effect of daylength on dry matter partitioning between fern and storage roots (Chapter Two), the second aspect was remobilization of soluble carbohydrates (fructans) from different roots during spear harvest (Chapter Three), and the last aspect was the effect of cutting height at harvest on spear yield and quality and stimulation of additional spears using hormone treatment (Chapter Four).

The experiments using controlled climate growth chambers were conducted to evaluate the effect of daylength on dry matter partitioning in asparagus plants. The treatments were long constant daylength (15.5 h) and reducing daylength. The results showed that partitioning carbohydrates between fern and crown was influenced by daylength. Plants exposed to reducing daylength showed reductions in plant height, shoot number, number of lateral per shoot, length of lateral, and fern dry weight. This reduction in fern growth was followed by decreasing NAR (net assimilation rate) except at daylengths around 14 hours. However under reducing daylength relatively more carbohydrate partitioned to crown than to fern, as indicated by root:shoot dry weight ratio and allometric ratio between crown and fern. The results also suggested that daylengths around 13.5 to 14 hours seem to be particularly favorable for storing carbohydrates in the roots. In addition, cultivar differences exist in the response to daylength. 'Jersey Giant' was more responsive to daylength than 'UC157' and 'Italian Hybrid' showed a little or no response to daylength.

Radioactive labeling using $^{14}\text{CO}_2$ was used to study fructan remobilization from different roots during spear harvest and fructans separation was done using HPLC System. The results showed that spear growth utilized carbohydrate, not only from the nearest roots, but also from more distant new roots. Spears also utilized carbohydrates from distant old roots during harvest but not to the some extent as from new roots. The HPLC system used in this work was able to separate fructans up to a degree of polymerization of 10 (DP10) and produced a single large peak of long chain fructans. The source of

carbohydrates used to support spear growth was mainly from long chain fructans (DP more than 10) as long chain fructans decreased sharply during spear growth while short chain fructans (DP3 to DP10) only decreased slightly. Changes in individual fructans suggested that hydrolysis rates of DP4 and DP3 seem to be a limiting process during fructan hydrolysis.

Finally, utilization of carbohydrate reserves and buds to produce marketable spears was studied by the application of cutting height at harvest. The results showed that marketable yield (both first class and total marketable yields) showed an optimum cutting height. The marketable yield increased with increasing cutting height and reached a maximum marketable yield at certain height then decreased with increasing cutting height. The optimum cutting heights for first class marketable yield (quality 1 spears) was lower than those for total marketable yield and varied with cultivars so that variation in cutting height during spear harvest should be used for cultivar evaluation. In addition the application of hormone mixture (BA and GA₃), when spear production had nearly ceased, induced additional spear production indicating that spear production was not limited by bud number but probably by carbohydrate level in storage roots, under the conditions of these experiments. However, considerable variation existed between individual plants.

ACKNOWLEDGEMENTS

It would not have been possible to complete this thesis without direct and indirect contributions of many others. I appreciate and would like to thank the following:

my supervisors Dr. D. J. Woolley and Dr. M. A. Nichols for their inspiration for getting this thesis started, along with their expert advice and encouragement throughout the year;

staff at the Plant Growth Unit (PGU), Ray Johnstone, Lindsey Sylva, and Lesley Taylor, for their assistance in preparing everything required for the experiments;

Chris Rawlingson and Judith Kidd for their assistance in laboratory work;

staff members at the International Students Office for their friendly cooperation;

Indonesian and New Zealand Governments, through the Ministry of Foreign affairs and Trade's Overseas Development Aid program, for the financial assistance that made this study possible;

my parents and family in Indonesia for their encouragement and most valued moral support;

finally, my wife Sri Hartini and my young wonderful sons Andika Rikarno Putra and Sindunuraga Rikarno Putra for their patience and persistent encouragement.

TABLE OF CONTENTS

Abstract	ii
Acknowledgements	iv
Table of contents	v
List of figures	x
List of tables	xiv
Chapter one : General introduction	1
1.1. Overview and rationale for thesis	1
1.2. The asparagus plant	4
1.2.1. Fern	6
1.2.2. Crown and roots	6
1.2.3. Bud clusters	6
1.2.4. Spears	7
1.3. Spear yield and quality in asparagus	7
1.3.1. Introduction	7
1.3.2. Yield and quality of asparagus spears	8
1.3.3. Factors affecting yield and quality of asparagus spears	9
1.3.3.1. Pre-harvest factors	9
1.3.3.1.1. Water stress	9
1.3.3.1.2. Planting dept	11
1.3.3.1.3. Plant density	12
1.3.3.1.4. Temperature	13
1.3.3.2. Harvest factors	14
1.3.3.2.1. Harvest pressure	14
1.3.3.2.2. Harvest height	15
1.3.3.2.3. Harvest time	16

1.4. Source-sink relationships and dry matter partitioning	18
1.4.1. Introduction	18
1.4.2. Source strength	19
1.4.3. Sink strength	20
1.4.4. Dry matter partitioning	22
1.4.5. Effect of source on dry matter partitioning	22
1.4.6. Effect of sink on dry matter partitioning	24
1.4.7. Effect of daylength on dry matter partitioning	26
1.5. Fructan metabolism	27
1.5.1. Introduction	27
1.5.2. Occurrence of fructans in plants	28
1.5.3. Chemical structures of fructans	30
1.5.4. Fructan biosynthesis	31
1.5.5. Fructan hydrolysis	34

Chapter two : Effects of daylength on drymatter partitioning of asparagus

(<i>Asparagus officinalis</i> L.)	36
2.1. Introduction	36
2.2. Materials and methods	37
2.2.1. Introduction	37
2.2.2. The first experiment	38
2.2.2.1. Propagation and plant raising	38
2.2.2.2. Growth chambers conditions	39
2.2.2.3. Experimental design	40
2.2.2.3.1. Daylength treatments	40
2.2.2.3.1.1. Constant daylength	40
2.2.2.3.1.2. Reducing daylength	41
2.2.2.3.2. Cultivar treatments	42
2.2.2.4. Data collection	42
2.2.2.5. Total soluble carbohydrate analysis	42
2.2.2.6. Statistical analysis	43

2.2.2.7. Allometric ratio	43
2.2.3. The second experiment	44
2.3. Results	45
2.3.1. The first experiment	45
2.3.1.1. Shoot, root and bud number	45
2.3.1.2. Plant height, lateral branch number and length	50
2.3.1.3. Fern dry weight	51
2.3.1.4. Crown dry weight	51
2.3.1.5. Relative Growth Rate (RGR), Leaf Weight Ratio (LWR) and Net Assimilation Rate (NAR)	54
2.3.1.6. Total soluble carbohydrate	54
2.3.1.7. Root:shoot dry weight ratio	57
2.3.1.8. Allometric ratio	57
2.3.2. The second experiment	60
2.3.2.1. Fern dry weight	60
2.3.2.2. Crown dry weight	60
2.3.2.3. Root:shoot dry weight ratio	63
2.3.2.4. Allometric ratio	63
2.4. Discussion	66
2.4.1. Effects of daylength on seedling growth of asparagus	66
2.4.2. Effects of daylength on dry matter partitioning of asparagus seedlings	67

Chapter three : Remobilization of soluble carbohydrates (fructans) from asparagus roots during spear harvest	73
3.1. Introduction	73
3.2. Materials and methods	74
3.2.1. Plant materials	74
3.2.2. Radioactive labeling	75
3.2.3. Experimental design	78
3.2.4. Total soluble carbohydrate analysis	78

3.2.5. Radioactive analysis	78
3.2.6. Fructan analysis	79
3.2.7. Data analysis	80
3.3. Results	83
3.3.1. Spear harvest	83
3.3.2. Total soluble carbohydrate and radioactivity of different roots during spear harvest	84
3.3.3. Fructose, glucose, and sucrose contents of different roots during spear harvest	86
3.3.4. Changes in fructan composition of different roots during spear harvest	88
3.4. Discussion	92
3.4.1. Changes in total soluble carbohydrates and radioactivity	92
3.4.2. Fructose, glucose and sucrose contents of roots during spear harvest	93
3.4.3. Changes in fructan concentrations in roots during spear harvest	95
Chapter four : Effect of cutting height on spear yield and quality of asparagus, and stimulation of additional spears using hormone treatment	97
4.1. Introduction	97
4.2. Materials and methods	98
4.2.1. Introduction	98
4.2.2. Experiment 1	98
4.2.2.1. Plant material	98
4.2.2.2. Experimental design	98
4.2.2.3. Data collection	99
4.2.2.4. Data analysis	99
4.2.3. Experiment 2	101
4.2.3.1. Plant material	101
4.2.3.2. Experimental design	101
4.2.3.3. Data collection	102
4.2.3.4. Data analysis	102

4.3. Results	103
4.3.1. Experiment 1	103
4.3.1.1. Spear yield	103
4.3.1.1.1. Fresh weight of spears	103
4.3.1.1.2. Dry weight of spears	106
4.3.1.2. Spear quality	107
4.3.1.3. Spear number and diameter	112
4.3.1.4. The relationship between cutting height and total yield and marketable yield	115
4.3.2. Experiment 2	118
4.3.2.1. Analysis of variance	118
4.3.2.2. Effects of cultivar	119
4.3.2.3. Effects of cutting height	120
4.3.2.4. Effects of hormone treatment	121
4.4. Discussion	123
4.4.1. Effects of cutting height on spear yield and spear number	123
4.4.2. Effects of cutting height on spear quality	125
4.4.3. Marketable yield versus total yield	126
4.4.4. Effects of hormone treatment on additional spear production	127
Chapter five : General discussion	129
5.1. Carbohydrate partitioning in relation to daylength, remobilization, and utilization of carbohydrate for spear production	129
5.2. Further work	132
References	133
Appendix 1. Statistical analysis of the effect of daylength on crown dry weight	144

LIST OF FIGURES

- Figure 1.1. The structure of an asparagus plant (Nichols 1996) 5
- Figure 1.2. Chemical structure of frutan from asparagus roots (Shiomi 1993) 31
- Figure 2.1. Main effect of daylength on shoot number of asparagus seedlings (A) and response of cultivars to daylength (B, C, D) (Quadratic curve fit). Harvest axes on C and D correspond to reducing daylength axes on A and B 46
- Figure 2.2. Main effect of daylength on root number of asparagus seedlings (A) and response of cultivars to daylength (B, C, D) (Quadratic curve fit). Harvest axes on C and D correspond to reducing daylength axes on A and B 47
- Figure 2.3. Main effect of daylength on root:shoot number ratio of asparagus seedlings (A) and response of cultivars to daylength (B, C, D) (Quadratic curve fit). Harvest axes on C and D correspond to reducing daylength axes on A and B 48
- Figure 2.4. Main effect of daylength on bud number of asparagus seedlings (A) and response of cultivars to daylength (B, C, D) (Quadratic curve fit). Harvest axes on C and D correspond to reducing daylength axes on A and B 49
- Figure 2.5. Main effect of daylength on fern dry weight of asparagus seedlings (A) and response of cultivars to daylength (B, C, D) (Quadratic curve fit). Harvest axes on C and D correspond to reducing daylength axes on A and B 52

Figure 2.6. Main effect of daylength on crown dry weight of asparagus seedlings (A) and response of cultivars to daylength (B, C, D) (Sigmoid curve fit). Harvest axes on C and D correspond to reducing daylength axes on A and B	53
Figure 2.7. Effects of daylength on Relative Growth Rate (RGR), Leaf Weight Ratio (LWR) and Net Assimilation Rate (NAR)	55
Figure 2.8. Total soluble carbohydrate of asparagus roots as affected by daylength (A) and cultivar within daylength (B). Legend : R=reducing daylength, C=constant daylength, UC='UC157', JG='Jersey Giant', IH='Italian Hybrid'	54
Figure 2.9. Main effect of daylength on root:shoot dry weight ratio of asparagus seedlings (A) and response of cultivars to daylength (B, C, D). Harvest axes on C and D correspond to reducing daylength axes on A and B	58
Figure 2.10. Allometric ratio between fern and crown dry weight of 'UC157', 'Jersey Giant' and 'Italian Hybrid' under reducing and constant daylength	59
Figure 2.11. Main effect of daylength on fern dry weight of asparagus seedlings (A) and response of cultivars to daylength (B, C) (Quadratic curve fit). Harvest axes correspond to reducing daylength axes	61
Figure 2.12. Main effect of daylength on crown dry weight of asparagus seedlings (A) and response of cultivars to daylength (B, C) (Quadratic curve fit). Harvest axes correspond to reducing daylength axes	62
Figure 2.13. Main effect of daylength on root:shoot dry weight ratio of asparagus seedlings (A) and response of cultivars to daylength (B, C) (Quadratic curve fit). Harvest axes correspond to reducing daylength axes	64

Figure 2.14. Allometric ratio between fern and crown dry weight of 'UC157' and 'Jersey Giant' under reducing and constant daylength	65
Figure 3.1. Radioactive (^{14}C) labeling of asparagus plant within chamber (top) and Binos II Infra Red Gas Analyzer (IRGA) with circulating pump to monitor CO_2 uptake (below)	76
Figure 3.2. The washed crown at root harvest, the old roots were tagged using green twist ties (top). The crown was separated into rhizome, new roots (consisting of new roots and new roots associated with spears), and old roots (consisting of old empty roots and old full roots) (below)	77
Figure 3.3. Chromatograms of standards	81
Figure 3.4. Chromatogram of extract from asparagus roots. Numbers indicate degree of polymerization (DP) of fructans and times indicate retention times of the peaks	82
Figure 3.5. Spear dry weight (A) and spear number (B) at different harvest treatments. Vertical bars indicate standard errors	83
Figure 3.6. Total soluble carbohydrate content and radioactivity of different roots during spear harvest. Radioactivity means followed by the same letter are not significantly different at $p=0.05$, data log transformed	85
Figure 3.7. Fructose, glucose, and sucrose contents of different roots during spear harvest	87
Figure 3.8. Changes in fructan composition (DP3 to DP6) of different roots during spear harvest	89

Figure 3.9. Changes in fructan composition (DP7 to DP10) of different roots during spear harvest	90
Figure 3.10. Changes in short chain (sum of DP3 to DP10) and long chain (sum of DP more than 10) fructans of different roots during spear harvest	91
Figure 4.1. Three classes grading system for spear head tightness : (A) Tight (Quality 1), (B) Medium (Quality 2), and (C) Seedy (Quality 3)	100
Figure 4.2. Cumulative spear fresh yield of four asparagus cultivars harvested at different cutting heights during 8 weeks harvest period	105
Figure 4.3. Spear quality of four asparagus cultivars harvested at different cutting heights	111
Figure 4.4. Total spear number (per plant) of four asparagus cultivars harvested at different cutting heights	113
Figure 4.5. The distribution of spear diameter of four asparagus cultivars harvested at different cutting heights	114
Figure 4.6. The relationship between cutting height and total yield and first class marketable yield of four asparagus cultivars	116
Figure 4.7. The relationship between cutting height and total marketable yield of four asparagus cultivars	117
Figure 4.8. Effects of hormone treatment on bud number remaining in relation to soluble carbohydrate remaining in storage roots	122

LIST OF TABLES

Table 2.1. Conditions of Controlled Climate Growth Chamber. Irradiance was measured as mol quanta between 400 and 700 nm	39
Table 2.2. Timetable of daylength reduction for experiment 1	41
Table 2.3. Timetable of daylength reduction for experiment 2	44
Table 2.4. Effects of daylength on plant height, lateral branch number and length of three asparagus cultivars	50
Table 4.1. Total fresh weight (gram/plant) of spears of four asparagus cultivars harvested at different cutting height	103
Table 4.2. Total dry weight (gram/plant) of spears of four asparagus cultivars harvested at different height of cutting	106
Table 4.3. Quality 1 spears (gram/plant) of four asparagus cultivars harvested at different height of cutting	108
Table 4.4. Quality 2 spears (gram/plant) of four asparagus cultivars harvested at different height of cutting	109
Table 4.5. Quality 3 spears (gram/plant) of four asparagus cultivars harvested at different height of cutting	110
Table 4.5. Total spear number of four asparagus cultivars harvested at different cutting height	112

Table 4.6. Main and interaction effects of cultivar, cutting height, and hormone treatment on additional spear number, spear fresh weight, bud number remaining, and total soluble carbohydrate of roots	118
Table 4.7. Effects of cultivar on additional spear number, spear fresh weight, bud number remaining, and total soluble carbohydrate of roots	119
Table 4.8. Effects of cutting height on additional spear number, spear fresh weight, bud number remaining, and total soluble carbohydrate of roots	120
Table 4.9. Effects of hormone treatment on additional spear number, spear fresh weight, bud number remaining, and total soluble carbohydrate of roots	119

CHAPTER ONE

GENERAL INTRODUCTION

1.1. Overview and rationale for thesis

Asparagus (*Asparagus officinalis L.*) is a nutritious and appetizing vegetable belonging to the genus *Asparagus* of the family Liliaceae. It is a dioecious perennial plant which is grown for its edible shoots (spears). Asparagus is indigenous to Europe and Asia and has been used as a food since ancient Greeks and Romans (Salunke and Desai 1984). However, nowadays asparagus is widespread around the world and has become an important international commodity. At least 61 countries worldwide produce asparagus with an estimated total area of 218,335 hectares. From Europe, France, Germany, Spain, Italy, and Hungary are big asparagus producing countries while in Asia, asparagus production is dominated by China and followed by Japan and Thailand. In addition, Australia and New Zealand are important asparagus producing countries with estimated area of about 4,500 and 2,500 hectares, respectively (Benson 1999).

In temperate climates, the life cycle of asparagus is controlled by the season. It is dormant during winter and in spring buds are released from dormancy and start to grow to produce spears. Thus, the growth of spears depends on the carbohydrate source in the storage roots. In term of carbohydrate balance, Nichols (1996) divided the growth of asparagus into four stages : the fern renewal stage, the carbohydrate accumulation stage, the dormancy stage, and the spear production stage. The new fern growth uses large quantities of carbohydrate from storage roots so that the major loss of carbohydrate from the plant occurs during the fern renewal stage. The fern acts as strong sink until the fern opens out and starts to produce substantial amounts of carbohydrate through photosynthetic activity. Carbohydrate accumulation is very important as the storage carbohydrate will be used to support spear and fern growth in the following season. In addition, further storage roots, buds, and bud clusters are produced during this stage when photosynthesis is very active.

However, in the autumn photosynthesis decreases, the fern dies back, and the plant becomes dormant. During the dormancy stage in winter the fern dies down and is removed. This results in a minor loss of carbohydrate. The spear production stage occurs in the spring when the buds are released from dormancy and starts to grow by using storage carbohydrate from storage roots.

Most storage carbohydrates in the roots or crown of asparagus are in the form of fructans. Shelton and Lacy (1980) showed seasonal changes in the fructan levels of asparagus roots. Fructan content in the roots increased during maturation and senescence and the level of fructans decreased during harvest and continued to decrease after harvest during fern production. Therefore, fructans stored during maturation and senescence play an important role in spear growth.

The structures of fructans from asparagus roots have been studied. Shiomi (1993) showed that fructans prepared from asparagus roots consisted of polymers of fructose residues linked by β -2,1 bonds and a non-terminal glucose residue bond with fructose residue at C1 and C6 positions, that is, $1^F(1-\beta\text{-D-fructofuranosyl})_m-6^G(1-\beta\text{-D-fructofuranosyl})_n$ sucrose. She confirmed that the fructans from asparagus roots had high degree of polymerization (DP) ranging from 12 to 22 with the predominant size being DP13-16. This was in contradiction to the previous finding that most fructans in the storage roots were low molecular weight fructans (Martin and Hartmann 1990). Pressman et al. (1993) also found that the highest DP of fructans in 'UC157' was 11 while in 'Junon' was 10.

Little attention has been given to the distribution and specific type of fructans that may be the major influencing factor in spear growth. Woolley et al. (1999) showed that the dry weight loss from the crown was relatively low during spear growth and harvest, but ^{14}C recovered in the crown decreased rapidly during spear growth and harvest. It appears that specific fructans may be required during spear growth. Thus information on the distribution of fructans in crown and remobilization of specific fructans, or fructans from specific roots, to the growing buds during spear production would be very important in

explaining and manipulating spear growth of asparagus so that asparagus production could be increased.

In addition, the partitioning of carbohydrate between fern and crown in the transition stage, between the fern renewal and the carbohydrate accumulation stages, affects the amount of carbohydrate stored in the crown, which in turn may determine spear growth and yield. Sudjarmiko et al. (1997) analyzed the allometric relationship between \log_e shoot and \log_e crown dry weight of 'Jersey Giant' asparagus planted from September to December and harvested every 4 weeks. The results indicated that the allometric relationship from October to January showed a constant slope indicating a constant ratio between relative growth rates of shoot and crown. However, in February harvest, an abrupt change in the allometric relationship occurred. The relative growth rate of the shoot fell relative to that of the crown indicating that more dry matter was partitioned to the crown. The authors suggested that this change was response to a change in environmental factors as the change in partitioning carbohydrate occurred irrespective of the age of the plant. It was hypothesized that the environmental signal affecting this partitioning of carbohydrate may be a change in day length as temperature change at that time was relatively small (17.6°C in January and 16.8°C in February). Woolley et al. (1999) also showed that carbon (^{14}C) partitioning changed abruptly between mid-summer (daylength 14h 29 min to 13h 27min) when 70 % of ^{14}C partitioned to the shoot, and the late summer (daylength 13h 27 min to 12h 27 min), when 74 % partitioned to the crown.

It is also unclear to what extent bud number may limit yield in comparison to levels of storage fructans. Drost and Wilcox-Lee (1990) reported that both carbohydrate reserves in storage roots and bud number decreased with decreasing soil water potential. Consequently, smaller bud number and low carbohydrate levels resulted in low spear yield and quality. Drost (1999) also showed that reducing the amount of water to mature asparagus linearly reduced all growth parameters including root number and root fresh weight, bud number and spear number. As a result, spear yield decreased linearly with decreasing irrigation. Besides, the percentage of marketable spears decreased as irrigation rate decreased. However, these results do not necessarily show whether the

limiting factor on spear yield is carbohydrate reserves or bud number. A possible method for determining the relative limitation to yield of carbohydrate reserves versus bud numbers would be to harvest spears at different heights. Thus varying amounts of reserve carbohydrate would be utilized for each bud for spear growth.

Therefore, this research project was conducted to determine the relative importance of storage carbohydrate compared with bud number in controlling spear yield and provide a strategy to obtain better partitioning of carbohydrate to storage roots in relation to daylength, with specific objectives : (1) to determine the effects of daylength on partitioning carbohydrate between fern and storage roots (Chapter Two); (2) to determine the remobilization of soluble carbohydrates (fructans) from different roots during spear harvest (Chapter Three); and (3) to analyze the effects of cutting height at harvest on spear yield and quality and stimulation of additional spears using hormone treatment (Chapter Four).

1.2. The asparagus plant

Asparagus is a perennial plant with over 50 % of the plant permanently below the ground. The major parts of a mature asparagus plant are spears, buds, rhizome, thick storage roots, and thin feeding roots (Figure 1.1) (Nichols 1996). Basically, these parts can be grouped into two portions: the foliage (aboveground portion) known as the fern and the underground portion of the plant known as the crown.

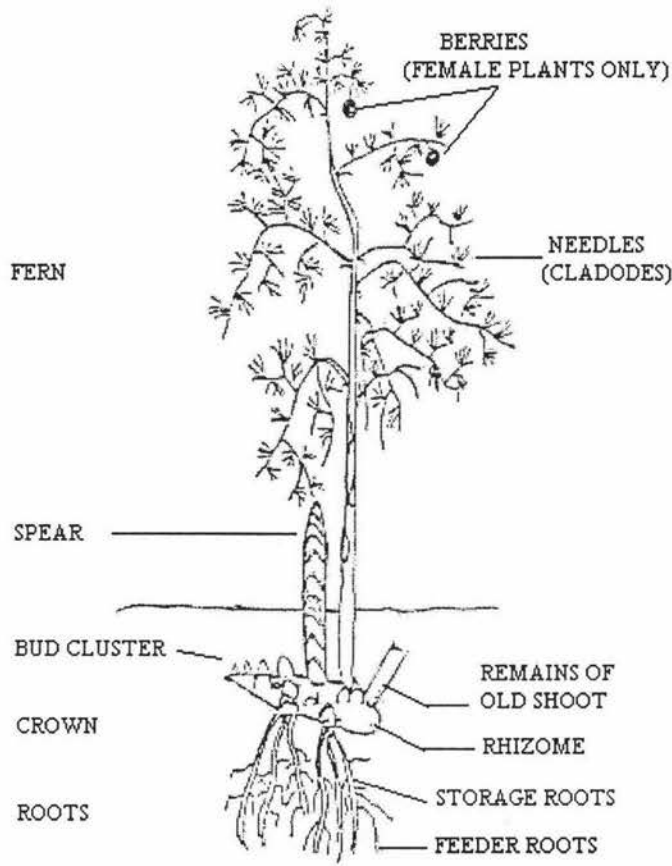


Figure 1.1. The structure of an asparagus plant (Nichols 1996).

1.2.1. Fern

Many shoots, each of which has developed from a separate bud on the rhizome, establish the fern of mature asparagus. Each shoot consists of a central stem which supports many fine, needle-like branches or cladophylls (Robb 1983). Asparagus does not have real true leaves (Feher 1992), instead, the leaves of asparagus have been reduced in size to form very small scales on the cladophylls. These thin, needle-like, green phyllocladia are situated in whorls and play a major role in photosynthesis. All stems, side-stems and leaf-like branches are smooth and hairless.

1.2.2. Crown and roots

The crown consists of rhizome, storage roots, and feeding roots. The storage roots are cylindrical, unbranched, and fleshy with diameter up to 6 mm. These roots develop from the lower surface of the rhizome, an underground stem, which is normally 10-20 cm below the soil surface. On mature plants, the storage roots can grow up to 2 m in length, depending on the soil type. Then, the fibrous feeding roots develop as a lateral roots on the storage roots. These fibrous roots are used to extract water and nutrients from the soil (Robb 1983). Asparagus seedlings grown in containers for 180 days had 19-31 storage roots, 6.5-40 cm long, with 17-191 feeder roots per storage root. These storage roots increase in thickness as the plant ages and can survive for several years (Feher 1992). The crown size increases with age and the storage roots play a major role in maintaining carbohydrate reserves (Douglas 1990).

1.2.3. Bud clusters

Bud clusters develop on the rhizome and have connections to thick storage roots. Each bud cluster comprises many buds which get younger and smaller as the bud is positioned further from the center of the crown. Within the cluster, bud growth starts from the oldest basal bud followed sometimes by the next basal bud; however, the next bud on the

cluster will not grow until apical dominance is lost due to shoot harvest or growth into fern. Thus within a bud cluster there were no more than two spears growing at the same time (Nichols and Woolley 1985). In the mature plants, lateral bud clusters on the rhizome develop a new axis of growth and the older parts of the crown decay so that the plants have several clusters of ferns which grow independently of each other (Robb 1983).

1.2.4. Spears

Spears are the shoots growing under (in white asparagus) or above ground (in green asparagus) and have no branches. The tip of spears are covered by scales which overlap each other. These spears are edible and the most important part of the asparagus plants as spears are the final marketable product. The scales open and branches are formed if the spears are not harvested (Feher 1992).

1.3. Spear yield and quality in asparagus

1.3.1. Introduction

In a temperate climate, the life cycle of asparagus is controlled by seasons. Asparagus is dormant during winter and in spring buds are released from eco-dormancy and start to grow to produce spears. Thus, the carbohydrate source in the storage roots plays an important role on spear growth and production. The life cycle of asparagus consists of four stages : the fern renewal stage, the carbohydrate accumulation stage, the dormancy stage, and the spear production stage (Nichols 1996).

New fern growth uses large quantities of carbohydrate from storage roots so that major loss of carbohydrate from the plant occurs during fern renewal stage. Carbohydrate accumulation stage starts once the fern has been renewed. Carbohydrate is accumulated rapidly during this stage. This carbohydrate accumulation is very important as the storage carbohydrate will be used to support spear and fern growth in the following season. In

the autumn photosynthesis stops, the fern dies back, and the plant becomes eco-dormant and possibly endo-dormant (Hughes 1992). Spear production stage occurs in the spring when the buds are released from dormancy and starts to grow by using storage carbohydrate from storage roots.

Thus, spear production depends mainly on two stages. Firstly, the ability of plants to accumulate carbohydrate in the storage roots through photosynthetic activity and secondly, the ability of plants to remobilize these carbohydrate reserves to produce spears in the harvest season (Nichols and Woolley 1985). These two stages determine both yield and quality of asparagus spears (see section 1.3.2). Consequently, any pre-harvest factor affecting asparagus growth will affect spear yield and quality. In addition, harvest factors such as harvest pressure and harvest time affect spear production and quality as these factors determine the use and depletion of storage carbohydrate during spear growth and production.

1.3.2. Yield and quality of asparagus spears

Asparagus harvest is usually done by cutting growing spears at certain length during spring until early summer. Important parameters of spear production are spear weight, spear length and spear diameter. However, not all total yield is suitable for sale. Thus, asparagus spears can be divided into marketable yield and unmarketable yield based on their quality.

Spear quality is determined from spear conditions including spear length, spear diameter, tip tightness, spear weight, spear fibrousness and spear health. However, the grading standard for quality assessment is not uniform among researchers. For example, Dean (1993) graded spears based on diameter sizes into small (6 – 9.5 mm), medium (10 – 12.5 mm), large (12.6 – 15.9 mm), and jumbo (> 16 mm), while Jayamangkala (1992) used three classes of spear diameter : class A (>10 mm), class B (8 – 10 mm), and class C (< 8 mm, unmarketable yield). McCormick and Thomsen (1990) recorded spear quality into three classes as follows : (1) Spears <9 mm butt diameter or otherwise bent or blemished,

(2) Clean, straight spears >9 mm butt diameter with moderately well closed heads, (3) Spears as for class 2 but with tightly closed heads showing no seediness. Class 1 is regarded as unmarketable yield, while classes 2 plus 3 are referred to as marketable yield and class 3 as export marketable yield. In addition, Verberne (1990) included insect damage as a parameter. Although other parameters are important, most classifications are based on spear diameter.

Thus, good quality for export refers to many parameters including freshness, spear size (length and diameter), health (freedom from diseases and pests), and tip tightness. To obtain the desired quality standard for export is not a simple process. The quality of the spears is affected by all the production processes, including pre-harvest and harvest factors.

1.3.3. Factors affecting yield and quality of asparagus spears

1.3.3.1. Pre-harvest factors

Pre-harvest factors relate to plant growth and carbohydrate accumulation that eventually affect spear growth and yield. These factors include water stress, planting depth, plant density, and temperature.

1.3.3.1.1. Water stress

Although asparagus is regarded as a relatively drought tolerant plant, many studies indicate that the application of irrigation increased plant growth and spear production. Wilcox (1985) suggested that the growth of asparagus is most limited by water stress when soil matric potential is reduced in the range between -0.05 MPa and -0.10 MPa, indicating that irrigation would be beneficial in asparagus plantings. In this direction, Sterrett et al. (1990) showed that vegetative growth was enhanced by supplemental irrigation. Stem diameter, number of shoots per plant and plant height were increased by

irrigation so that plant produced more vigorous fern. This vigorous fern has been associated with improved carbohydrate accumulation in storage roots. As a result spear yield was significantly increased. Irrigated plant produced about 130 thousands spears per hectare with mean spear weight around 23 gram per spear at year 4 compared to about 100 thousands spears per hectare with mean spear weight about 19 gram per spear for non-irrigated plants.

Similarly, Hartmann (1985) showed that irrigation during the growing season induced plant growth that resulted in an increase of the spear yield in the following harvest. The number of thick spears (16 – 22 mm) of plants grown in sandy soil with irrigation was 40.3 % compared to 30.3 % for non-irrigated plant. The comparable figures for plants grown in heavy soil (loamy sand) were 45.7 % and 51.1 %. According to Hartmann, asparagus growing in heavy soil showed higher spear yield and quality because of a higher proportion of available water.

Decreasing soil water potential reduced total root dry weight, the number of fleshy roots and bud number with the largest reduction being in the -0.50 MPa compared to -0.05 MPa (Drost and Wilcox-Lee 1990). This may relate to the reduction of photosynthetic activity as a result of the reduction in fern xylem water potential. Wilcox-Lee and Drost (1990) reported that decreasing soil moisture from -0.05 MPa to -0.30 MPa significantly reduced xylem water potential and photosynthetic activity of asparagus. Consequently, carbohydrate accumulation was reduced so that fleshy roots and root dry weight decreased. Besides, smaller bud number and bud size resulted in low spear yield and low quality as spear diameter decreased.

Drost (1999) also showed that reducing the amount of water to mature asparagus linearly reduced growth parameters including root number and root fresh weight, bud number and spear number. As the amount of irrigation reduced, there was a linear decrease in spear yield. Besides, the percentage of marketable spears decreased as irrigation rate decreased. However, these results do not necessarily show whether the limiting factor on spear yield is carbohydrate reserves or bud number.

In addition there was also a tendency for increasing soil water content to increase fiber content of spears but this trend was very weak (Keulder and Riedel 1990). They showed the relationship between rainfall and fiber content was very low and non significant ($r=0.22$) so that the effect of soil water potential on fibrousness of asparagus spears was negligible.

1.3.3.1.2. Planting depth

The depth at which asparagus crowns are placed in the soil has been shown to affect spear yield and quality. Takatori et al. (1974) reported that planting depth affected many aspects of spear production, including earliness of production, spear number per acre, spear weight and spear size. Shallow plantings resulted in spear production considerably earlier than deeper plantings. The 2 inches planting depth produced 10,000 marketable spears per acre (1 ha = 2.471 acres) one week earlier than the 6 inches depth and two weeks earlier than the 12 inches depth. Besides, the number of spears decreased with increasing planting depth. Planting depth of 2 inches (1 inch = 2.54 cm) resulted in 176,814 spears per acre while 6 and 12 inches planting depth produced 153,648 and 100,940 spears per acre respectively. However, spear size increased considerably with planting depth. When spears were separated by diameter into large, medium and small, plants with 2 inches planting depth only produced 3.75 % large spears and most spears (59.18 %) fell into the small category. The figures for 6 and 12 inches planting depth were 10.38 % and 16.02 % large spears respectively and 40.6 % and 34.30 % small spears respectively. Overall, The 6 inches plantings resulted in the highest total yield expressed in weight. This planting depth produced nearly as many spears as the 2 inches depth while the spear size was almost as large as in the 12 inches depth.

Lindgren (1990) found similar findings of planting depth effects on annual yield of asparagus. The results indicated that increasing planting depth from 5 cm to 10, 15 and 20 cm reduced the number of spears significantly from 72.3 spears/plot in 5 cm depth to 59.6 spears/plot in 10 cm depth and to 47.4 spears/plot in 20 cm depth. However, spear weight increased significantly with increasing planting depth. For example, 5 cm

planting depth produced spears with mean weight of 13.3 gram/spear while 20 cm planting depth resulted in larger spears (16.6 grams/spear).

Although deeper plantings initially produced fewer spears and less total yield the situation was gradually reversed with increasing crop age. Initially, crowns planted at 10 cm depth were significantly more productive than crowns planted at deeper or shallower depth. However, as the plants aged, plants with deeper depth produced larger spear size and became as productive as those planted at 10 cm while shallow planted become relatively lower yielding (McCormick and Thomsen 1990). They suggested that for improving spear quality, asparagus should be planted at 20 cm depth so that high proportion of marketable spears could be achieved over the life of the asparagus.

1.3.3.1.3. Plant density

When using direct seeding method, asparagus growers tend to sow at very high rates. However there is a clear optimum density for maximum yield (Takatori et al. 1975; Kaufmann and Orth 1990). At first harvest, two years after sowing, both total number of spears per acre and total weight of spears per acre increased with increasing plant density from 40,000 to 120,000 plants per acre. However, both parameters decreased when plant density was increased to 160,000 plants per acre. Besides, spear quality indicated by spear size decreased gradually with increasing plant density (Takakori et al. 1975).

Using crowns as plant material, McCormick and Thomsen (1990) reported similar findings, typically spear number and total yield increased while mean spear weight decreased as plant density increased. At plant density of 19,000 crowns/ha, spear production was 180,000 spears/ha with mean spear weight of 22.1 grams/spear. This achieved total yield of 3940 kg/ha. Increasing plant density to 33,000 and 44,000 crowns per hectares increased yield significantly to 4650 and 5030 kg/ha respectively, while spear weight decreased non-significantly to 21.3 and 20.3 grams/spear respectively. The authors suggested that as spear numbers increased more rapidly than their weight declined, increasing plant density would be of great benefit. The major benefit of the

extra 25,000 plants/ha was to increase total yield by 30 %. The above results indicates that plant density used in this study is still below the maximum plant density, thus further study with plant density more than 44,000 crowns/ha should be conducted to achieve the maximum plant population producing optimum spear yield.

Kaufmann and Orth (1990) suggested that maximum yield could be obtained with optimum plant density of 92,000 crowns per hectare. Plant density of more than 92,000 plants per hectare would decrease the marketable yield. This may be due to high competition between plants to get light, water and nutrient so that plant growth and production are limited.

1.3.3.1.4. Temperature

Effects of temperature on spear growth and production have been reported. For example, there was no spear growth at 5°C but spear grew normally when temperature was increased to 15°C. Increasing temperature from 10 to 30°C, with 5°C interval, increased spear growth. The time needed to growth from 1 to 10 cm was much longer than that required to grow from 10 to 20 cm so that spear growth appeared to be exponential at all temperatures (Nichols and Woolley 1985). This result was consistent with Krarup and Henzi (1992) who found that weekly yield of spears increased gradually with increasing temperature starting with a mean weekly temperature of 9°C to 16°C.

However, high temperatures have detrimental effects on spear quality in some cultivars. In cv. Boonlim, increasing temperature due to plastic cover reduced spear weight significantly from 16.2 to 14.9 grams/spear but seem to have no or little effect in cv. Jersey Giant (Poll 1990). In addition, Poll and Kruistum (1990) reported that increasing temperature from 19°C to 31°C, with 4°C interval, reduced spear quality in many asparagus cultivars including Geynlim, Limbras, and Boonlim. In this case, Nigh (1996) explained that high temperature prior to harvest induces both respiration and transpiration. High rate of respiration process reduces carbohydrate reserve in both storage roots and spears. As a result, spear weight decreases and tip breakdown occurs.

In addition, increased transpiration leads to a reduction of spear weight by the loss of water.

Poll (1996) suggested that splitting of white asparagus spears was affected by both air and soil temperature. When the soil temperature was 15°C, air temperature of 25°C resulted in 47.2 % split spears compared with 15.4 % for air temperature of 15°C. However, at a soil temperature of 25°C a significantly higher percentage of split spears (56.0 %) was obtained from low air temperature (15°C) compared to 36.9 % for high air temperature (25°C). Thus, equal air and soil temperature resulted in significantly less split spears compared with a large difference in temperature between air and soil and lower temperature produced less split spears than higher temperature.

1.3.3.2. Harvest factors

Spear yield and quality is affected by harvest factors through their effects on carbohydrate accumulation and remobilization for spear growth.

1.3.3.2.1. Harvest pressure

Spear yield of asparagus is mainly affected by the ability of plant to store carbohydrate in the roots during carbohydrate accumulation stage and remobilization of this carbohydrate reserve to support spear growth and production during the harvest period. These two stages affect each other as low carbohydrate accumulation results in poor spear yield while high harvest pressure leads to low carbohydrate accumulation. Thus, the levels of harvest pressure are always a question for asparagus growers. A long harvest time may lead to reduced future yield while a short harvest period may reduce current yield. Takakori et al. (1970) analyzed the effect of harvest duration from 30 to 120 days on spear yield. The results indicated that spear yield was optimum at 60 days of harvest. Sanders (1985) suggested that 60 and 70 days of harvest tended to produce higher yield than 50 days, however, 70 days of harvest produced more small spears so that 60 days of

harvest was optimum for spear yield and provided a greater carbohydrate reserve in roots for next season.

Nichols (1996) illustrated that that an overly long harvest period not only depletes carbohydrate reserves for fern renewal but also reduces the time available for the accumulation of carbohydrate in storage roots in preparation for the following harvest season. As a consequence, carbohydrate reserve within storage roots of over-harvested plant would be much lower than that of normal-harvested plant. In this condition, it is expected that over-harvested plant would produce lower spear yield than normal-harvested plant in the next harvest.

The above illustration agrees with the findings of Shelton and Lacy (1980). Asparagus harvested for 8 or 10 weeks resulted in significantly lower yield at the next harvest season than plant harvested for 4 and 6 weeks. The higher the harvest pressure the lower spear yield in the next harvest. The results indicated that percentage of marketable size spears (more than one cm in diameter 12 cm from the tip) was lowest for plant harvested for 10 weeks (55 %) compared to 65 % for plant harvested for 4 and 6 weeks in the previous year. In addition, they found that severe harvest pressure reduced storage carbohydrates (% of dry weight) significantly. Total storage root dry weight from plants harvested for 6 weeks was significantly less than that from plants harvested for 0 or 3 weeks.

1.3.3.2.2. Harvest height

The effects of harvest height would be similar to those of harvest pressure as height of spears at harvest relates to the depletion of carbohydrate reserves in the storage roots and buds available to produce spears. The higher the cutting height the more carbohydrate depleted from storage roots.

In a cultivar trial conducted in New Zealand spear yield varied markedly between cultivars on the same site. For example, 'Jersey Giant' produced total spear yield about 3

times than that of 'Brunetto' but produced about 7 times marketable yield than 'Brunetto' (Nichols 1992). Bussell et al. (1996) suggested that differences between cultivars varied with cutting height. Nichols and Fisher (1999) illustrated that the relationship between total spear yield and cutting height up to 30 cm was essentially linear; however, marketable spears decreased after a certain cutting height.

For processing purpose, asparagus spears are usually harvested at 18 cm cutting height, while for fresh export 23 cm spear length is preferable. However, spear quality indicated by tip tightness varied between 18 and 23 cm spear length (Nichols and Fisher 1999). They proposed that asparagus plants harvested at 18 cm do not provide information on how the plants might behave at higher cutting height as this involves the availability of both storage carbohydrates and bud numbers in the crown. Thus, the effects of cutting height on spear production should be considered in asparagus cultivar trials.

The effects of cutting height on spear yield and quality is discussed in chapter four of this thesis.

1.3.3.2.3. Harvest time

After released from winter dormancy, asparagus begins to emerge in spring when soil temperatures are warm enough. In temperate region such as New Zealand, asparagus is traditionally harvested by cutting all the spears that emerge in Spring. Asparagus growers usually stop harvesting in early December to allow the plant to produce sufficient carbohydrate to support the following year's crop. As asparagus cannot be stored for a long duration, very little fresh asparagus is available during December for the pre-Christmas market so that the price of fresh asparagus increases (Robb 1986). If, in the spring, asparagus spears are not harvested and allowed to grow, asparagus harvest can be done in autumn and summer by cutting down the fern and re-watering the soil so that spear emergence will occur. This practice of producing asparagus in summer has been attempted as the price of asparagus increases (Dufault 1991), but many results indicate

that this difference in harvest time leads to significant decreases in yield and quality of asparagus spears.

Robb (1986) showed that the yield and quality of the summer harvested asparagus was significantly worse than the spring harvested crops. Spear yield at summer harvest was 3.56 t/ha compared to 4.57 t/ha for spring harvest. Besides, mean spear weight and spear diameter and tip quality were significantly worse for summer harvest than spring harvest, and the percentage of marketable yield of summer harvest was only about 50 % of spring harvest. Within each harvest time, spear weight and spear diameter gradually decreased as the season progresses. The author suggested that the quality of summer harvested spears was comparable to that of spring harvest only during the first 1-2 weeks of the harvest, so that shifting the harvest time from spring to summer was not feasible.

The poor quality of summer harvested spears compared with the traditional spring product has been attributed to low carbohydrate levels in the storage root systems and this may be responsible for poor yield performance of summer-harvested plants. Thus, extended periods of fern growth after harvesting seasons are necessary for plant to recover and recharge the crowns with storage carbohydrates for the following seasons harvest (Dufault 1990). In addition, feathering of spear tips and unacceptable stand reduction are other problems encountered with forcing asparagus.

However, Dufault (1996) suggested that harvesting asparagus in summer has some potential commercial value compared with normal spring harvest (first spear emergence varied from January to March) in South Carolina, USA. Commercial summer asparagus production would be profitable provided harvest happens during periods of high market demand and there is adequate time for fern to replenish crown carbohydrates to ensure acceptable yields in the subsequent harvest season. Thus harvest timing in forced asparagus is very important. The author indicated that harvesting asparagus in July and August resulted in better spear production than in May and June or September and October. Harvesting in May or June reduced yield potential because fern growth in the months before fern cutting in either May or June forcing decreases crown carbohydrate

levels so that recovery ability and stands were significantly reduced. Harvesting in September and October produced low yield because low temperatures inhibited spear emergence so much that this harvest time was not commercially acceptable. Besides, decreasing soil temperature prevented fern regrowth for carbohydrate recovery. On the other hand, forcing in July and August produced yield significantly higher than the other forcing months because the plants have adequate time elapsed from first emergence in spring until fern cutting to recover from carbohydrate drain so that plants forced in July and August had higher yield potential.

In summary, spring harvest showed the greatest marketable spear production, followed by July and August forcing which produced 13% and 28% less than normal spring production respectively. Other forcing times were significantly lower (by %) than spring harvests as follows: June and September (40%), May (54%) and October (87%). Although summer yields were less than the normal spring harvest, the price of fresh asparagus during late summer is much higher than spring production so that summer production is more desirable and profitable.

1.4. Source-sink relationships and dry matter partitioning

1.4.1. Introduction

An asparagus plant can be thought as a collection of photosynthetic sources and growing sinks. All growing sinks require carbohydrate supply from the mature exporting source leaves and this relationship determines the crop yield. In considering plant growth and development in relation to crop yield, not only should the photosynthetic rate of individual leaves be considered, but also the overall rate of dry matter production by the whole plant, which is dependent on both photosynthetic rate, leaf area available for light interception and respiration. Thus, it is important to distinguish between the total capacity of the plant to produce assimilate (source strength) and the capacity of the growth centers and storage organs to attract assimilates (sink strength) (Wareing 1979).

The quantity of assimilate available for partitioning to sinks depends on both source and sink properties in a complex way. For instance, although leaf development plays an important role in determining source strength, the young growing leaves also act as sinks. These developing leaves constitute a major sink for assimilate until they commence to export assimilates themselves. Then for a developing leaf, photosynthetic assimilate is partitioned within the leaf itself for further leaf growth. Later carbohydrate may be temporarily stored in the leaves followed by export to competing sink organs. Regulation of the partitioning of dry matter between sinks at the whole plant level, particularly the integration of source processes and sink requirements, is still poorly understood (Gifford and Evans 1981; Wardlaw, 1990). This inadequacy of understanding of the control of dry matter partitioning within plants is indicated by the development of empirical descriptions based on descriptive allometry, priority concepts for sinks, functional equilibrium between organs or resistance and teleonomic models (Gifford and Evans 1981). Most of the models include the role of source, assimilate transport, and sink properties, but the most appropriate viewpoint will be dependent on purpose of the model and the interest. In the mean time, there is much evidence showing the effects of the environmental factors such as daylength on dry matter partitioning (see section 1.4.7).

1.4.2. Source strength

A source can be defined as an organ that is net exporter of carbon assimilates while source strength refers to the total capacity of the plant to produce assimilates (Wilson 1972 ; Wareing and Patrick 1975; Wareing 1979). *Source strength* can be defined as 'source size x source activity'. This can be expressed as

$$\begin{array}{ccc} \text{rate of assimilation} & = & \text{leaf area} \times \text{net assimilation rate} \\ \text{per plant} & & \text{per plant} \quad \text{per unit leaf area.} \\ (\text{g.week}^{-1}) & & (\text{m}^2) \quad (\text{g.m}^{-2}.\text{week}^{-1}) \end{array}$$

Thus, source strength is determined by light interception by leaves and the efficiency of energy conversion to dry matter which is dependent on the efficiency of photosynthesis and whole plant respiration. The ability of the leaves to produce dry weight gain per unit

Ho et al. (1989) suggested that the net accumulation rate of dry matter is a measure of 'net sink strength'. This does not account for respiratory losses which may be substantial as up to 50 % of the gross carbon produced by photosynthesis are by respiration over the course of growing season (Amthor 1984). Ho (1976) found that due to respiratory losses of assimilates in the sink organ, the absolute growth rate of dry matter in a sink organ underestimates the total amounts of assimilates received by a sink organ. Thus, Ho et al. (1989) suggested that the net gain of dry matter plus respiratory loss of dry matter is a measure of 'gross sink strength'.

A plant may be source limited, when the actual rate of assimilate production is less than the potential maximum rate of consumption, or sink limited, when the potential rate of production is greater than the actual rate of consumption (Wareing 1979). Identification of plant conditions whether organ growth is either source limited (limited by assimilate supply) or sink limited (saturated by assimilate supply) is very important, because this might have implications for the regulation of organ growth and serial yield (Patrick 1988).

In this regard, Wareing and Patrick (1975) and Wolswinkel (1985) proposed that sink strength refers to the potential capacity of a sink to accumulate assimilates. This potential capacity reflects the essential ability of the sink to receive or attract assimilates, which is a critical determinant of organ growth. Therefore, more precisely, sink strength can be quantified as the potential capacity to import assimilates into the phloem of the sink region and to transport the imported substances from the phloem into the cells of the sink organ (Wolswinkel 1985). The potential sink strength can be measured by calculating the potential growth rate of a sink under conditions of non-limiting assimilate supply. For instance, conditions for potential growth can be created by growing plants at a high irradiance and/or reducing the number of sinks on a plant. Marcelis (1996) suggested that the value of potential growth rate is not a static parameter, but may change with, for example, developmental stage or temperature, but not with factors such as light intensity or CO₂ concentration which are assumed to affect only the availability of assimilates.

This may apply to asparagus plant. Potential growth of both shoots and roots may change with development stages of the plant and environmental changes such as daylength, that in turn, would affect dry matter partitioning between root and shoot.

1.4.4. Dry matter partitioning

The term dry matter partitioning is often used to explain the distribution of dry matter between organs of the plants in the concept of source-sink relationships. Although there is much information available on the operation of individual processes in plants such as photosynthesis, sugar metabolism, translocation, and cell expansion, the controls which actually regulate the partitioning of dry matter at the whole plant level are still only poorly understood (Wardlaw, 1990).

In terms of root and shoot activity, the dry matter partitioning between root and shoot has been described as a functional equilibrium between root activity (water and nutrient uptake) and shoot activity (photosynthesis); thus, in a constant environment, the ratio of shoot-to-root dry matter would be proportional to the ratio of shoot-to-root activity. Although in this way the ratio between shoot and root dry weight can often be estimated fairly well in vegetative plants, the mechanism underlying this equilibrium is quite complicated and not well understood (Farrar, 1992). Thus, the process of dry matter partitioning is not simple, but involves all aspects of assimilate production within source organs (mainly leaves), assimilate movement via phloem transport, and assimilate influx into sink organs such as storage roots.

1.4.5. Effect of source on dry matter partitioning

The dominant primary sources of assimilate are leaves through photosynthetic activity, although green stems and floral organs can sometimes make substantial contributions. It is well known that environmental factors, particularly light, have strong direct effect on photosynthesis and hence on source strength. For example, a high source strength due to high irradiance strongly enhances the total dry matter production and plant growth;

however, information on the effect of source strength on the dry matter partitioning between the plant organs is inadequate. In some crops (e.g. tomato and soybean), there was no significant effect of source strength on the dry matter partitioning between reproductive organs such as fruits and vegetative parts such as leaves and roots (Egli, 1988; Cockshull et al. 1992). Daie (1985) also suggested that the source is not likely to control dry matter partitioning. In addition, Marcelis (1992) found no significant relationship between solar radiation and dry matter partitioning into the fruits during a growing season of cucumber, but he noticed that a large change in solar radiation seemed to induce a change in dry matter partitioning to the fruits, but the time lag between the change in radiation and the change in partitioning was variable. However, in this thesis the focus is on dry matter partitioning between roots and shoots.

It is therefore of interest that Minchin et al. (1994) found that the distribution of available assimilate between roots and shoots is affected by both source supply and sink function. They presented data showing that photosynthate partitioning between the root and the shoot of a barley seedling is affected by the supply of photosynthate from the source leaf. When the supply of photosynthate is reduced (by reducing the PAR from 400 to 100 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$ which reduced the photosynthetic rate of the labeled leaf by 80 %), an increased fraction of the exported photosynthate went to the shoot while photosynthate transferred to the roots was reduced. Thus, reduced photosynthesis resulted in an increase in growth of the shoots relative to the roots in order to compensate for the reduction of assimilates. Conversely, when the roots were cooled a short time before reducing the supply of photosynthate, the effect of a reduced supply upon partitioning was reversed with an increased fraction then going to the root. It is important to note that these effects are dealing with short-term responses, responses solely due to the mechanisms functioning at the time of treatments. Over a period of time, acclimation can be expected to occur leading to changes in the relative importance and sink to source signaling may have a specific role. These results suggested that root:shoot ratio was affected by environmental factors over short periods of time.

Measurement of photosynthate flow into a variety of sink types has been described by a sink hierarchy based upon the ability of the sinks in attracting photosynthate (Wardlaw 1990). The accepted hierarchy in order of decreasing dominance is: fruit > vegetative shoot > root. Thus, the effects of source on dry matter partitioning between organs would be determined by sink properties. For instance, some organs such as fruits, seeds or underground storage organs have priority and suffer less from a reduction in assimilate supply than other organs.

Organ initiation often decreases and organ abortion increases with decreasing source strength (Wardlaw, 1990). As a consequence in the long term the number of sink organs may change considerably and hence the dry matter partitioning. Marcelis (1993) suggested that the effect of source strength on dry matter partitioning was an indirect effect via an increase in number of sink organs on the plant than a direct effect on dry matter partitioning. Thus, in the long term dry matter partitioning would change due to a change in number of sinks and thus in amount of machinery. This can be interpreted by suggesting that source strength exerts coarse control but not fine control on dry matter partitioning (Farrar 1992).

1.4.6. Effect of sink on dry matter partitioning

To understand the regulation of dry matter partitioning by the sinks, there has been substantial interest in sink characteristics. As stated before, sink organs for assimilate are net importers of assimilate. Essentially, all plant organs at some stages of plant development would act as sinks, receivers of assimilate. In terms of assimilate transport, the ability of a sink organ to import assimilate is the sink strength. However, the proportion of imported assimilate used for respiration by sink organs can be substantial. Thus, sink strength of a sink organ, measured as an absolute growth rate or net accumulation rate of dry matter, fails to assess the true ability of a sink organ to receive assimilate and is a measure of 'net sink strength'. Therefore, the import rate of assimilate, measured as the sum of the net carbon gain and respiratory carbon loss by a sink organ, should give a more appropriate estimate of the actual sink strength.

Although actual sink strength would be affected by the availability of assimilate supply (source properties) and the assimilate transport from the source to the sink through phloem, the most critical determinant is the intrinsic ability of the sink to receive or attract assimilate (sink properties) (Cook and Evans 1983). This intrinsic ability of a sink is the potential sink strength. Marcelis (1996) suggested that dry matter partitioning is dependent on the sink strengths as differences between sink organs depend on the differences in sink strengths.

Thus, when there are more than one sink organs, the availability of partitioning assimilates to a particular sink may be determined by the competition between sink organs. New growing leaves act as a sink until they are 30 to 50 % expanded so that net export of photoassimilates commences (Wareing and Patrick 1975). As a source usually supplies the nearest sink, photoassimilates are initially exported to younger leaves and the shoot apex. The position of a leaf relative to the shoot apex changes during plant growth and an increasing amount of assimilates are exported to the plant roots. The development of reproductive or storage organs which usually have a high sink strength can change this sequence. Ho (1988) notes that when dry-matter production in flowering tomato plants was reduced, the limited amount of mobile assimilate was mainly imported by the developing young leaves to sustain growth, and the initiating inflorescence was aborted. The higher priority of importing assimilate by developing leaves over inflorescence remained, even when extra assimilate was made available. Import to the inflorescence was only improved after the demand by the developing leaves had been met. Although sink competition may be amplified when assimilate supply is limited, the priority of partitioning is consistent at each stage of plant development. In tomato, a developing inflorescence is a weaker sink for assimilate than the expanding leaves, but a truss with growing fruit is a stronger sink than young leaves and roots.

Effects of sinks on dry matter partitioning have been clearly demonstrated by experiments on several crops. Heuvelink and Buiskool (1995) showed that an increase in number of generative sinks increased the generative:vegetative ratio, but decreased the

partitioning into the individual generative sinks. the sink strength (competitive ability to attract assimilates) is not only correlated with the number of sinks, but also with the weight of the individual sink organs.

Although generative sinks may not relevance to asparagus plant, this could apply to vegetative sinks in asparagus. For example, during carbohydrate accumulation stage, asparagus roots act as strong sinks; however, there is no information about partitioning carbohydrate to different individual root such as new growing roots, new developed roots, old full roots, and old empty roots. In this case, new growing roots may act as a strong sink as in generative sinks.

Therefore, sink properties indicated by sink strength play an important role in dry matter partitioning in plants. The partitioning of assimilates among sinks has often been suggested to be regulated primarily by the sinks themselves.

1.4.7. Effect of daylength on dry matter partitioning

Many experiments showed that exposure of plants to longer days, without increasing the supply of photosynthetically active radiation (PAR), resulted in substantial increases in dry-matter production up to 200 %, and even greater percentage increases in leaf area (Eagles 1971; Heide at al. 1985a; Heide at al. 1985b).

Eagles (1971) found that for two cocksfoot populations adapted to widely different environments, daylength extension induced an increase of plant leafiness (LAR being leaf area per unit of plant dry weight) compared with plants held in short days, but this was associated with reductions in NAR (the net assimilation per unit of leaf area). In addition, shoot : root ratio has been found to be significantly affected under daylength extension. The results showed that plants under daylength extension had higher shoot-root ratio, indicating that significantly more dry matter was accumulated in the leaves with long day treatment. There were also different responses between the Portuguese and the Norwegian populations. In both short day and long day treatments, the Portuguese

population showed higher shoot-root ratio at 5°C than the Norwegian, whilst the reverse situation was found at higher temperature. These results together with the above fact that relative growth rate (RGR) of the Norwegian plants was higher at low temperatures than the Portuguese plants suggested the specific adaptation to the combination of long day and cool temperatures at high latitude and emphasized the importance of dry matter distribution in determining differences in the growth of these two natural populations from contrasting climatic regions (Eagles 1971).

Heide et al. (1985a and b) also found that both extended daylength and increasing temperature had a strong effect on height growth of *Poa pratensis*, *Pleum pratense* L and *Bromus inermis*. The rapid height growth in long days (LD) was mainly due to increases of leaf blade and sheath length. They suggested that the increase in both plant height and leaf area were the result of increased cell size and number, that in turn, increased the dimensions of the leaf sheaths and blades.

In asparagus plants, some existing evidence suggested changes in dry matter partitioning induced by daylength. Sudjatkiko et al. (1997) found an abrupt change in allometric relationship between fern and crown of asparagus seedlings harvested in February. The relative growth rate of the shoot fell relative to that of the crown indicating that more dry matter was partitioned to the crown. As temperature change at that time was relatively small (17.6°C in January and 16.8°C in February), they suggested that this change was due to a change in day length . In addition, using radioactive label, Woolley at al. (1999) showed that carbon (¹⁴C) partitioning changed abruptly between mid-summer, when 70 % of ¹⁴C partitioned to the shoot, and late summer when 74 % partitioned to the crown.

1.5. Fructan metabolism

1.5.1. Introduction

Starch is the most common form of storage carbohydrate in higher plants, however, fructans occur as a complementary form in a considerable number of plant species such

as cheatgrass, ryegrass, wheat, barley, oat, and asparagus. Hendry (1993) reported that approximately 15 % of the angiosperm plants store fructans as the principal reserve carbohydrate.

The role of fructans in these plants has been considered to be mainly as storage carbohydrates. In some plants, this reserve carbohydrate is used for both long-term storage to support plant growth in the following season and short-term storage to maintain the supply of carbohydrate in growing organs, especially when carbohydrate production is limited.

Because of the importance of fructans both as a reserve carbohydrate in vegetative organs of numerous plants and in plant adaptation, fructan metabolism has received increasing attention over the past years. Most results suggested that fructan biosynthesis is catalyzed by enzymes such as sucrose:sucrose fructosyl transferase (SST) and fructan:fructan fructosyl transferase (FFT), while fructan hydrolysis prior to mobilization is catalyzed by fructan exohydrolase (FEH) (Henson and Livingston 1996; Bonnet and Simpson 1995). These enzymes enable plants to accumulate fructans when photosynthate is produced in amount exceeding the requirements for respiration and growth and enable plants to hydrolyze and mobilize stored fructans when they are needed for growth.

As fructans may have a major role in plant growth and production, particularly for fructan-storing plants, any information regarding fructan behavior would be useful for manipulating fructan accumulation so that plant production could be improved.

1.5.2. Occurrence of fuctans in plants

Fructans are non structural carbohydrate polymers which consist primarily of fructose and one glucose group that accumulate in the vegetative tissues of many temperate plant species such as wheat (*Triticum aestivum* L.), oat (*Avena spp.*), barley (*Hordenum vulgare* L.), cheatgrass (*Bromus tectorum* L.), and asparagus (*Asparagus officinalis* L.). Fructans usually occur in large amounts in vegetative storage tissues such as tubers and

rhizomes but in lesser amounts in leaf and stem tissues. However, large amounts of fructans are produced in photosynthetic tissues including leaves of various plants when environmental conditions, such as cool temperature, lead to a situation where carbohydrate production through photosynthesis exceeds the rates of carbohydrate translocation and utilization. For example, in low ambient temperatures during early spring and autumn fructans may comprise 20 to 30 % of the dry weight of the leaves of cereal and forage crops (Chatterton et al. 1993).

In temperate grasses, most carbohydrates are stored in the form of fructans that are utilized for both short-term and long-term storage. For short term period, fructan accumulation shows a diurnal pattern, fructans are accumulated in fully expanded leaves toward the end of each light period and are mobilized during the dark period (Simpson and Bonnet 1993). In addition, pasture grasses also accumulate fructans in the base of pseudostem during vegetative growth and this reserve of carbohydrate is mobilized to support the early stage of regrowth after defoliation (Yamamoto and Mino 1987).

In cereal crops, fructans are accumulated from prior to anthesis until up to four weeks after anthesis and are mobilized for use as a source of assimilate for developing grains (Bell and Incoll 1990). Bancal and Triboi (1993) showed the important role of fructans during grain filling in wheat (*Triticum aestivum* L.) grown under different temperature. Day and night temperature were 18 and 10°C respectively in crop 1, whereas they were shifted to 28 and 20°C 5 days after anthesis in crop 2. The results indicated that fructans were accumulated up to 150 mg culm⁻¹ 2 weeks after anthesis in crop 1, but ceased to accumulate in crop 2 as soon as high temperature was applied. In this case, wheat grown under mild temperature exhibited a photosynthetic capacity exceeding requirements for plant respiration and grain filling. Dry matter was then stored in vegetative parts of the plant, partly as fructans in the culm.

Fructans also occur as the major carbohydrate reserve in storage roots of asparagus (*Asparagus officinalis* L.). Fructans are accumulated during carbohydrate accumulation stage starting once the fern has expanded so that fructan levels increases rapidly during this stage. This carbohydrate storage is very important as the fructans will be used to

support spear and fern growth in the following season (Nichols 1996). Shelton and Lacy (1990) also showed seasonal changes in the fructan levels of asparagus roots. Fructan level in the roots increases during maturation and senescence and the level of fructans decreases at the end of dormancy that is followed by spear growth. Nichols (1996) suggested that in winter approximately 60 % of root dry weight comprise fructans that can be mobilized to produce spears, fern and new roots after asparagus is released from winter dormancy.

1.5.3. Chemical structures of fructans

Chemical structures of fructans have been related to sucrose from which they are derived. These fructan polysaccharides are formed through a glycosidic linkage of fructose to one of the three primary hydroxyl groups of sucrose. Thus, there are three basic structure of fructans with three degree of polymerization : 1-kestose (also called isokestose, 6-kestose (also called kestose) and neokestose. Fructose linked to carbon 1 of the fructose moiety of sucrose results in 1-kestose while 6-kestose is formed when fructose is linked to carbon 6 of the fructose moiety of sucrose. Both 1-kestose and 6-kestose have a terminal glucose and a terminal fructose. Similarly, neokestose is formed by adding fructose to carbon 6 of the glucose moiety. This trisaccharide has both end groups being fructose (Nelson and Spollen 1987). *Asparagus officinalis* stores fructans of both the 1-kestose and neokestose series (Smith 1993).

Shiomi (1993) reported that fructan polysaccharides isolated from asparagus roots consist of polymers possessing approximately 11 to 21 fructose residues linked by β -(2 \rightarrow 1) bonds and a non-terminal glucose residue linked with fructose residues at C₁ and C₆ positions (Figure 1.2).

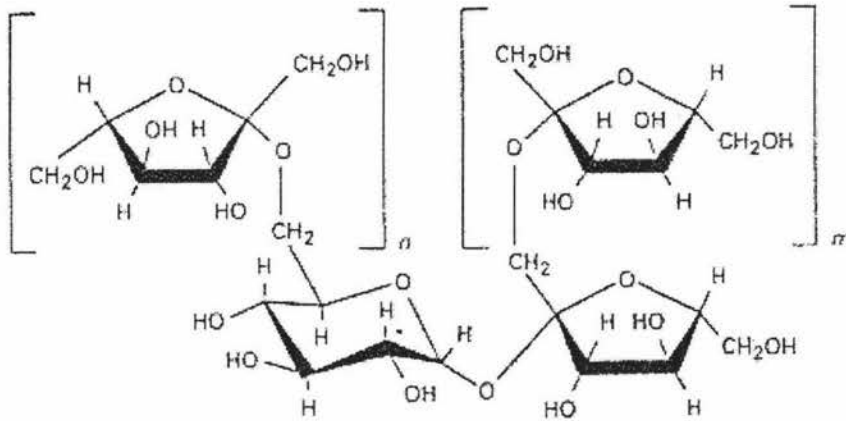


Figure 1.2. Chemical structure of frutan from asparagus roots (Shiomi 1993).

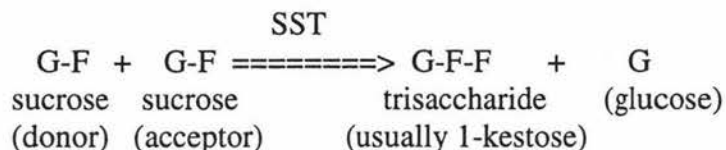
In addition, varying amounts of short chain fructans (degree of polymerization of 2 to 10) are also present (Martin and Hartmann 1990; Dean 1996; Woolley et al. 1999).

1.5.4. Fructan biosynthesis

In higher plants including asparagus plant, fructan biosynthesis is catalyzed by enzyme sucrose:sucrose fructosyl transferase (SST) and fructan:fructan fructosyl transferase (FFT) (Shiomi 1992; Cairns 1993; Smith 1993), while in bacteria it is catalyzed by sucrose enzymes. Cairns (1993) summarized the role of these enzymes in fructan synthesis as follows:

Sucrose:sucrose fructosyl transferase (SST)

SST catalyzes the initial step in fructan synthesis in plants by forming a trisaccharide intermediate, 1-kestose (isokestose), from two sucrose molecules in the following fructosyl transfer reaction:

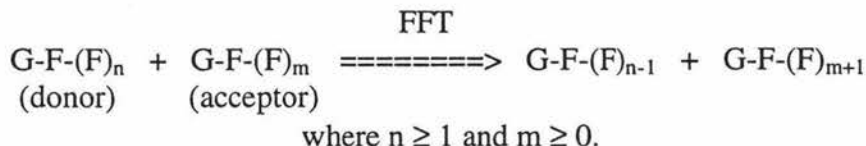


In this process, fructan synthesis may be measured by directly monitoring the formation of trisaccharide or indirectly by calculating glucose release.

The K_m for SST isolated from asparagus roots is 0.11 M indicating that a substantial amount of sucrose has to occur before the enzyme will begin to start reactions in the region of its maximum velocity (Smith 1993).

Fructan:fructan fructosyl transferase (FFT)

This enzyme utilizes 1-kestose produced by SST as a substrate in the synthesis of fructans with higher degree of polymerization (DP) by transferring fructosyl between fructan molecules in the following reaction:

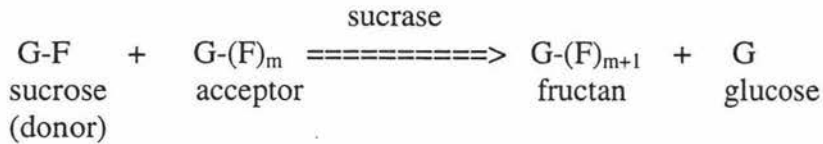


The activity of FFT is generally measured by the chromatographic determination of larger fructan formed from a preformed fructan substrate, usually 1-kestose.

Two fructosyl transferase activities have been identified in asparagus: 1-F-fructosyl transferase and 6-G-fructosyl transferase. 1-F-fructosyl transferase produces β -2,1 chains of fructose using both neokestose and neokestose and neokestose-oligosaccharides as acceptors to produce fructan polymers of the neokestose series. The second enzyme (6-G-fructosyl transferase) catalyses transfer of a terminal β -2,1-linked fructose residue from a donor to the 6 OH group of the glucosyl residue of the acceptor. Isokestose is used as donor to produce neokestose when sucrose is the acceptor (Smith 1993).

Sucrase

In bacteria, fructan synthesis is performed through the direct transfer of fructose from donor-sucrose to sucrose and fructans acceptor, in the following fructosyl transferase reaction :



where $m \geq 1$.

Fructan synthesis by sucrase can be determined directly by quantifying of total fructan synthesis or indirectly by measuring glucose release.

From above reactions, it can be seen important qualitative differences between SST and sucrase reactions and FFT reaction. Both SST and sucrase produce fructans from sucrose called de novo net synthesis of fructan. On the other hand, FFT requires preformed fructan substrates and catalyzes the redistribution of terminal fructose between fructan chains so that there is no net synthesis of fructans. In this case, FFT reaction may be categorized as non-synthetic fructosyl transfer, as distinct from synthetic reactions of SST and sucrase (Cairns 1993).

However, there are two special cases of FFT reactions. First is trisaccharide synthesis, when sucrose and high DP fructans and sucrose are used simultaneously as substrates. In this reaction, transfer of fructose to sucrose produces a net synthesis of trisaccharide due to the incorporation of sucrose into trisaccharide and shortening of fructan chains. Second is degradative fructosyl transfer if trisaccharide is the sole substrate. In this reaction, tetrasaccharide is formed accompanied by the release of sucrose so that the total mass of fructan is reduced.

Sims et al. (1993) analyzed fructan biosynthesis in excised leaves of *Lolium tumelentum* L. by using $^{13}\text{CO}_2$ generated from $\text{Na}_2^{13}\text{CO}_3$ and 2 M HCl. The leaves were fed $^{13}\text{CO}_2$ for 6 hours and harvested at 0, 4, 8, 16, and 24 hours following the end of feeding. The

results indicated that sucrose was the only carbohydrate detected at the end of the feeding period, 6 hours after leaf excision, while trisaccharide materials were detectable 10 hours after leaf excision. Most of trisaccharide materials were in the form of 1-kestose, while 6-kestose was only present in trace amount. Fructans with DP 4 such as nystose were present at 14 hours after excision, while oligosaccharides of progressively higher DP and high molecular weight material appeared between 10 hours and 26 hours after excision. These results suggested that the amount of labeled material in fructo-oligosaccharides with DP 3, 4 and 5, and in fructan above DP 5 showed that there was a progressive movement of ^{13}C from sucrose to oligosaccharides and then into high molecular weight fructan. This suggested that each oligosaccharide is the immediate precursor of the next member of the series and that oligosaccharide material is the precursor of high-DP fructans. Besides, the concentration of the tri-, tetra-, and pentasaccharides fell during the accumulation of higher- DP fructan. This suggested that high-DP fructan was accumulated at the expense of oligosaccharides as high molecular weight material is more effective acceptor of fructosyl residues than material of a lower weight. The concentration of monosaccharides and sucrose were stable during the time-course. The authors suggested that, in addition to trisaccharide synthesis from sucrose by the action of sucrose:sucrose fructosyl transferase (SST), sucrose can also act as an effective acceptor of fructosyl residues by the action of fructan:fructan fructosyl transferase (FFT).

1.5.5. Fructan hydrolysis

Many studies in fructan biosynthesis show that fructans are generally synthesized in plants when photosynthesis exceeds consumption of carbohydrate for growth. In many cases, fructans are accumulated in leaves of plants during exposure of plants to low temperature and continuous illumination (Guerrand et al. 1996), in stems just prior to anthesis and after anthesis during the early part of grain filling (Bancal and Triboi 1993), in leaves on plants in which sink activity has been reduced (Simpson et al. 1991), or in storage roots of asparagus during carbohydrate accumulation stage (Nichols 1996).

Conversely, fructans are hydrolyzed and mobilized from their reserves when carbohydrate consumption in sink tissues exceeds supply of photosynthate from current photosynthesis. Thus, fructan mobilization occurs as plants grow in warmer condition following a cool period (Pollock 1982), during the latter stage of seed-filling in cereal crops as photosynthetic activity of leaves decreases (Borrell et al. 1989), or during bud growth in spring of perennial grasses (Pollock and Jones 1979) and asparagus (Nichols 1996). Fructan hydrolysis also occurs earlier when leaves of cereal crops are shaded after anthesis, as leaf photosynthetic capacity drops considerably (Kuhbauch and Thome 1989).

Many studies show that fructan hydrolysis in higher plants prior to mobilization is catalyzed by fructan exohydrolase (FEH). The enzyme is a β -fructofuranosidase that catalyses sequential removal of terminal fructose residues from fructans (Smith 1993). Henson and Livingston (1996) suggested that catalysis by this enzyme was exolytic and by multiple chain attack. This hydrolytic enzyme has been isolated from oats (Henson and Livingston 1996), *Lolium rigidum* (Bonnett and Simpson 1995), wheat (Yukawa et al. 1995), *Dactylis glomerata* (Yamamoto and Mino 1987), and *Lolium temulentum* (Simpson et al. 1991).

Generally, the magnitude of FEH activity is inversely related to the magnitude of fructan concentration in plant tissues (Simpson and Bonnett 1993). Yakawa et al. (1995) showed that wheat growing under cold condition increased fructan accumulation as sucrose:sucrose fructosyl transferase (SST) increased but fructan exohydrolase (FEH) decreased. When FEH activity increased, fructan accumulation in plant decreased. Similarly, Ende and Laere (1996) found that during field-growth of chicory (*Cichorium intybus* L), the activity of SST decreased continuously to essentially disappear in October. In contrast, FEH activity increased continuously and increased rapidly after mid-October as fructans were mobilized intensively for growth. Besides, FEH activity increased substantially in forage grasses when fructans were mobilized after defoliation (Yamamoto and Mino 1987).