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

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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 $^{14}CO_2$ 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 frutans 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 GA_3), 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.

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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, $1^{F}(1\text{-}\beta\text{-}_{D}\text{-fructofuranosyl})_{m}\text{-}6^{G}(1\text{-}\beta\text{-}_{D}\text{-fructofuranosyl})_{n}$ 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. Sudjatmiko 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 at 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 extend 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.

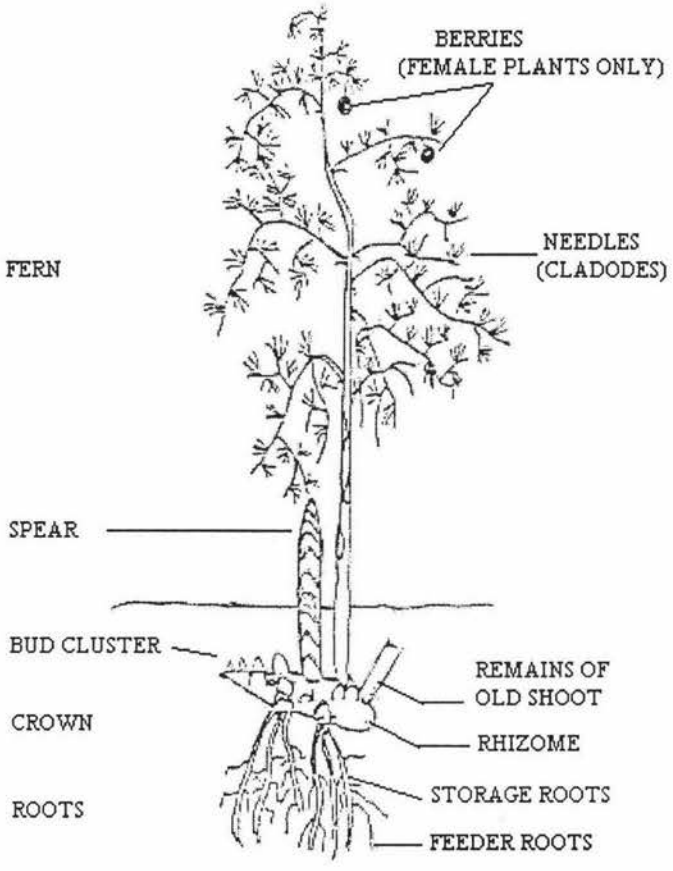


Figure 1.1. The structure of an asparagus plant (Nichols 1996).

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 – 95 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 telenomic 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). S*ource strength* can be defined as '*source size x source activity*'. This can be expressed as

rate of assimilation	=	*leaf area*	x	*net assimilation rate*
per plant		*per plant*		*per unit leaf area.*
$(g.week^{-1})$		(m^2)		$(g.m^{-2}.week^{-1})$

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

leaf area per unit time is measured as net assimilation rate (NAR). It can be calculated from gross photosynthesis and respiration as follows :

Net assimilation rate (NAR) = gross photosynthesis – (dark respiration + photorespiration of the leaves + respiration of the rest of the plant)

From the value of NAR and leaf area per plant, source strength can be calculated.

In this case, expanded leaves play a major role in source strength. Leaf photosynthesis is generally the main source of carbon for growth and storage in other parts of the plants (Wardlaw 1990). Considering factors affecting photosynthesis directly, it is clear that the photosynthetic rate may be modified by various aspects of development, including changes occurring during the development and subsequent ageing of the individual leaf. Thus, leaf development plays an important role in determining source strength as the rate of leaf development will affect the efficiency of light interception that affects dry matter production. On the other hand, young leaves also act as sinks because developing leaves constitute a major sink for assimilates until they commence to export assimilates themselves (Wareing 1979). In asparagus, the new fern growth acts as strong sink and uses large quantities of carbohydrate from storage roots. The fern becomes a source after it opens out and starts to produce carbohydrate through photosynthetic activity (Nichols 1996).

1.4.3. Sink strength

By analogy to source strength,

sink strength = sink size x sink activity

where sink activity may be defined as the rate of uptake or incorporation of assimilate per unit weight of sink tissue (Wilson 1972 ; Wareing and Patrick 1975; Wareing 1979). This can be quantified as follows :

'absolute growth rate of particular sink = *dry weight* (g) x *relative growth rate (RGR)*'

($g.week^{-1}$) ($g.g^{-1}.week^{-1}$)

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 CO_2 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 μmol photons $m^{-2}s^{-1}$ 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. Sudjatmiko 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 (^{14}C) partitioning changed abruptly between mid-summer, when 70 % of ^{14}C 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 gown 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 polysaccarides 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→1) bonds and a non-terminal glucose residue linked with fructose residues at C_1 and C_6 positions (Figure 1.2).

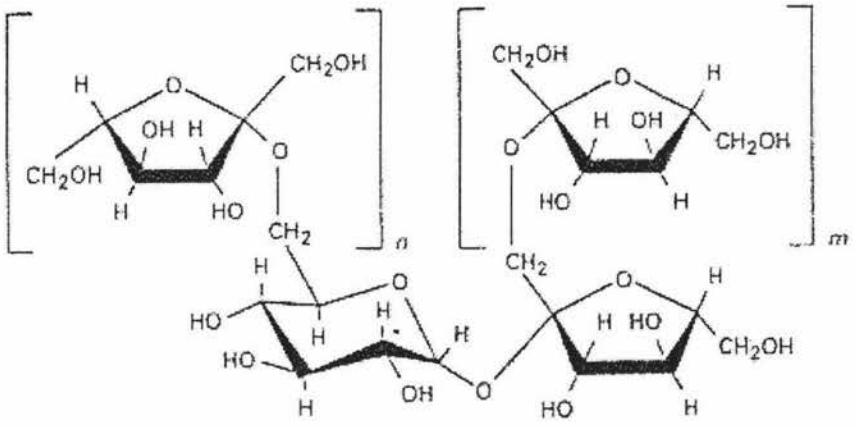


Figure 1.2. Chemical structure of frutan from asparagus roots (Shiomi 1993).

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 at 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 sucrase 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:

$$\underset{\substack{\text{sucrose}\\(\text{donor})}}{\text{G-F}} + \underset{\substack{\text{sucrose}\\(\text{acceptor})}}{\text{G-F}} \xrightarrow{\text{SST}} \underset{\substack{\text{trisaccharide}\\(\text{usually 1-kestose})}}{\text{G-F-F}} + \underset{(\text{glucose})}{\text{G}}$$

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:

$$\underset{(\text{donor})}{\text{G-F-(F)}_n} + \underset{(\text{acceptor})}{\text{G-F-(F)}_m} \xrightarrow{\text{FFT}} \text{G-F-(F)}_{n-1} + \text{G-F-(F)}_{m+1}$$

where $n \geq 1$ and $m \geq 0$.

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 trasferase. 1-F-fructosyl trasferase 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 tranferase reaction :

$$\underset{\substack{\text{sucrose}\\(\text{donor})}}{\text{G-F}} + \underset{\text{acceptor}}{\text{G-(F)}_m} \xrightarrow{\text{sucrase}} \underset{\text{fructan}}{\text{G-(F)}_{m+1}} + \underset{\text{glucose}}{\text{G}}$$

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 $^{13}CO_2$ generated from $Na_2{}^{13}CO_3$ and 2 M HCl. The leaves were fed $^{13}CO_2$ 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 was 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 oligosaccarides as high molecular weight material is more effective acceptor of fructosyl residues than material of a lower weight. The concentration of monosaccarides and sucrose were stable during the time-course. The authors suggested that, in addition to trisaccaride 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 exolitic 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).

CHAPTER TWO

EFFECTS OF DAYLENGTH ON DRY MATTER PARTITIONING OF ASPARAGUS (*Asparagus officinalis L.*)

2.1. Introduction

Carbohydrate balance which involves source sink relationship between fern and crown is a major issue in asparagus production. The new fern growth acts as a strong sink using large quantities of carbohydrate from storage roots. Once the fern opens out and starts to produce carbohydrate through photosynthetic activity, it acts as source that transported carbohydrate to crown. The ability of plants to partition carbohydrate to storage roots tends to determine yield in asparagus plants as spear production depends on this carbohydrate reserves in storage roots. The extend to which bud number on the crown may limit yield compared with carbohydrate levels is unclear.

Dry matter partitioning is not a simple and static process, but a dynamic process involving 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 (Patrick 1988). Although the controls which regulate the partitioning of dry matter at the whole plant level are still poorly understood (Wardlaw, 1990), some evidence indicated a role for daylength in controlling dry matter partitioning within the plants.

Eagles (1971) showed that plants (*Dactylis glomerata* L.) under short daylength has significantly higher root:shoot ratio, indicating that under short daylength treatment significantly more dry matter was partitioned to the roots. In addition, Hay and Heide (1983) plants (*Poa pratensis*) exposed to long daylength showed an increase in leaf area ratio so that the plants had higher relative growth rate (RGR) than those under short daylength.

Existing evidence in asparagus plant also suggested an effect of daylength on dry matter partitioning between root and shoot. For example, Sudjatmiko 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 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, the abrupt change in 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. They hypothesized 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. The environmental signal affecting this partitioning 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). In addition, Woolley at al. (1999) showed that 70 % of carbon (^{14}C) partitioned to the shoot at mid-summer (daylength 14h 29 min to 13h 27min); however, ^{14}C partitioning changed abruptly late summer (daylength 13h 27 min to 12h 27 min), when 74 % partitioned to the crown.

This experiment was conducted to determine the effects of daylength on partitioning of carbohydrate between fern and storage roots in asparagus seedlings.

2.2. Materials and methods

2.2.1. Introduction

The work in this chapter is divided into two experiments. The first experiment was conducted from 16 December 1997 to 18 February 1998 using three asparagus cultivars: 'UC157', 'Jersey Giant' and 'Italian Hybrid' while the second experiment was run from 2 March 1998 to 11 May 1998 using two cultivars: 'UC157' and 'Jersey Giant'. The second experiment was done using bigger plants to support the results in the first experiment.

2.2.2. The first experiment

2.2.2.1. Propagation and plant raising

The plants were propagated by sowing seeds of 'UC157', 'Jersey Giant' and 'Italian Hybrid' in 14 October 1997. The seeds were sown in 1:1:1 bark:peat:pumice mix contained in white hydroponic type cell trays. The cells had a volume of 55 cm^3 and the seeds were sown one seed per cell. Base fertilizer of 150 g Osmocote (16N-3.5P-10.8K) /100 liters was added to the growing media.

The seeds were germinated on a heated bench (25°C) in glasshouse 28 at the Plant Growth Unit, Massey University. Temperatures in the glasshouse were controlled by heating to 16°C, with fan ventilation operating at 25°C. Water was applied once a day and the trays were covered by newspaper to retain moisture for about one week The seedlings were raised in the glass house for 9 weeks. The seedlings with two shoots were selected and repotted into 220 cm^3 pots on 16 December 1997 with the same base fertilizer as above and transferred to growth chambers for the experiment.

During the experiment, the plants were watered every day and fertilized once a week with water soluble NPK Fertilizer (Peters) 0.5 g/l consisting of 100 ppm N, 37 ppm P and 83 ppm K. A second re-potting into 560 cm^3 pots was carried out on the 25 January 1998.

2.2.2.2. Growth chambers conditions

The experiment was conducted in four controlled climate growth chambers in Level One and Two, Institute of Natural Resources, Massey University. Each chamber was lit by two different sources of light : main light (high intensity) and extension light (low intensity). High light intensity was provided by 2 x 1000W high pressure discharge lamps and 6 x 1000W halogen lamps, while low light intensity was provided by 2 x 100W incandescent lamps and one 20W high efficiency long life tube lamp. Temperature within the chambers was set constant at 20°C.

Table 2.1. Conditions of Controlled Climate Growth Chamber. Irradiance was measured as mol quanta between 400 and 700 nm.

Growth Chamber	Temperature	High Intensity ($m\ sec^{-1}\ m^{-2}$)	Low Intensity ($m\ sec^{-1}\ m^{-2}$)	Daylength Treatment
A	20°C	481	4.8	Reducing
B	20°C	470	4.8	Constant
C	20°C	422	4.8	Reducing
D	20°C	416	4.8	Constant

2.2.2.3. Experimental design

A nested design with 3 asparagus cultivars ('UC157', 'Jersey Giant', and 'Italian Hybrid') nested within 2 daylengths (reducing daylength and constant daylength) was used as follows.

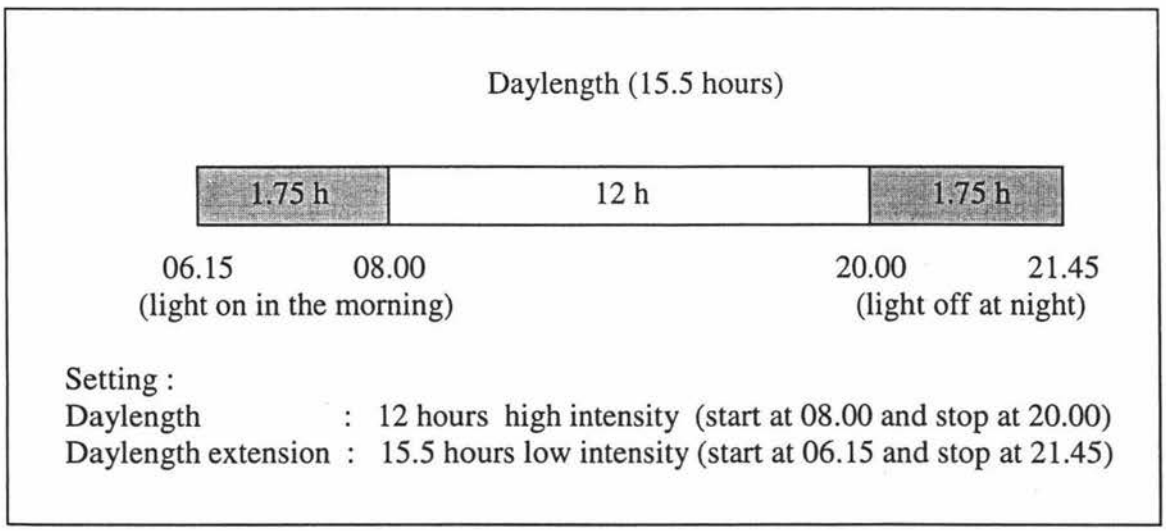
2.2.2.3.1. Daylength treatments

Both daylength treatments received similar amounts of radiant energy. Two pairs of chambers were used for each daylength. The chamber for each pair were matched for light intensity so that chamber A and B was one pair and chamber C and B was the other (see Table 2.1). Within each pair of chambers, the reducing daylength treatment was the slightly higher light intensity in order to compensate for the low light intensity energy of the long photoperiod. In this way the total photosynthetically active radiation (PAR) was kept more or less constant for each pair of cabinets.

2.2.2.3.1.1. Constant daylength

Constant daylength was set up as shown in Diagram 2.1 :

Diagram 2.1. The arrangement of constant daylength treatment.



2.2.2.3.1.2. Reducing daylength

In this treatment, daylength was reduced linearly from 15.5 h to 12 h with a reduction of 15 minutes per 4 days. The daylength reduction was done alternately for the morning and night low light intensity. Thus, both daylength treatments received 12 hours of high intensity light. The timetable of daylength reduction can be seen in the following table.

Table 2.2. Timetable of daylength reduction for experiment 1.

Harvest	Date	Reduce 15 minutes / 4 days		Daylength (Hours)	Note
		Morning	Night		
0	16 Dec '97	06.15	21.45	15.50	Start
	24 Dec '97	06.30	-	15.25	
	28 Dec '97	-	21.30	15.00	
1	01 Jan '97	06.45	-	14.75	
	05 Jan '97	-	21.15	14.50	
2	09 Jan '98	07.00	-	14.25	
	13 Jan '98	-	21.00	14.00	
3	17 Jan '98	07.15	-	13.75	
	21 Jan '98	-	20.45	13.50	
4	25 Jan '98	07.30	-	13.25	repotted
	29 Jan '98	-	20.30	13.00	
5	02 Feb '98	07.45	-	12.75	
	06 Feb '98	-	20.15	12.50	
6	10 Feb '98	08.00	-	12.25	
	14 Feb '98	-	20.00	12.00	
7	18 Feb '98	08.00	20.00	12.00	
8	26 Feb '98	08.00	20.00	12.00	Finish

2.2.2.3.2. Cultivar treatments

Three asparagus cultivars : 'UC157', 'Jersey Giant' and 'Italian Hybrid' were completely randomized within each growth chambers with 5 single plant replications. Total plant number was 120 plants per chamber for 8 harvest times, excluding initial harvest.

2.2.2.4. Data collection

Five replicates of plants from every cultivar within each chamber were destructively harvested at 8 days intervals as indicated in Table 2.2. After harvest, the plants were washed to remove soil. Number of shoots, roots and buds were recorded. Each plant was then cut into crown (rhizome and roots) and shoots for fresh weight recording before drying in a freeze dryer for 3 days. After dry weight of crown and shoots were recorded, the plant samples were ground for soluble carbohydrate analysis.

2.2.2.5. Total soluble carbohydrate analysis

Total soluble carbohydrate was determined using the anthrone reaction. Anthrone reagent was made by dissolving 10 g thiourea and 0.5 g anthrone (9,10-dihydro-9-oxoanthracene) in one liter of 66 % (v/v) H_2SO_4 and warming the mixture to 80-90°C. The reagent was store at 0-4°C. The color of the reagent increases slowly with time and the color yields tend to decrease after 2 weeks (Southgate 1991).

Fifty mg dried ground sample was extracted in 5 ml distilled water at 95°C (water bath) for 30 minutes in a glass tube. The sample was centrifuged at 700 g for 15 minutes and then the supernatant was taken for analysis. A standard fructose solution was diluted to give standards in the range of 250 – 7000 µg/l.

Blank and standard amounts of fructose were carried through with each series of unknown samples. Fifty µl of the test solution was pipetted into a glass test-tube and 5 ml anthrone reagent was added. The tube was swirled to mix the content and left in the dark.

The absorbance was measured after 25 minutes at 620 nm using a spectrophotometer (U-2000, Hitachi).

2.2.2.6. Statistical analysis

Statistical analysis was performed using the general linear model (proc glm) of SAS Package version 6.12.

2.2.2.7. Allometric ratio

Allometry in plant growth involves the study of the growth rate of one part of the plant in relation to the other (Hunt 1987). Thus, the allometric ratio between root and shoot dry weight can be used to analyze the ratio of the relative growth rate of shoot to root. This approach can eliminate problems caused by confounding the root to shoot ratio with changes in plant size as the plant grows.

The allometric ratio is calculated using linear regression of $\log_e$ of root dry weight against $\log_e$ shoot dry weight as follows :

$$\log_e Y = \log_e c + k \log_e X$$

where : $\log_e c$ is a constant;

k is the allometric constant (the slope);

$\log_e Y$ is natural logaritm of shoot dry weight;

$\log_e X$ is natural logaritm of root dry weight.

The value of k can summarize the whole behavior of both root and shoot across any harvest periods so that allometric ratio is of considerable practical value. A bigger value of k indicates more partitioning of dry matter to roots while lower value of k suggests more dry matter partitioning to shoot. The value of k is usually constant during

vegetative growth under constant environmental condition. However, k is strongly affected by environment and genotype variation (Hunt 1987).

2.2.3. The second experiment

The procedure of this experiment was similar to the previous one but with modifications. Firstly, there were only two chambers used in this experiment, one chamber for constant daylength (15.5 h) and another chamber for reduced daylength starting from 14.5 hours (Table 2.3). Secondly, only two asparagus cultivars ('UC157' and 'Jersey Giant') were used and thirdly, bigger pot sizes were used. The seeds were sown on 25 November 1998 using white propagating trays (55 cm^3 cell size) and then repotted to 530 cm3 pots at 30 January 1998. The plants were transferred to growth chambers at 2 March 1998 (Harvest 0, Table 3). Before the last two harvests (15 April 1998), the plants were repotted to 2400 cm^3 pots.

Constant daylength treatment was the same as the previous experiment (15.5 h).

Table 2.3. Timetable of daylength reduction for experiment 2.

Harvest	Date	Reduce 30 minutes / harvest		Daylength (Hours)	Note
		Morning	Night		
0	2 Mar '98	06.45	21.15	14.50	Start
1	16 Mar '98	06.45	21.15	14.50	
2	23 Mar '98	07.00	21.00	14.00	
3	30 Mar '98	07.15	20.45	13.50	
4	6 Apr '98	07.30	20.30	13.00	
5	13 Apr '98	07.45	20.15	12.50	repotted
6	27 Apr '98	08.00	20.00	12.00	
7	11 May '98	08.00	20.00	12.00	Finish

2.3. Results

2.3.1. The first experiment

2.3.1.1. Shoot, root and bud number

Under constant daylength, shoot number of asparagus seedlings increased linearly as harvest progressed. The plants under reducing daylength also showed a similar increase until harvest 5; however shoot production slowed down after harvest 5 to produce a curvilinear effect. Although shoot number was statistically not different between daylength treatments, plants under constant daylength always produced more shoots at later harvests. These responses were clearly shown by 'UC157' and 'Jersey Giant' but not 'Italian Hybrid' (Figure 2.1).

Root and bud number also increased with harvest times, but were not significantly affected by daylength treatment in any harvest. Under constant daylength 'UC157' and 'Jersey Giant' produced slightly higher root and bud number than under reducing daylength, but 'Italian Hybrid' showed no indication of a response (Figure 2.2 and 2.4).

In contrast, the effects of cultivar on shoot, root and bud number were significant at most harvest. 'UC157' produced significantly higher numbers of roots and buds than the other cultivars at all harvest but 'Jersey Giant' produced higher shoot number than 'UC157' at harvest 1 to 4 and harvest 7 to 8. The response was shown by root to shoot number ratio (Figure 2.3) where 'UC157' showed higher ratio in both constant and reducing daylength compared with 'Jersey Giant' and 'Italian Hybrid'. In all cases, 'Italian Hybrid' was inferior compared with 'Jersey Giant' and 'UC157'. More importantly daylength appeared to affect the root:shoot number ratio with both 'UC157' and 'Jersey Giant' showing a daylength effect while Italian Hybrid showing little or no effect.

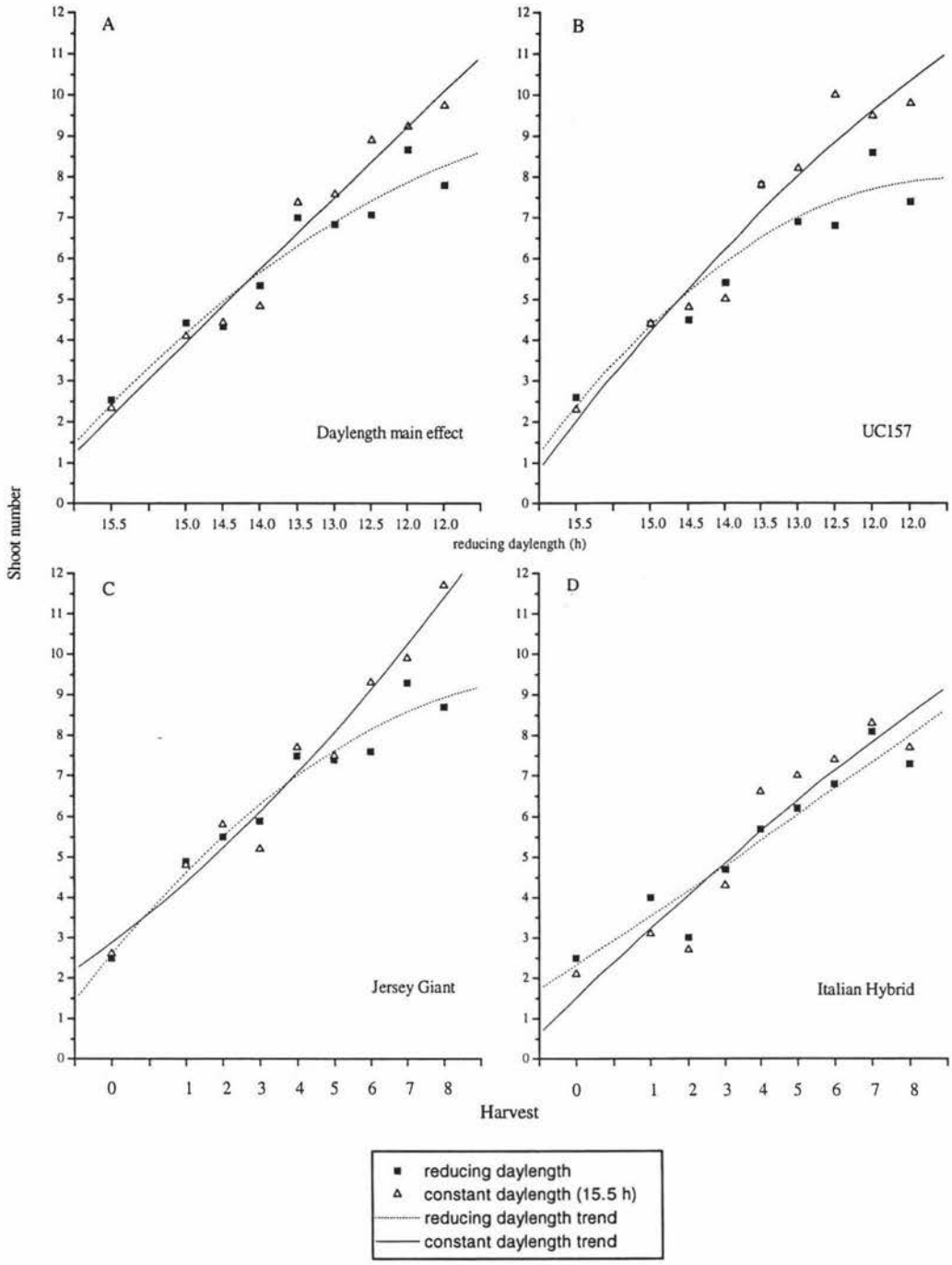


Figure 2.1. Main effect of daylength on shoot number of asparagus seedlings (A) and response of cultivars to daylength (B, C, D) (Quadratic curve fit). Harvest axes on C and D correspond to reducing daylength axes on A and B.

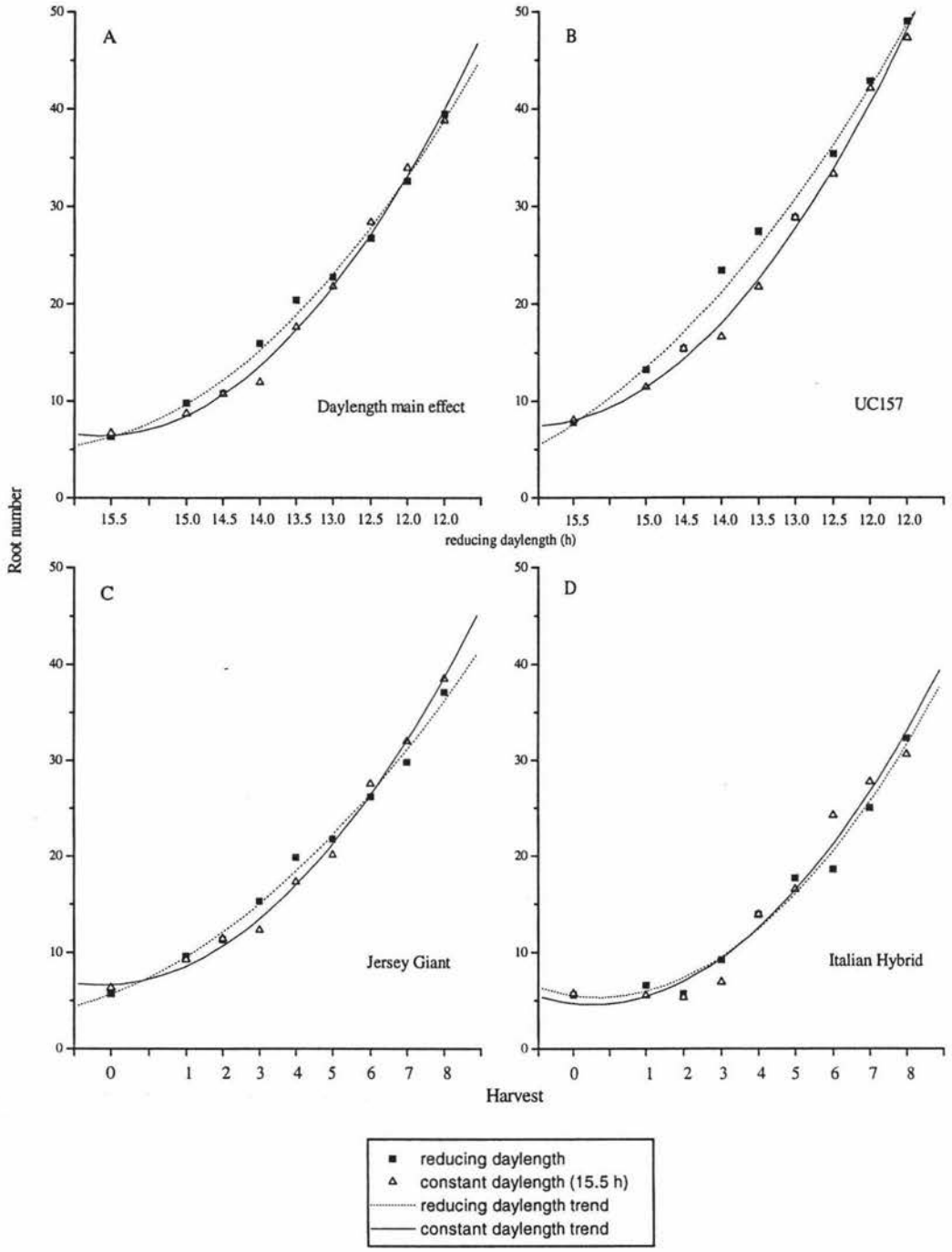


Figure 2.2. Main effect of daylength on root number of asparagus seedlings (A) and response of cultivars to daylength (B, C, D) (Quadratic curve fit). Harvest axes on C and D correspond to reducing daylength axes on A and B.

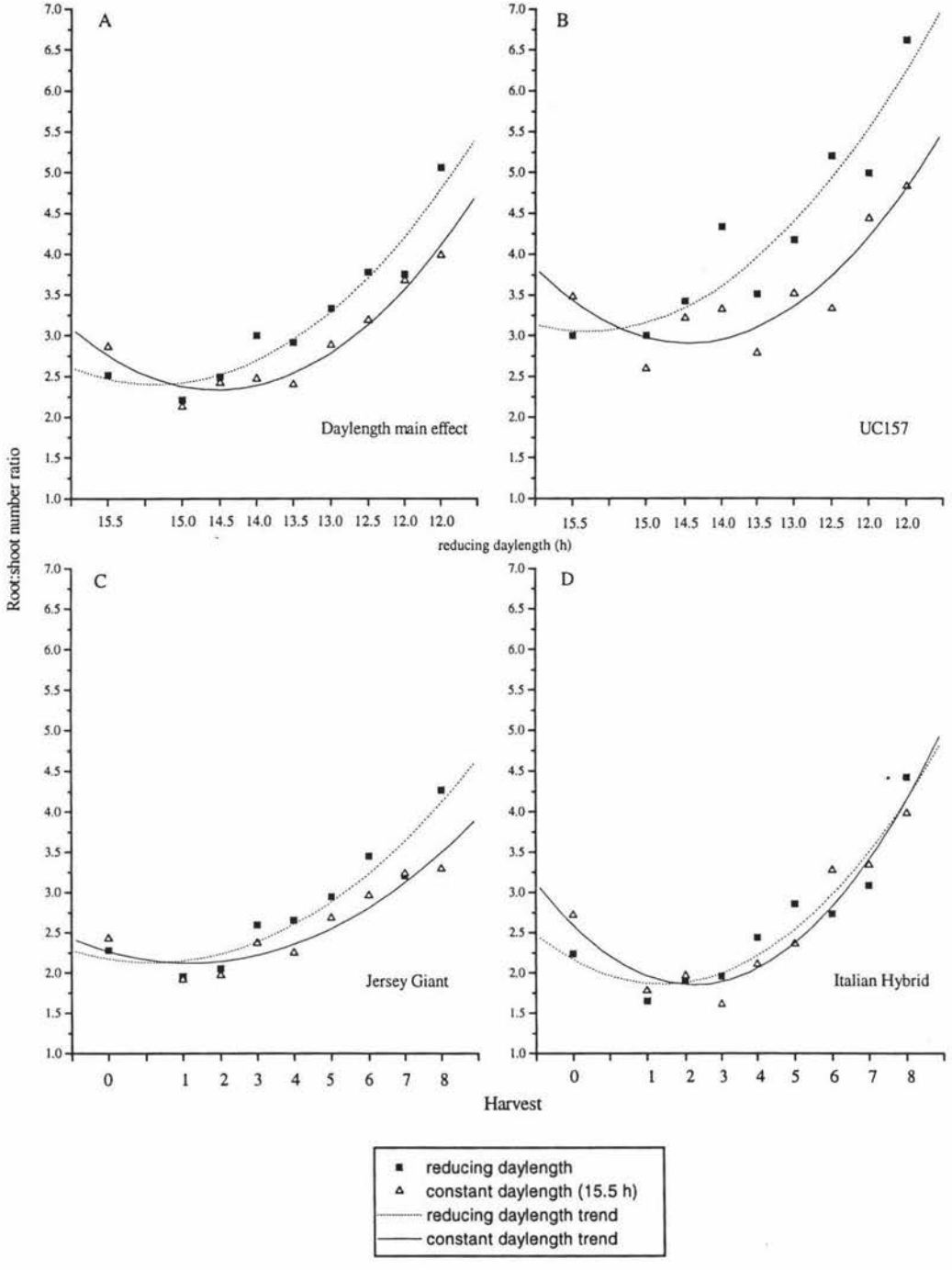


Figure 2.3. Main effect of daylength on root:shoot number ratio of asparagus seedlings (A) and response of cultivars to daylength (B, C, D) (Quadratic curve fit). Harvest axes on C and D correspond to reducing daylength axes on A and B.

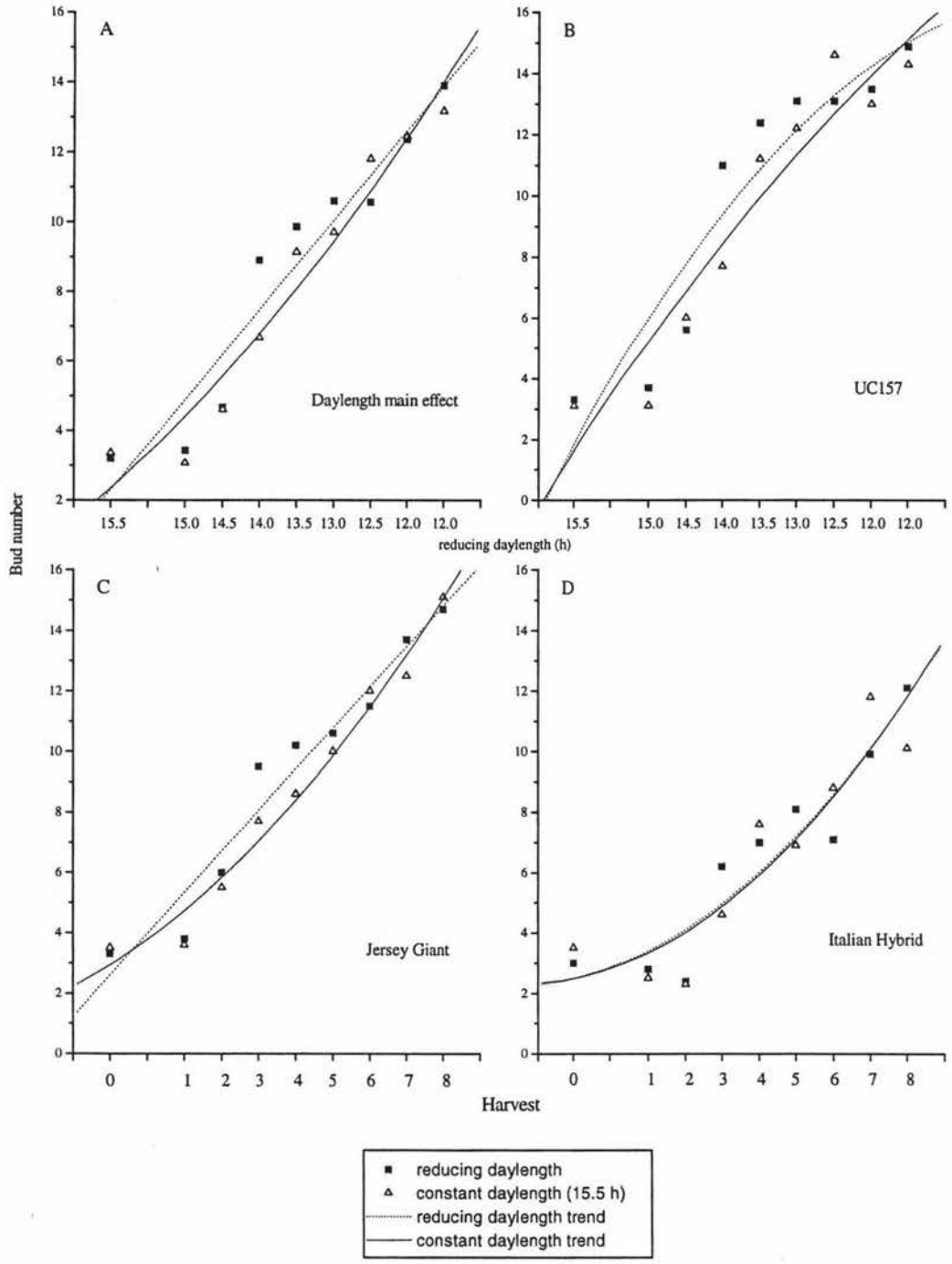


Figure 2.4. Main effect of daylength on bud number of asparagus seedlings (A) and response of cultivars to daylength (B, C, D) (Quadratic curve fit). Harvest axes on C and D correspond to reducing daylength axes on A and B.

2.3.1.2. Plant height, lateral branch number and length

Plant height, lateral branch number and length were observed at harvest 7 and lateral length was measured from lateral number 5 from base.

The results showed that plant height was significantly affected by daylength, constant long daylength resulted in significantly higher plants than reducing daylength. 'Italian Hybrid' was shorter than 'Jersey Giant' under both constant and reducing daylength. In addition, plants under constant long daylength had significantly higher lateral branch number, 21 compared to 15 for reducing daylength. Also plants under constant daylength showed significantly longer lateral branch length (13.7 cm) than those under reducing daylength (10.4 cm) (Table 2.4).

Table 2.4. Effects of daylength on plant height, lateral branch number and length of three asparagus cultivars.

Cultivar	Plant height (cm)		Lateral branch number (per shoot)		Lateral branch length (cm)	
	Reducing	Constant	Reducing	Constant	Reducing	Constant
UC 157	43.8 cd	60.5 a	15.2 b	21.0 a	10.8 b	14.5 a
Jersey Giant	45.2 c	60.2 a	15.1 b	20.4 a	10.2 b	13.5 a
Italian Hybrid	40.4 d	51.7 b	13.9 b	20.4 a	9.3 b	13.0 a
Main effect	43.6 B	57.5 A	15.1 B	20.6 A	10.1 B	13.7 A

Within columns, means followed by the same small letter and means (main effect) followed by the same capital letter are not significantly different at p=0.05.

2.3.1.3. Fern dry weight

Fern growth under constant daylength and reduced daylength showed similar trends, fern dry weight increased as harvest progressed (Figure 2.5). Initially, the fern growth was not different; however, at later harvest plants under constant daylength showed significantly faster fern growth than plants under reduced daylength. For example, at harvest 4 fern dry weight of plants under both daylength treatments was about 1.1 g/plant; however at harvest 5, fern dry weight of plant under constant daylength was 1.9 g/plant which was significantly higher then that of plant under reduced daylength (1.5 g/plant).

The response of cultivars to daylength was fairly similar. However, under reducing daylength fern growth of 'UC157' was suppressed more than those of 'Jersey Giant' and 'Italian Hybrid', as indicated by the fern dry weight difference between constant and reducing daylength.

2.3.1.4. Crown dry weight

Response of asparagus to daylength treatments was similar between cultivars. Crown dry weight increased with the time and was significantly affected by daylength treatment, at most harvest times from harvest 3 to 5 (see Appendix 1 for table of value and statistical analysis). Under constant daylength where environmental conditions were constant, crown dry weight can be fitted accurately; however, under reducing daylength where environmental condition changed, perturbations of crown dry weight occurred so that data points have been both fitted and connected directly (Figure 2.6).

At early harvests, crown dry weights increased slowly and were not affected by daylength. However, starting from harvest 3 the crown grew very fast and in this stage daylength treatment resulted in significant effects on crown dry weight. Plants under reduced daylength produced significantly higher crown dry weight from harvest 3 to 5, but at harvest 6, 7 and 8 plants under constant daylength showed higher crown dry weight than those under reducing daylength.

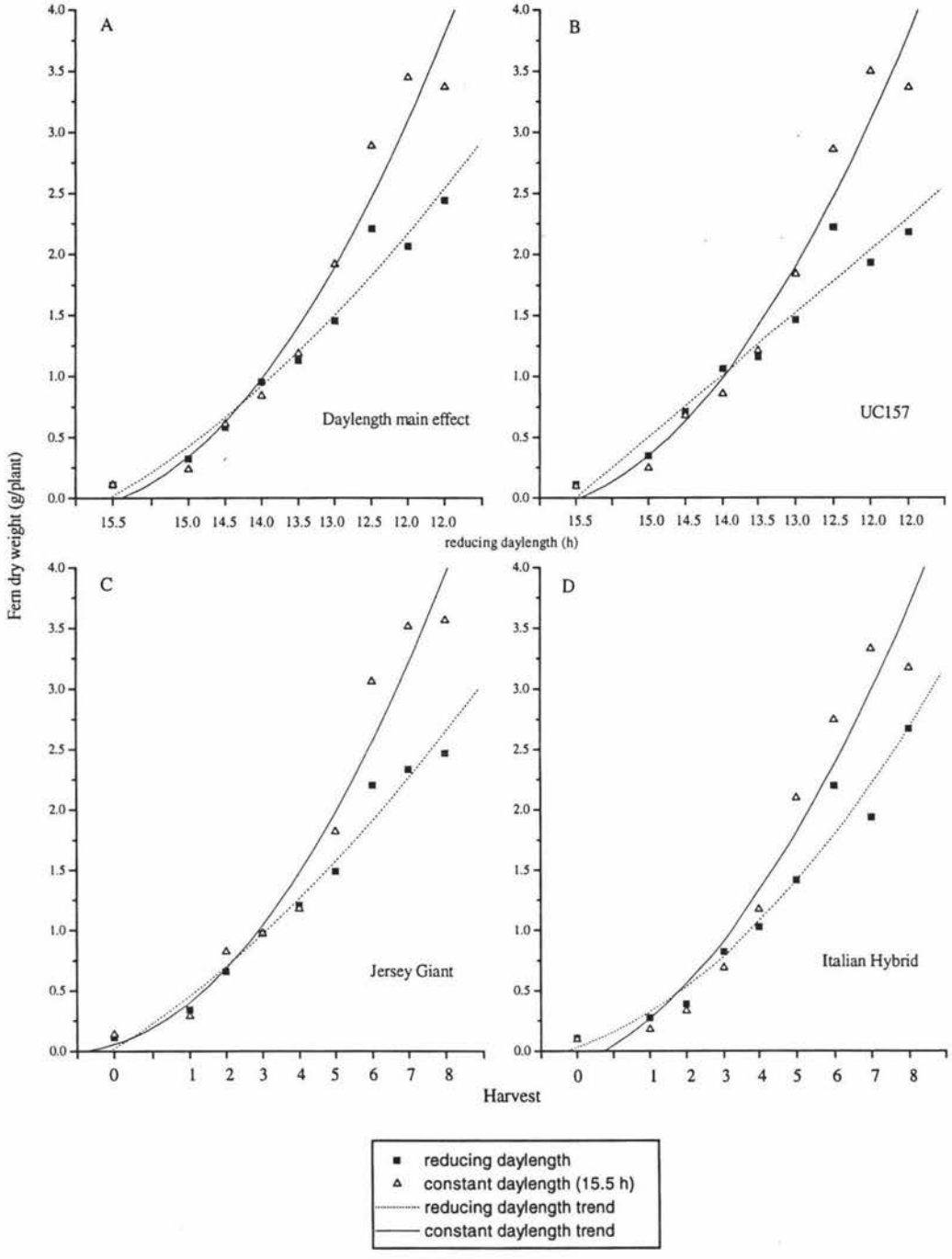


Figure 2.5. Main effect of daylength on fern dry weight of asparagus seedlings (A) and response of cultivars to daylength (B, C, D) (Quadratic curve fit). Harvest axes on C and D correspond to reducing daylength axes on A and B.

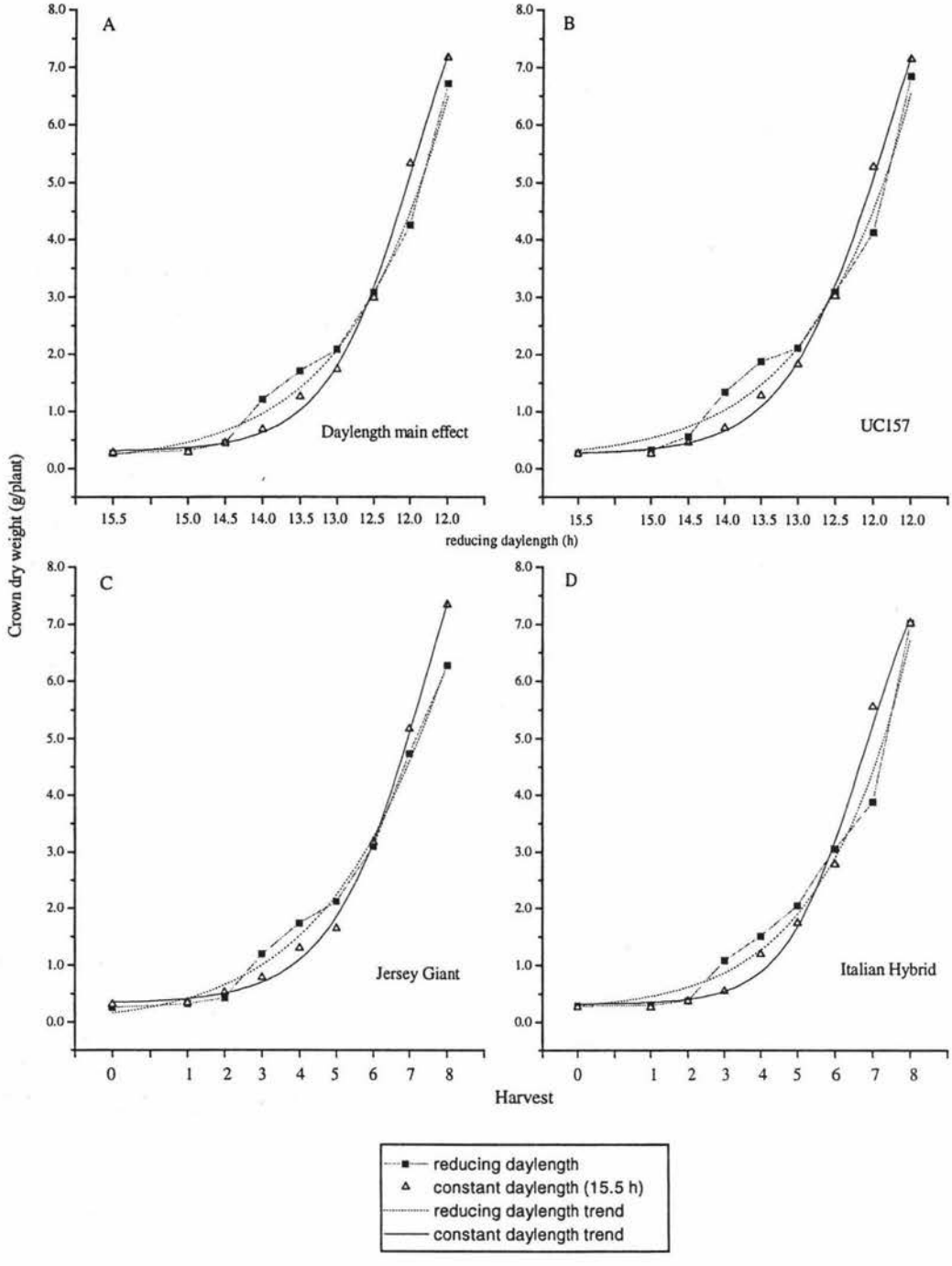


Figure 2.6. Main effect of daylength on crown dry weight of asparagus seedlings (A) and response of cultivars to daylength (B, C, D) (Sigmoid curve fit). Harvest axes on C and D correspond to reducing daylength axes on A and B.

2.3.1.5. Relative Growth Rate (RGR), Leaf Weight Ratio (LWR) and Net Assimilation Rate (NAR)

Plants under constant long daylength showed higher RGR (grams dry weight gained per gram of plant present per 8 days) than plants under reducing daylength. For example, at time 2 (16 days after treatment) RGR was 0.37 for constant daylength compared to 0.33 for reducing daylength. However, an abrupt change from 0.33 to 0.53 occurred at time 4 (corresponding to a daylength of 14 hours). Thereafter the RGR at a reducing daylength decreased to 0.29 which was lower than the RGR at a constant daylength (0.39).

Similarly, plants under constant daylength showed higher NAR (grams dry weight gain per gram dry weight of fern per 8 days) at most times except at time 4 when plants under reducing daylength had NAR of 1.2 g/g/8 days compared to 0.90 g/g/8 days for plants under constant daylength. NAR was calculated by dividing RGR with LWR (formula RGR=NAR x LAR but LAR (Leaf Area Ratio) was replaced by LWR (grams dry weight of fern per gram total dry weight) as LAR was not available for asparagus plant) (Figure 2.7).

2.3.1.6. Total soluble carbohydrate

There was no significant effect of daylength treatment on total soluble carbohydrate. At harvest 1 total soluble carbohydrate was around 18.5 % and increased to around 30.5 % at harvest 3. However, the total soluble carbohydrate decreased slightly to about 30.0 % at harvest 5 and followed by a sharp increase to around 45 % at harvest 7 (Figure 2.8.A).

Although total soluble carbohydrate was not significantly different between cultivars under reducing and constant daylength, it can be noticed that under reduced daylength at harvest 3 and 7 'Jersey Giant' showed higher soluble carbohydrate than other cultivars while 'Italian Hybrid' had the lowest soluble carbohydrate. In contrast, under constant daylength at harvest 3 and 5 'Italian Hybrid' had highest soluble carbohydrate, followed by 'Jersey Giant' and 'UC157' (Figure 2.8.B).

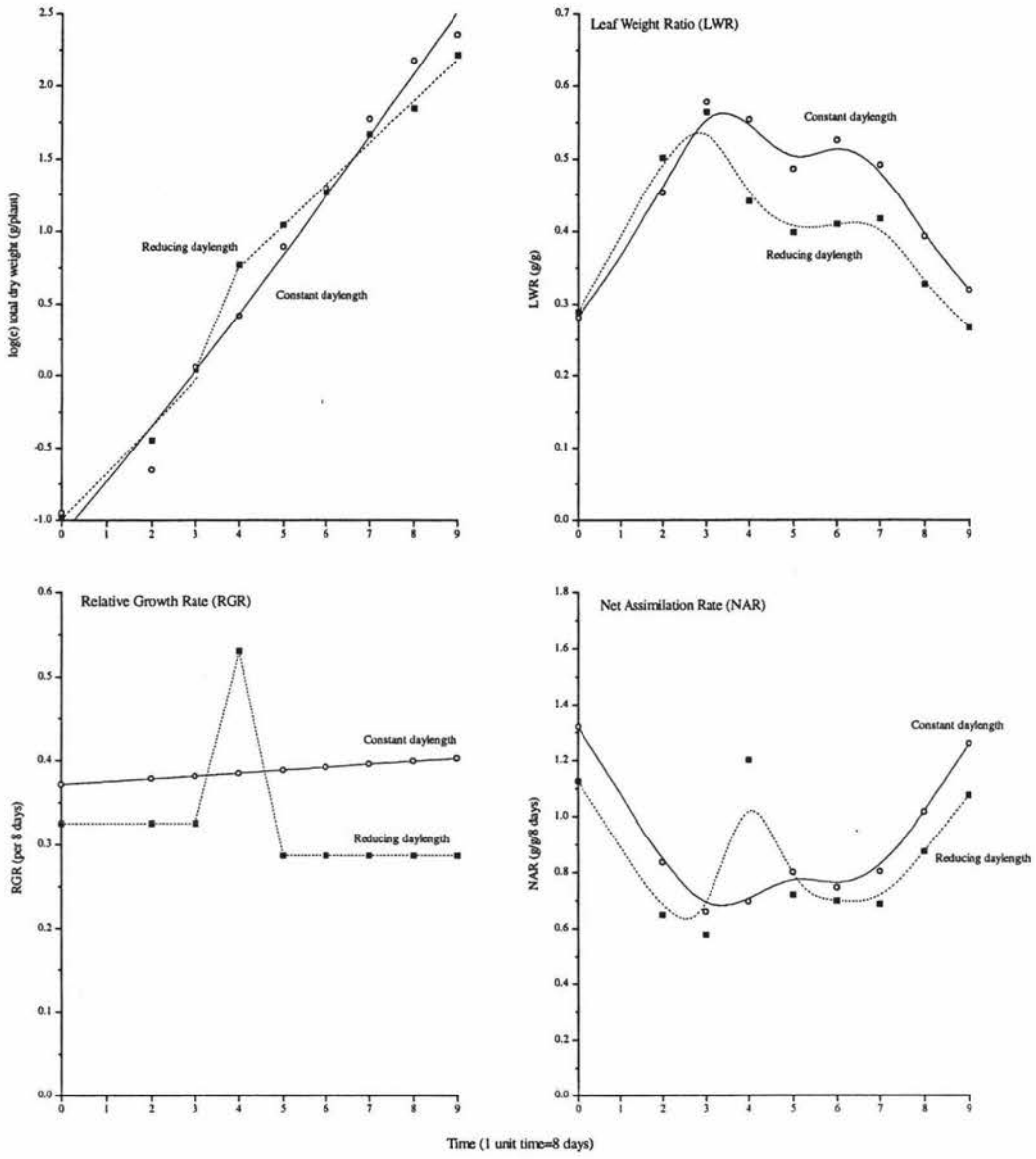


Figure 2.7. Effects of daylength on Relative Growth Rate (RGR), Leaf Weight Ratio (LWR) and Net Assimilation Rate (NAR).

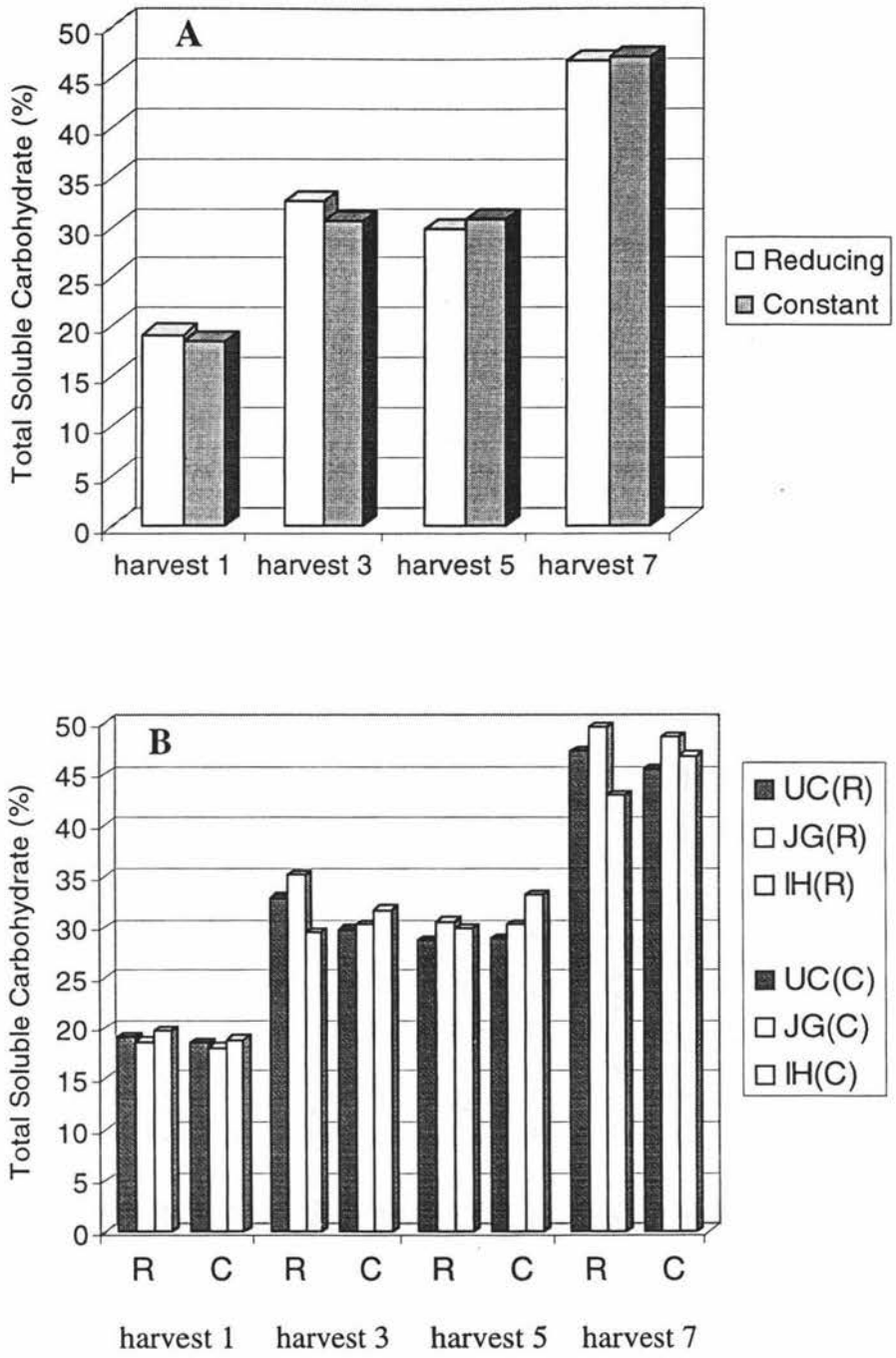


Figure 2.8. Total soluble carbohydrate of asparagus roots as affected by daylength (A) and cultivar within daylength (B). Legend : R=reducing daylength, C=constant daylength, UC='UC157', JG='Jersey Giant', IH='Italian Hybrid'.

2.3.1.7. Root:shoot dry weight ratio

In this section, the term 'root' represents crown dry weight (roots and rhizome) and 'shoot' represents fern dry weight.

Root:shoot ratio was significantly affected by daylength treatment beyond harvest 2 (Figure 2.9). The root to shoot ratio at initial harvest (harvest 0) was about 2.5 and then decreased to about 1.1 at harvest 1. The ratio continued to decrease to about 0.8 at harvest 2. After harvest 2, the effect of daylength was significant. Plants under reduced daylength showed significantly higher root:shoot ratio than those under constant daylength. For example, at harvest 4 the ratio of plants under reduced daylength was 1.52 compared to 1.06 for plants under constant daylength. At harvest 7 the ratio went up to 2.09 for reduced daylength which was significantly higher than that for constant daylength (1.57). The effects of cultivar on root:shoot ratio was only significant at harvest 2 and 3 where 'Italian Hybrid' showed a higher ratio than 'Jersey Giant' and 'UC157'. However, at harvest 8 'UC157' grown under reduced daylength had root:shoot ratio about 3.14 which was significantly higher than that of 'Italian Hybrid' and 'Jersey Giant' (Figure 2.9).

2.3.1.8. Allometric ratio

The allometric ratios were plotted for harvest 1, 3, 5 and 7 using linear regression of $\ln y = k \ln x + \ln a$, where y is fern dry weight; x is crown dry weight; and k is the value of slope. The results indicated that all cultivars ('UC157', 'Jersey Giant' and 'Italian Hybrid') showed similar trend. Plants under constant daylength had a steeper slop (k higher) than those under reduced daylength. For example, the slope of 'UC157' under constant daylength is 0.8602 while the slope is 0.6948 for reduced daylength. The slopes of 'Jersey Giant' and 'Italian Hybrid' under constant daylength are 0.8836 and 0.9174 respectively while under reduced daylength are 0.7254 and 0.7204 respectively (Figure 2.10). However, the difference was not statistically significant for all cultivars and the constant daylength treatment indicated a curvilinear trend.

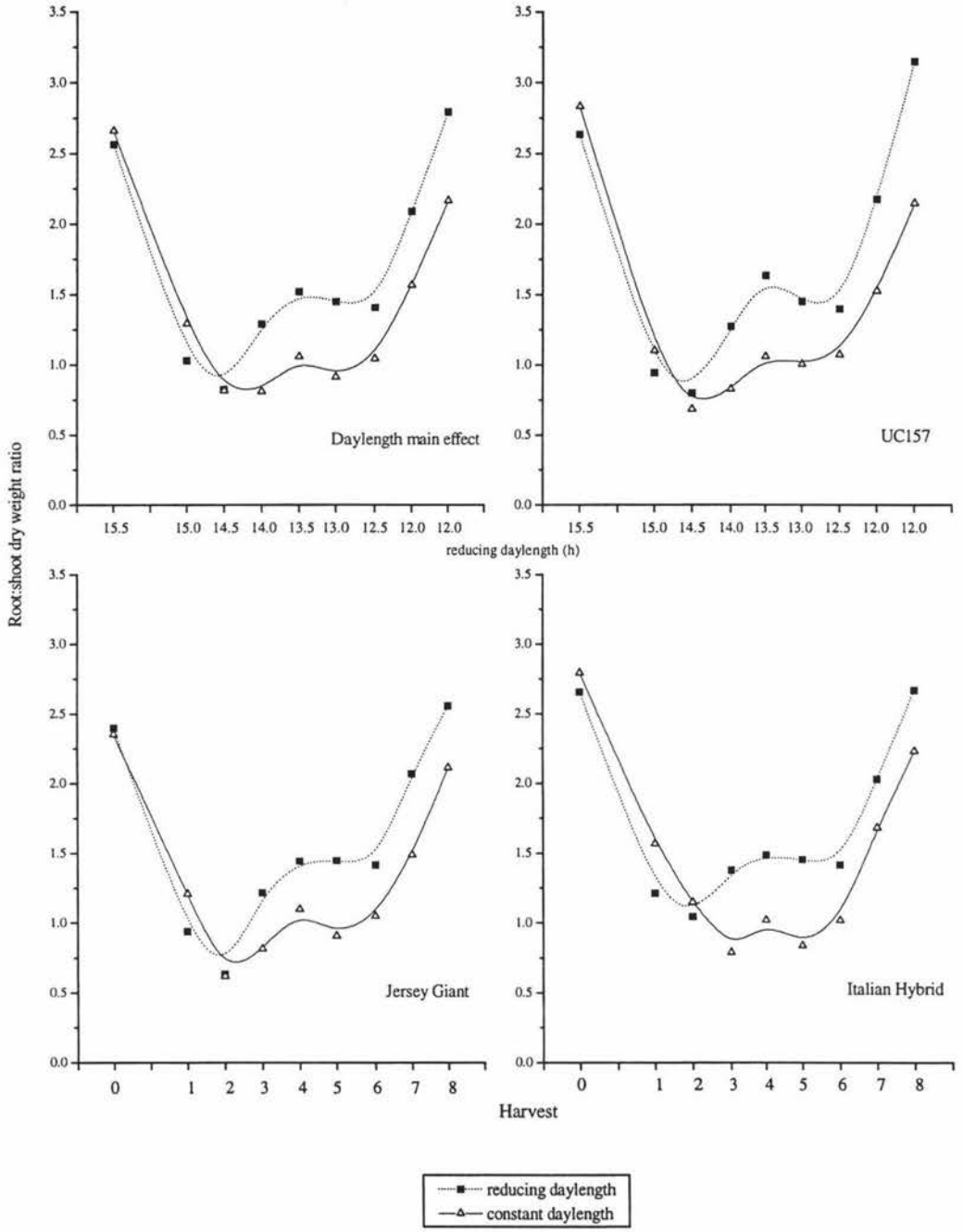


Figure 2.9. Main effect of daylength on root:shoot dry weight ratio of asparagus seedlings (A) and response of cultivars to daylength (B, C, D). Harvest axes on C and D correspond to reducing daylength axes on A and B.

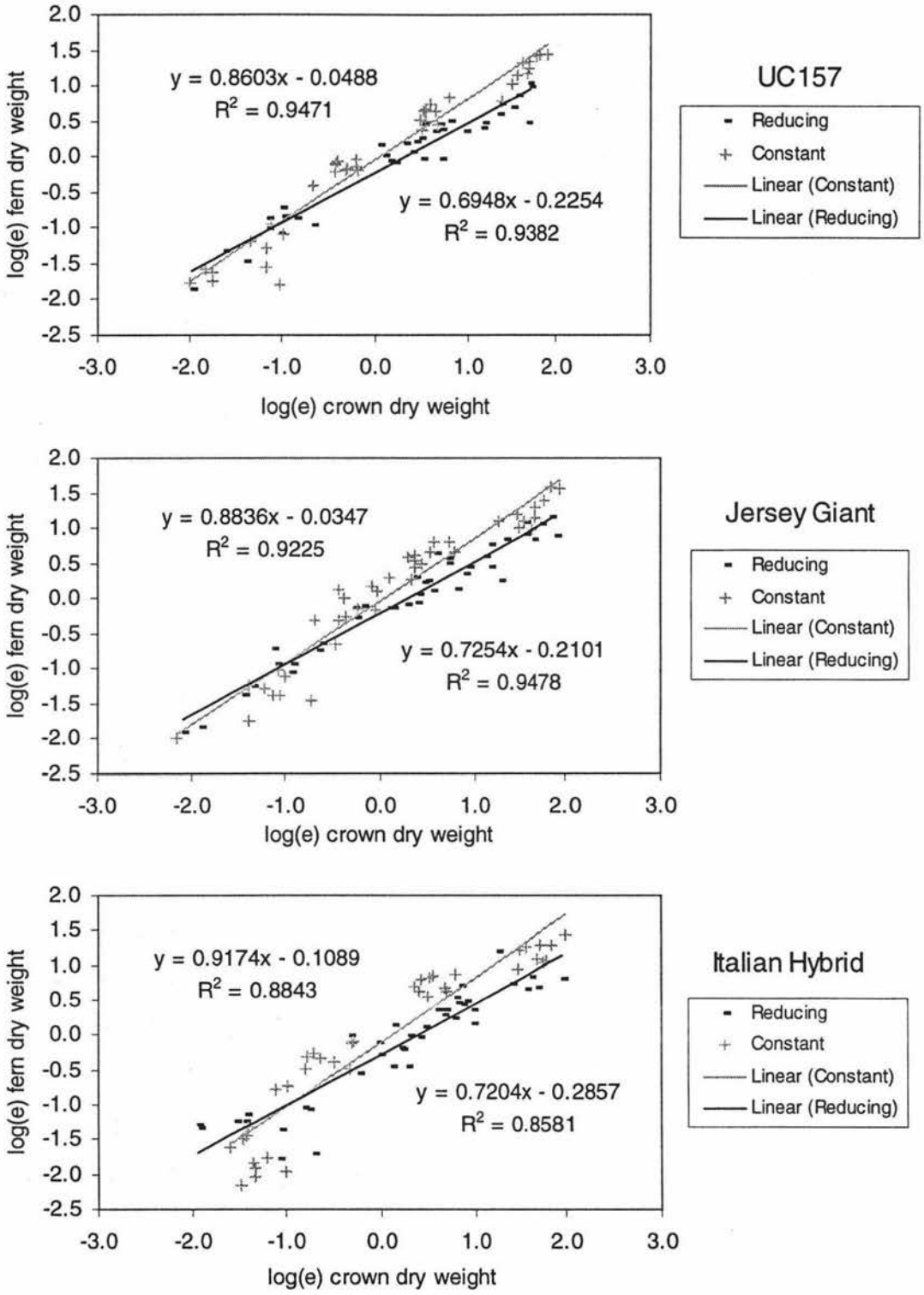


Figure 2.10. Allometric ratio between fern and crown dry weight of 'UC157', 'Jersey Giant' and 'Italian Hybrid' under reducing and constant daylength.

2.3.2. The second experiment

2.3.2.1. Fern dry weight

Fern growth under constant daylength and reduced daylength showed similar trends, both fern fresh and dry weight increased slowly until harvest 5 but increased sharply after that (Figure 2.11). The figures indicate that the fern growth of both 'UC157' and 'Jersey Giant' was not different from harvest 0 to harvest 5; however, after harvest 5, fern under constant daylength grew faster than that under reduced daylength so that fern dry weight was significantly different after harvest 6. In addition, the results showed that 'Jersey Giant' produced higher fern dry weight in both reduced and constant daylength than 'UC157'.

2.3.2.2. Crown dry weight

In the second experiment, plants under reduced daylength produced higher crown dry weight at most harvest times compared to plants under constant daylength. However, the response of cultivars to daylength was different. (Figure 2.12).

Under constant daylength, crown dry weight of 'UC157' and 'Jersey Giant' under daylength treatment was similar. Crown size increased slowly until harvest 5 and then increased sharply after that. In this constant daylength, 'Jersey Giant' produced slightly higher crown weight than 'UC157' but this was not significantly different. In contrast, under reduced daylength, 'Jersey Giant' showed significantly higher crown production than 'UC157'. For example, averaged over harvest 3 and 4 'Jersey Giant' showed a 37 % increase of crown dry weight when reducing daylength was between 13.5 and 13 h compared with a constant long daylength. In contrast, 'UC157' only showed a 7 % increase.

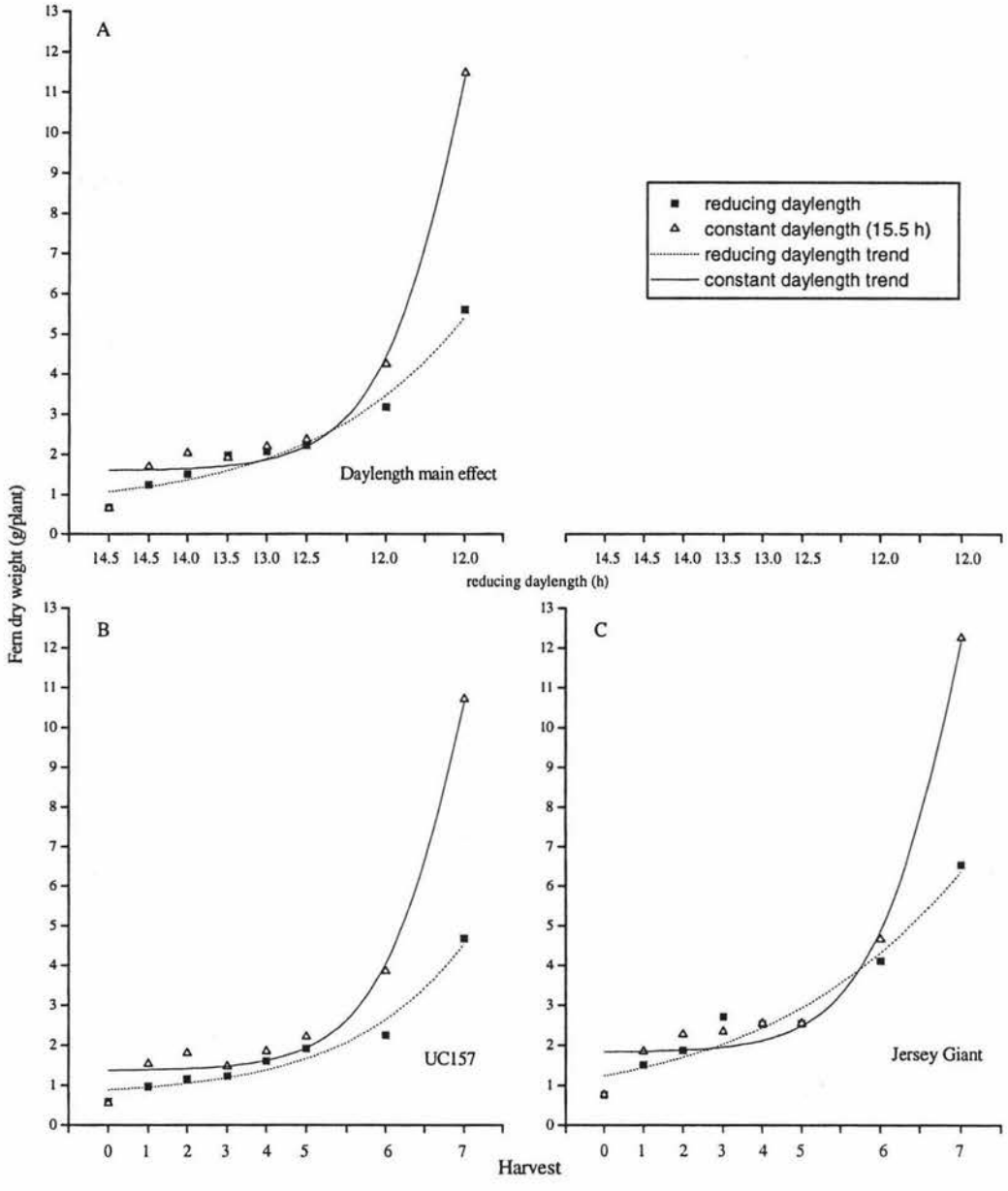


Figure 2.11. Main effect of daylength on fern dry weight of asparagus seedlings (A) and response of cultivars to daylength (B, C) (Quadratic curve fit). Harvest axes correspond to reducing daylength axes.

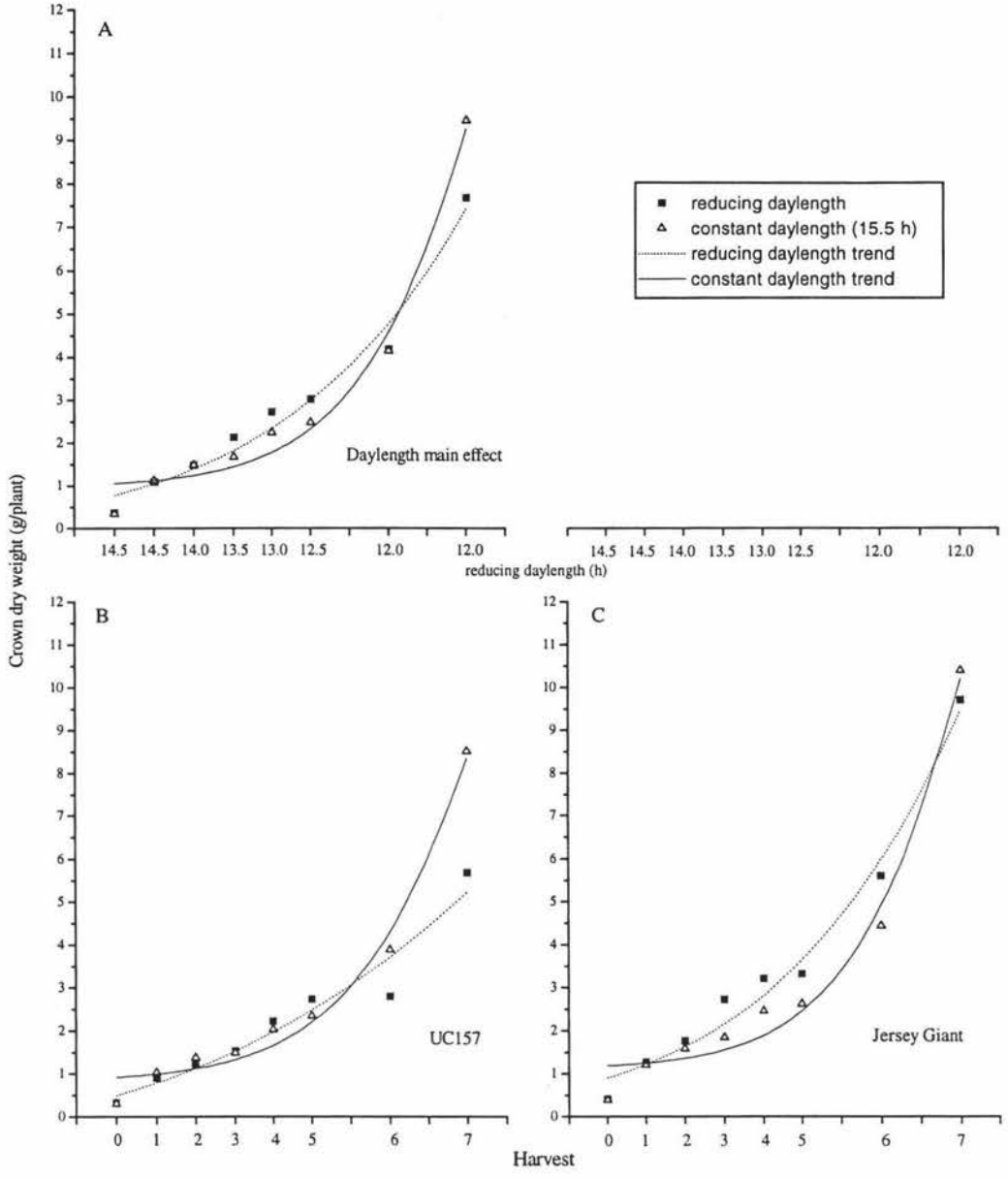


Figure 2.12. Main effect of daylength on crown dry weight of asparagus seedlings (A) and response of cultivars to daylength (B, C) (Quadratic curve fit). Harvest axes correspond to reducing daylength axes.

2.3.2.3. Root:shoot dry weight ratio

Initially, the root:shoot ratio was about 0.5 then it increased gradually until harvest 4 where the ratio of plants under reduced daylength was about 1.3 while plants under constant daylength was about 1.0. After harvest 4 the root:shoot ratio of plants under constant daylength went down to about 0.8 at harvest 7 while the root:shoot ratio of plants under reduced daylength remained constant until harvest 7. Thus, plants under reduced daylength maintained higher root:shoot ratio than those under constant daylength (Figure 2.13). However, 'UC157' and 'Jersey Giant' showed different trends of root:shoot ratio. 'UC157' showed higher root:shoot ratio until harvest 5 within both reduced and constant daylength; however, under reduced daylength , after harvest 5 root:shoot ratio of 'UC157' went down while the ratio of 'Jersey Giant' continued to increased until harvest 7 so that the ratio was significantly different at later harvests.

2.3.2.4. Allometric ratio

The allometric ratios were plotted from harvest 1, 3, 5 and 6 (14 days interval) using linear regression of $\ln y = k \ln x + \ln a$, where y is fern dry weight; x is crown dry weight; and k is the value of slope.

The results indicate that both 'UC157' and 'Jersey Giant' under constant daylength may have steeper slopes than those under reduced daylength. The slope of 'UC157' under constant daylength is 0.902 which is higher than that for reduced daylength (0.8283). The slopes of 'Jersey Giant' under constant daylength is 0.8484 while under reduced daylength is 0.6981 (Figure 2.14). This difference was not statistically significant. Once again, the results indicated a curvilinear component but this time for reducing daylength.

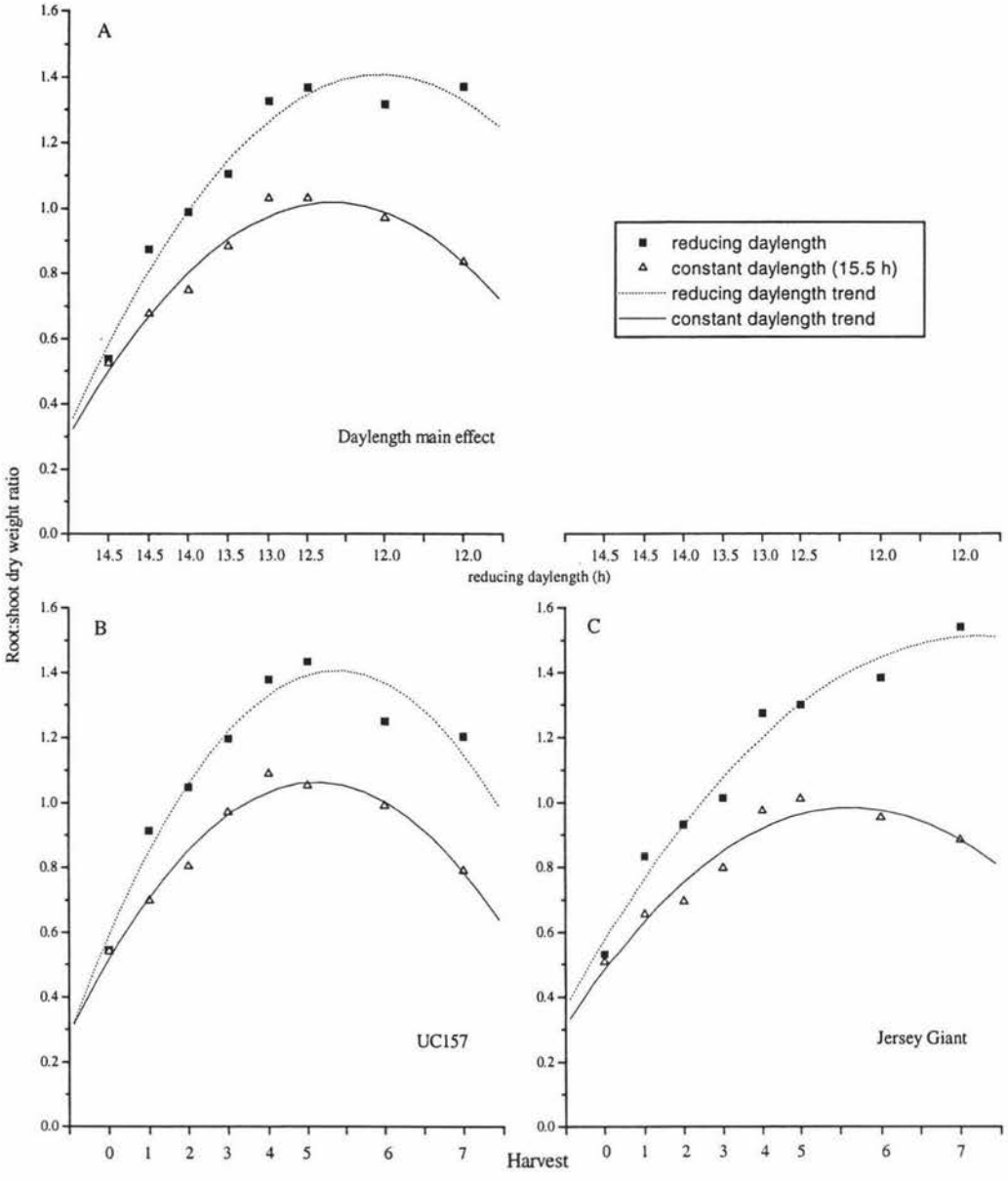


Figure 2.13. Main effect of daylength on root:shoot dry weight ratio of asparagus seedlings (A) and response of cultivars to daylength (B, C) (Quadratic curve fit). Harvest axes correspond to reducing daylength axes.

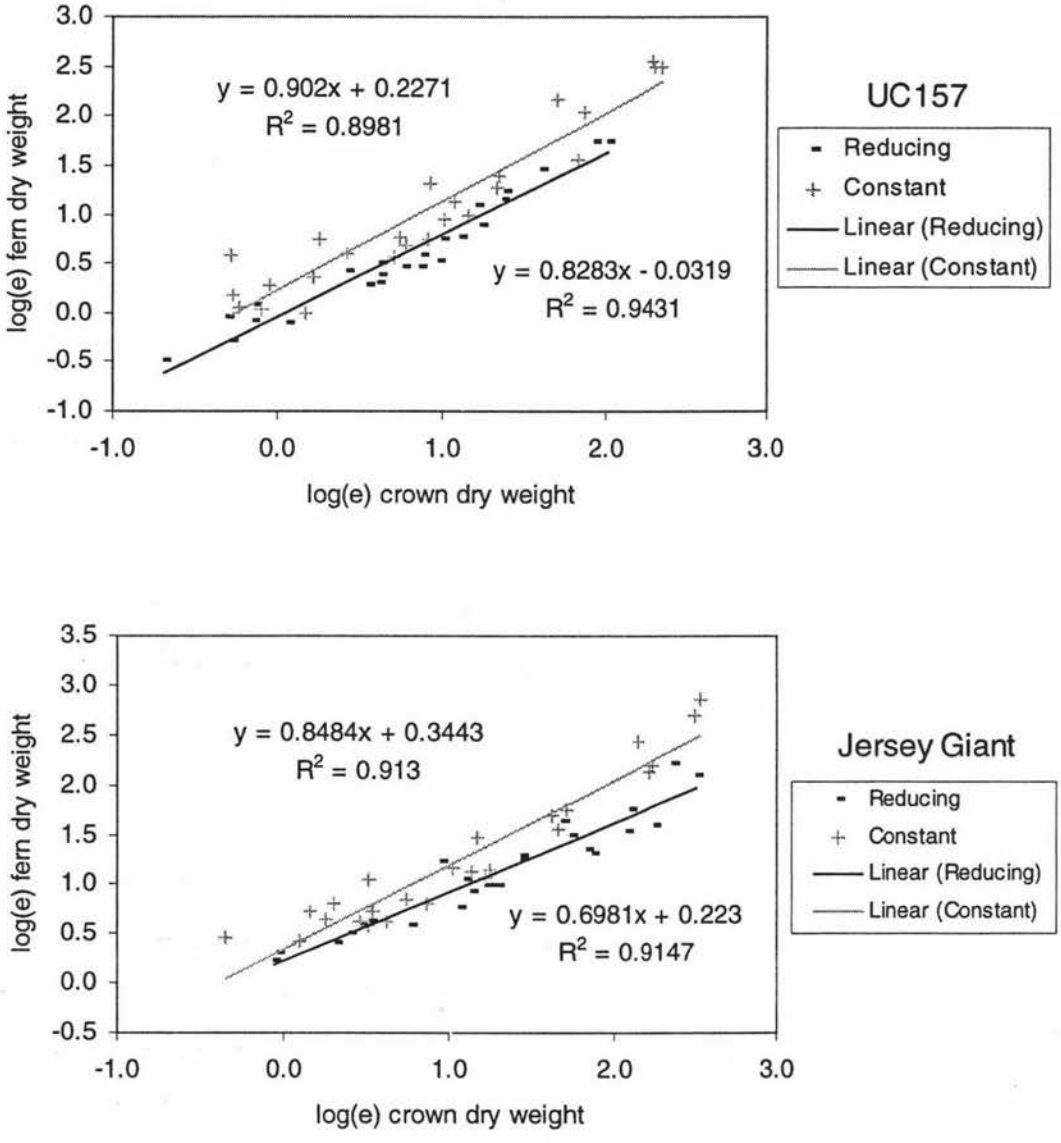


Figure 2.14. Allometric ratio between fern and crown dry weight of 'UC157' and 'Jersey Giant' under reducing and constant daylength.

2.4. Discussion

2.4.1. Effects of daylength on seedling growth of asparagus

The germination of asparagus seeds requires water, oxygen, and warm temperature with optimum at 25-30°C. In this optimum condition, the seeds absorb moisture and oxygen, and then the stored food within the seed is broken down to provide energy and the materials required for growth of the plant embryo. Thus, the initial seedling growth is mainly dependent on the stored food within the seeds until the shoot emerges from the ground, turn to green and begins to photosynthesize so that the plant becomes self sufficient (Robb 1983).

The results showed that root and shoot production was different between cultivars. Under both constant and reducing daylength 'Italian Hybrid' produced lower shoot and root number than 'Jersey Giant' and 'UC157'. Shoot number of 'Jersey Giant' and 'UC157' was more or less the same but 'UC157' produced more roots than 'Jersey Giant'. As a result, 'UC157' maintained higher root:shoot number ratio and the difference was clearer under reducing daylength. Initially, root:shoot number ratio was around 3. The ratio increased to around 4 at harvest 5 and continued to increased to more than 5 at later harvests. In contrast, the ratio increased to less than 4.5 under constant daylength. Thus, the ratio difference between reducing and constant daylength was quite large in 'UC157' but there was only a small difference in 'Jersey Giant' and nearly the same ratio in 'Italian Hybrid'. These results suggested the different responses of cultivars to daylength conditions. These results were different from Robb (1983) who stated that every shoot is followed by two roots so that root:shoot ratio of the asparagus seedlings is approximately 2:1.

Bud and root formation and shoot development are dynamic processes in asparagus growth. The growth of new asparagus roots is associated with fern establishment. The roots will not growth if the fern does not grow (McCormick and Franklin 1990). Stancanelli and Falavigna (1990) suggested a positive relationship between the number

of storage roots and the number of fern, as the seedling growth progresses throughout the growing season, more and more buds are developed on the crown and the buds develop to produce fern, eventually fern establishment supports bud formation.

The results indicated that the number of buds produced by plants was different between cultivars, 'Italian Hybrid' produced fewer buds than the other cultivars while 'UC157' and 'Jersey Giant' had similar bud production. In addition, plants under reducing daylength tended to have more buds than those under constant daylength as showed by 'UC157' and 'Jersey Giant'. 'Italian Hybrid' showed similar trends of bud number between reducing and constant daylength (Figure 2.4).

The fact that plants under constant daylength produced more ferns but had fewer buds than those under reducing daylength suggested an effect of daylength on bud break. Long constant day length induced bud break and shoot growth and conversely, bud break and shoot growth was suppressed under reducing daylength, but not bud formation.

2.4.2. Effects of daylength on dry matter partitioning of asparagus seedlings

The potential capacity of the crop to partition assimilates efficiently to the harvest or storage organ may be determined by environment factors such as daylength (photoperiod) during development. Light has both quantitative (amount of energy) and inductive (photoperiod or light quality) effects on plant growth and development (Patrick 1988). For example, total dry matter production is strongly affected by light radiation intercepted by the plant foliage so that light may act as a significant determinant of final yield. In addition, inductive stimuli, sensed as photoperiod can cause significant changes in dry matter partitioning within plants so that daylength can affect crop productivity significantly. Therefore, in investigation on daylength, it is necessary to ensure that the observed changes in dry-matter production are not simply the consequences of changes in the supply of radiant energy.

In the natural environment, the effect of daylength is confounded with the supply of radiant energy, and the spectral composition of light at the beginning and at the end of each day varies with latitude. Experimentally, however, although the establishment of environments which differ only in daylength is not easy, particularly for experiments which can last for several weeks, potential problems could be limited by several approaches. In recent studies, the required experimental treatments have been imposed in summer using matching daylit and dark phytotron chambers maintained at the same temperature and humidity (Hay and Heide 1983). All of the experimental plants can then be exposed to realistic irradiances for about 12 hours and photoperiod can be extended up to 24 h by very low irradiance illumination , usually within the range 1-2 W m^{-2} using incandescent lamps. Thus, the confounding of photoperiod and energy supply could be avoided by this approach.

This approach was applied in the present study. As described in Materials and Methods, daylength extension utilized two incandescent lamps and one long life tube lamp to produce low light intensity (about 4.8 $m\ sec^{-1}\ m^{-2}$) which was only around 1 % of high light intensity. The reducing daylength treatment was set at slightly higher light intensity in order to compensate for the low light intensity energy of the long photo period. In this way the total photosynthetically active radiation (PAR) was kept more or less constant for each pair of treatments.

The results showed that asparagus plants exposed to constant long daylength produced more fern at later harvests than those under reducing daylength. Fern dry weight of plants under constant long daylength was about 40 % higher than that of plants under reducing daylength. These results agree with other findings that exposure of plants to longer days, without increasing the supply of PAR, resulted in substantial increases in dry matter production up to 200 %, and even greater increases in leaf area. These effects, which were common to vegetative and reproductive plants, tended to be most marked at lower temperatures (Eagles 1971; Heide at al. 1985a; Heide at al. 1985b).

The present results also indicated that response of cultivars to daylength treatments were different. Fern growth of 'UC157' was suppressed more than that of 'Jersey Giant' and 'Italian Hybrid' had the least suppression of fern growth. These were indicated by the difference between fern dry weight under constant daylength and reducing daylength (Figure 2.5). This could be caused by different genetic backgrounds of the cultivars.

Similar results were found by Eagles (1971) who studied the effect of daylength and temperature on vegetative growth of two natural populations of cocksfoot (*Dactylis glomerata* L.) from contrasting climatic regions with different patterns of seasonal variation in daylength. One population was from Tjotta, Norway (latitude 63°N), with a range of daylength from 7 to 23 hours, and the other population was from Sintra, Portugal (latitude 39°N), with a narrower seasonal range of daylength from 11.5 to 16 hours. The seedlings were grown up to 42 days under two daylength treatments; all plants received and 8-h period of high intensity light (80 Wm^{-2} in the wavelength interval 400-700 nm) and half of the plants received 8-h light extension of low-intensity light from an incandescent source (2.3 Wm^{-2} in the wavelength interval 400-700 nm). They 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).

Besides increasing fern growth, the present results also showed that plants under constant long daylength were significantly taller, produced more longer lateral branches that those under reducing daylength. Besides, 'Italian Hybrid' was shorter than 'UC157' and 'Jersey Giant' under both constant and reducing daylength (Table 2.4). Increasing plant height, lateral branch number and length resulted in increasing fern growth of plants under constant long daylength.

The findings of Heide et al. (1985a) with *Poa pratensis* are consistent with the present results. In this vegetative plant, in which the shoot comprised leaf tissues only, with no true stem present, they found that both extended daylength and increasing temperature

had a strong effect on height growth. The rapid height growth in long days (LD) was mainly due to increases of leaf blade and sheath length. This is reflected in the large enhancement of leaf area at all temperatures (9, 15 and 21°C) at LD treatment. However, specific leaf weights were lower in longer days and higher temperatures, suggesting that the effects of LD on dry weight accumulation were not as large as those on leaf area. For instance, after three weeks total plant leaf area was increased by 125% due to daylength extension from 10 to 24 h, whereas dry weight increased by only 36%. This was associated with greatly reduced specific leaf weight in long photoperiods. The shoot-root ratio increased progressively with daylength up to 18 h and then levelled off. In addition, Heide et al. (1985b) found that the exposure of *Pleum pratense* L and *Bromus inermis* to extended daylength (long day) induced large and significant increases in dry weight, height, and leaf area compared with 8 h short days. 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.

The present results indicated that plants under a constant long day had higher RGR at most time except at time 4 (32 days after treatment), when reducing daylength treatment was 14 h (Figure 2.7). These results showed similarities to Heide et al. (1985a) who found that RGR was markedly higher in LD than in SD at all temperatures and harvests. The RGR was 31% higher in LD than in SD at 9°C and 15°C and 41% higher at 21°C, which also had highest absolute growth rate. The main change in all these parameters occurred between 14 and 18 h photoperiod.

The present results also showed that, except for around a 14 hour photoperiod, plants under constant long days had higher LWR and NAR than those under reducing daylength, so that plants under long constant daylength had higher RGR. These results are supported by the finding of Hay and Heide (1983) who found that plants of *Poa pratensis* cv. Holt, growing in daylight phytotron compartments, showed a short-term stimulation of net assimilation rate (NAR) when the plants were exposed to daylength extension (24 h). After a few days, the NAR decreased to approximately the same level as that of plants exposed in 8 h days at the same radiant energy supply, but, thereafter,

substantial increases in leaf area ratio maintained the higher RGR of the long-day plants compared to the corresponding 8 h plants.

However in contrast Eagles (1971) found that an increase of plant leafiness under long day was associated with reductions in NAR, compared with plants under short day. These findings are similar to the present results around a photoperiod of 14 hours when an abrupt change occurred resulting in a higher NAR than the constant long daylength treatment (Figure 2.7).

In the present study, daylength treatments also affected crown dry weight accumulation. In the first experiment all cultivars showed similar response, plants under reducing daylength had higher crown dry weight than those under constant daylength at harvest 3 when daylength was 14 h. The plants maintained higher crown dry weight until harvest 5 (12.5 h daylength). However, at later harvests plants under long constant daylength accumulated more dry weight in the crown and had higher relative growth rates over-all. This result was presumably due to the higher LWR and NAR of long day plants resulting in more total photosynthate production. Under these conditions, although the dry matter partitioning to the crown compared to the fern was relatively lower under constant daylength the absolute dry matter partitioned to the crown was higher than that under reducing daylength. Similar results were found in the second experiment for 'Jersey Giant' but 'UC157' showed less response to daylength for crown dry weight accumulation.

These results suggested that plants showed increased partitioning to the crown under reducing daylength starting at about 14 h. Analysis of total soluble carbohydrate also suggested that the changes occurred at 14 h daylength. At this daylength storage roots of 'UC157' and 'Jersey Giant' showed higher total soluble carbohydrate than under long constant daylength. Although the difference was not significant, this physiological change could produce large effects on plant growth if it represents increased flow of sucrose from the fern to the crown.

In addition, root:shoot dry weight ratio was affected by daylength treatments. The changes in the ratio were very different between experiment 1 (Figure 2.9) and experiment 2 (Figure 2.13) but in both experiments, plants under reducing daylength maintained higher root:shoot ratio than those under constant daylength. This suggested that relatively more dry matter was transported to the crown under reducing daylength than under constant daylength. This was confirmed by allometric relationship indicating that plants under reducing daylength showed lower value of slope than those under constant daylength. This suggested that relatively more dry matter was partitioned into the crown under reducing daylength.

These results are in agreement with other findings. For example, Sudjatmiko et al. (1997) found that asparagus seedlings harvested from October to January showed a constant allometric slope indicating a constant ratio between relative growth rates of shoot and crown. However, an abrupt change in relationship occurred in February harvest. The relative growth rate of the shoot fell relative to that of the crown indicating that more dry matter was partitioned to the crown. They suggested that this change was due to changes in day length as temperature changes at that time are relatively small (17.6°C in January and 16.8°C in February). In addition, Woolley at al. (1999) 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. Thus the results in this chapter strongly support the hypothesis that changes in partitioning found by Sudjatmiko et al. (1997) and Woolley at al. (1999) were due to changes in daylength. In addition, cultivar differences exist that could possibly be used to advantage. Furthermore, Eagles (1971) showed that the temperate grass, *Dactylis glomerata*, had a significantly higher root:shoot ratio under short days, indicating that significantly more dry matter was accumulated in the roots. All this evidence suggests an effect of daylength change on dry matter partitioning between shoots and roots.

CHAPTER THREE

REMOBILIZATION OF SOLUBLE CARBOHYDRATES (FRUCTANS) FROM ASPARAGUS ROOTS DURING SPEAR HARVEST

3.1. Introduction

Carbohydrate storage plays an important role in asparagus production. Seasonal changes in carbohydrate reserves have been reported (Shelton and Lacy 1980; Haynes 1987; Pressman et al. 1993; Nichols 1996). Storage carbohydrates decrease gradually during spear harvest in spring. After spear harvest, carbohydrate levels decline more rapidly to support fern growth until reaching the minimum levels. Once the fern has expanded, the carbohydrate accumulation starts so the carbohydrate reserves increase rapidly and reach maximum levels at the end of summer. In addition, further storage roots, buds, and bud clusters are produced while the photosynthetic process is very active. Thereafter, carbohydrate levels decrease gradually during winter and eventually the storage carbohydrates are re-mobilized to support spear growth in the following harvest season. Thus, spear yield of asparagus depends mainly on the ability of plants to store carbohydrates in the storage roots during fern growth and the ability of plants to re-mobilize these carbohydrate reserves to support spear growth during the harvest period.

However reports regarding the composition of soluble carbohydrates during spear growth conflict. Martin and Hartmann (1990) reported that most fructans in the storage roots were low molecular weight fructans. Shelton and Lacy (1980) found that soluble carbohydrate from asparagus roots were up to DP9, composed of 90 % fructose and 10 % glucose, while Presmann et al. (1993) showed that that the highest DP of fructans in 'UC157' was 11 while in 'Junon' was 10. In contrast, Shiomi (1993) found fructans with high a degree of polymerization (DP) ranging from 11 to 22 with the predominant size being DP13-16.

Woolley et al. (1999) found that the dry weight loss from the crown was relatively low during spear harvest. In contrast, ^{14}C recovered in the crown decreased rapidly during spear harvest. This result suggested that specific fructans, or fructans from specific roots, may be required in the spear growth. Thus, investigations on the distribution of fructans in storage roots and remobilization of the fructans to the growing buds during spear production could provide important information for explaining and manipulating the spear growth of asparagus in order to increase spear production. However, little attention has been given to the distribution and specific type of fructans that may be the major influencing factor in spear growth. Besides, there is no information about the role of different roots as the source of soluble carbohydrate for spear growth.

Therefore, the objective of this experiment is to determine the remobilization of soluble carbohydrates (fructans) from different roots during spear harvest

3.2. Materials and methods

3.2.1. Plant materials

Two year old 'UC 157' plants were used for this experiment. The plants were raised in growth chambers and then put in a cold room to become dormant. Crown size, number of bud clusters and number of storage roots were recorded from the dormant plants. The feeding roots were removed and all storage roots were tagged using green twist fasteners so that the old roots could be distinguished from later formed roots. On 16 October 1997, the plants were replanted into 20 liters plastic bags consisted of bark:peat:pumice mix (1:1:1), iron sulphate (50 g/100 l media), and Osmocote (16N-3.5P-10.8K) (400 g/100 l media). The plants were then grown in glasshouse 26 in Plant Growth Unit, Massey University, Palmerston North, New Zealand.

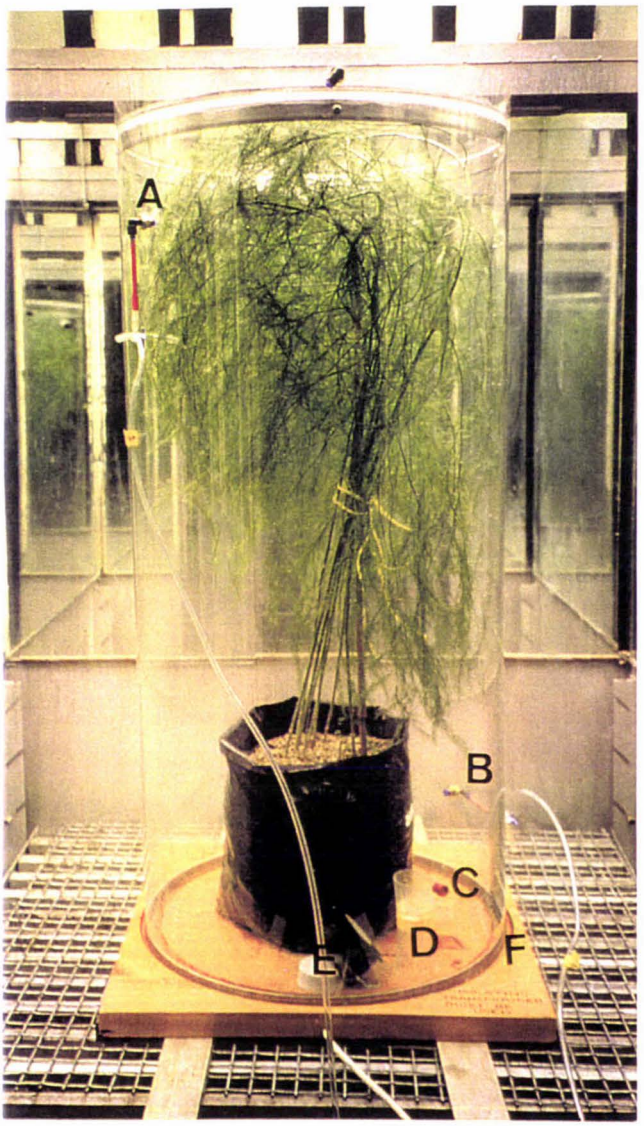
3.2.2. Radioactive labeling

Radioactive labeling using $^{14}CO_2$ was used to study soluble carbohydrates (fructans) distribution and remobilization. The $^{14}CO_2$ was applied to actively photosynthesizing plants. Nine plants were fed in March 1998 and 6 plants in June 1998.

The application of ^{14}C was done within Growth Cabinets which were set at 20°C in Level One, Block B, Institute of Natural Resources, Massey University. The plant to be labeled was put in an air tight perspex chamber containing a generating flask and a fan. The base of the chamber was polyurethaned wood with a water-filled circular groove to form a seal between the perspex and base. The chamber was connected to a Binos II Infra Red Gas Analyzer (IRGA) in a closed loop (Figure 3.1). The $^{14}CO_2$ was generated by injecting 0.5 ml 5 mCi of ^{14}C-sodium bicarbonate ($NaHCO_3$) through a rubber septum into a generating flask containing 30 ml of 80 % lactic acid ($CH_3.CHOH=COOH$). The fan was used to circulate air within the chamber and CO_2 uptake was monitored by IRGA.

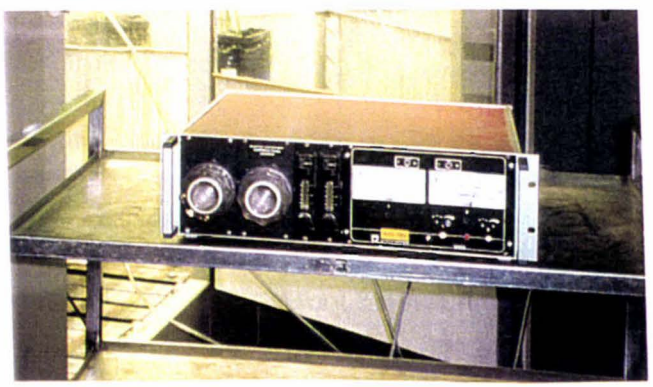
Initially, CO_2 concentration within the chamber was set at 400 parts per million (ppm). When the concentration reduced to about 100 ppm, additional CO_2 was added to maximize permanent fixation of $^{14}CO_2$. The process of fixation was stopped after two hours or when CO_2 concentration was less than 100 ppm.

After labeling, the plants were dried off in an open tunnel house over the winter period. Growth was re-started in 17 November 1998 by watering the plants.



Label :

A. Inlet
B. Outlet
C. Rubber septum
D. Lactic acid
E. Fan
F. Water filled groove in wooden base.



Binos II IRGA

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).

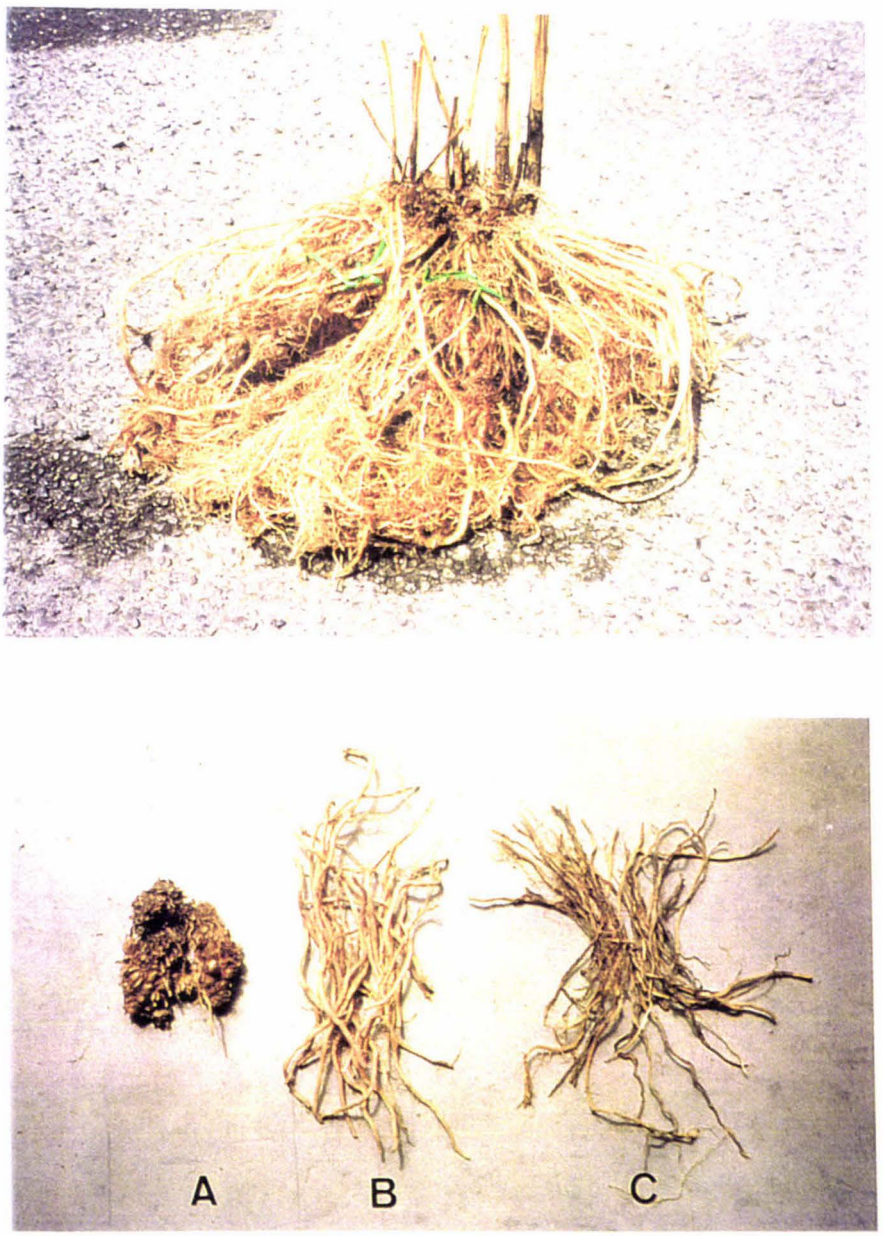


Figure 3.2. The washed crown at root harvest, the old roots were tagged using green twist ties (top). The crown was separated into rhizome, new roots (consisting of new roots and new roots associated with harvested spears), and old roots (consisting of old empty roots and old full roots) (below).

3.2.3. Experimental design

The labeled plants were harvested at different stages as follows :

NH : no harvest, the crown was destructively harvested before spear growth,

LH : light harvest, the spears were harvested for 2 weeks,

HH : heavy harvest, the spears were harvested for 4 weeks.

At harvest the crowns were divided (Figure 3.2) as follows :

N : new roots,

NS : new roots associated with harvested spears,

OE : old empty roots,

OF : old full roots.

Thus, a two factors factorial design with 5 replicates was used in this experiment. Because there were no spears harvested in the NH (no harvest) treatment, root categories for N (new roots) and NS (new roots associated with harvested spears) were the same for this treatment.

3.2.4. Total soluble carbohydrate analysis

The procedure for total soluble carbohydrate analysis was presented in Chapter Two.

3.2.5. Radioactive analysis

The supernatant from soluble carbohydrate analysis was used for radioactive analysis. The scintillation cocktail used was HiSafe (OptiPhase 'HiSafe'3; Wallac, an G&G Company) and the counting time was set at 300 seconds with DPM counting mode. Each sample was prepared by mixing 100 µl supernatant and 1000 µl HiSafe within an Eppendorf tube. Carbon-14 was assayed by scintillation counting (Wallac 1409/11 LSC Version 1.6).

3.2.6. Fructan analysis

Supernatant (from soluble carbohydrate analysis) was filtered through 0.45 micron Toyo Membrane Filters using a syringe and filter holder before analysis by HPLC (High Performance Liquid Chromatography).

Monosaccharides, disaccharides, and oligosaccharides were separated using a Waters 510 pump HPLC and a differential refractometer (Optilab 5922 RI Chromatography Module) as a detector. The detector was set at 50x attenuation. Supernatant of 40 µl was injected by auto sample injector (Waters Intelligent Sample Processor (WISP) Model 712) into the column (Rezex RSO Oligosaccharide, Phenomenex, Serial No. 217564). The mobile phase used was purified water at isocratic flow rate of 0.3 ml/minute. The water was purified by passing through a Water Purification System (SYBRON, Barnstead) set at 17.5 megohm-cm. The output signal was handled by Betaram Module (β-RAM Radio-HPLC Detector; IN/US System, Inc.) which produced retention time and peak area.

Using this HPLC system, fructans (fructo oligosaccharides) can be separated based on their degree of polymerization (DP). Because pure fructans were not available, glucose, fructose, sucrose, and malto oligosaccharides (DP2 to DP6) (Sigma Chemicals) were used as standards for the determination of fructan peaks. The chromatogram of these standards showed that glucose and fructose could be separated clearly while sucrose and maltose appeared as a single peak (similar retention time) (Figure 3.3). Besides, standards were also added to the sample as markers so that fructan peaks could be recognized. Based on these standards, fructans up to DP10 can be determined (Figure 3.4) and some samples showed a DP11 peak. Also the chromatogram showed a peak of long chain fructans. The hydrolysis of this peak resulted in fructose.

Glucose standard was used to quantify glucose; sucrose standard was used to quantify DP2; and fructose standard was used to quantify fructose and all fructans except DP2. In this chapter, the group of fructans from DP3 to DP10 is called 'short chain fructan' while

the group of fructans with DP higher than 10 is called 'long chain fructan' which was calculated from the difference between total soluble carbohydrate and the sum of fructans from DP1 to DP10.

3.2.7. Data analysis

Data obtained from this experiment were analyzed using general linear model procedure (proc glm) of SAS package version 6.12.

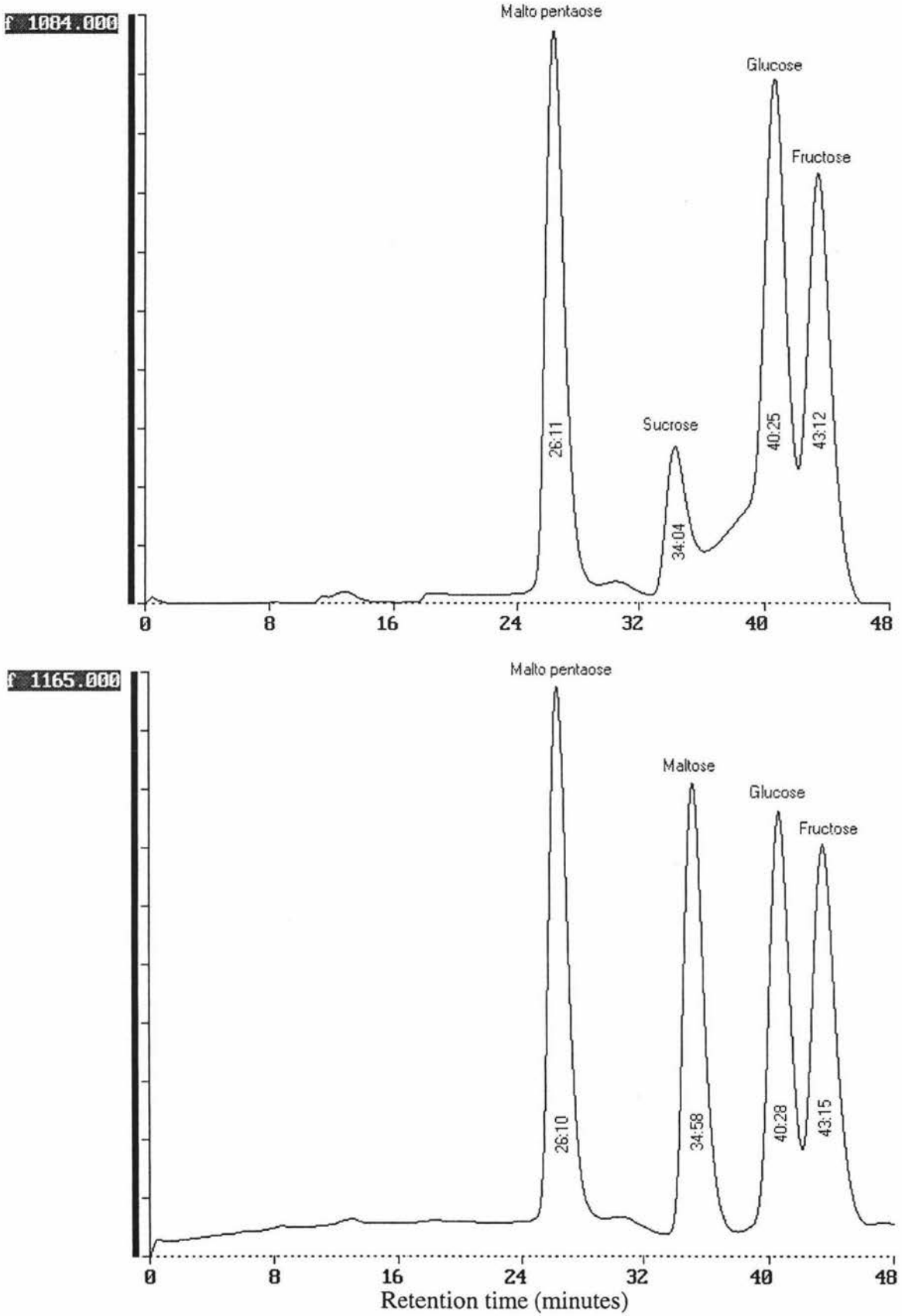


Figure 3.3. Chromatograms of standards.

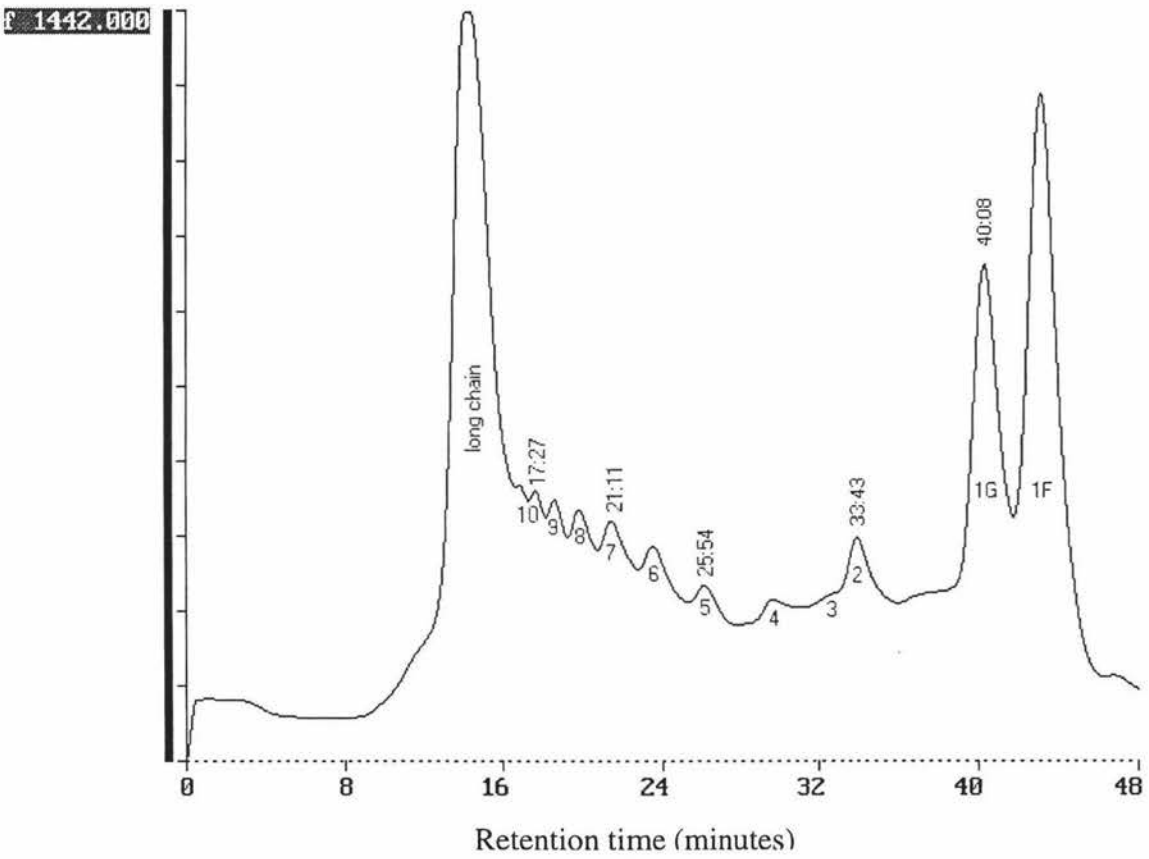


Figure 3.4. Chromatogram of extract from asparagus roots. Numbers indicate degree of polymerization (DP) of fructans and times indicate retention times of the peaks.

3.3. Results

3.3.1. Spear harvest

Spear dry weight and spear number at different harvest treatments are presented in Figure 3.5. At no harvest (NH), no spear was harvested and this provides a control for depletion studies. During light harvest (LH), about 11 spears were harvested per plant with average of 9.7 g dry weight per plant, while heavy harvest resulted in about 26 spears per plant with average of 21.2 g dry weight per plant.

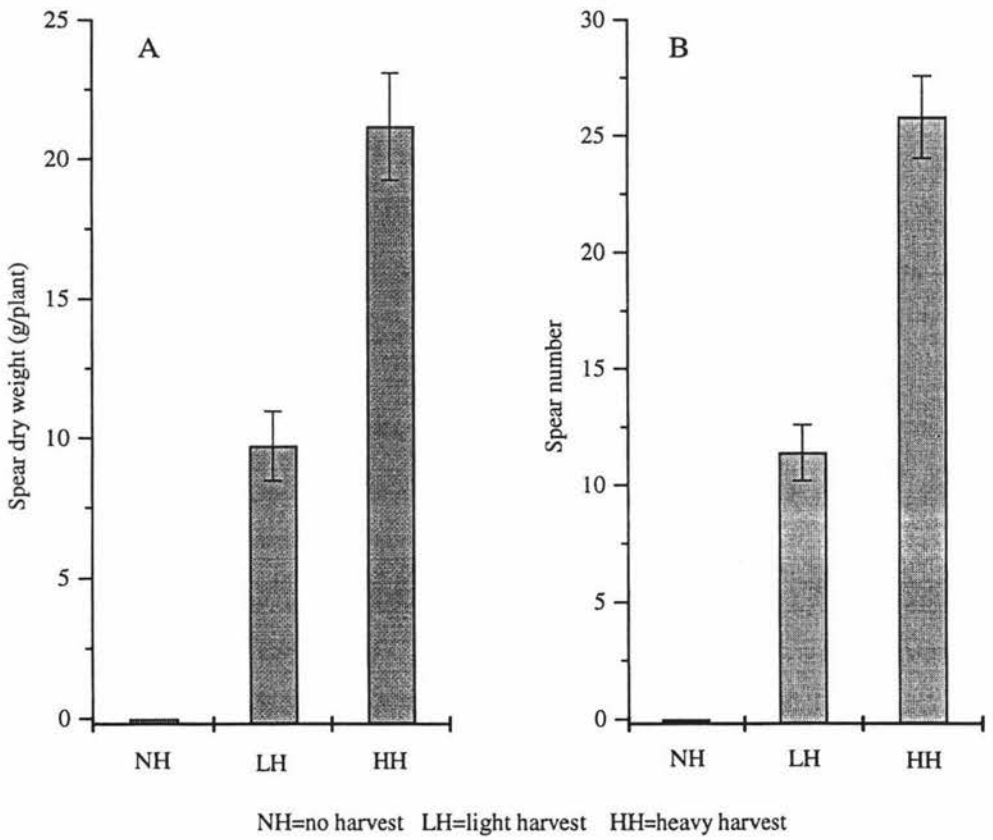


Figure 3.5. Spear dry weight (A) and spear number (B) at different harvest treatments. Vertical bars indicate standard errors.

3.3.2. Total soluble carbohydrate and radioactivity of different roots during spear harvest

Total soluble carbohydrate and radioactivity of all roots decreased as the spear harvest progressed; however, the intensity of decrease was different amongst the four root categories (Figure 3.6). Changes in new roots and new roots associated with harvested spears were similar for the light harvest. Total soluble carbohydrate decreased from 428.1 mg/g DW (dry weight) to 322.9 mg/g DW for new roots and to 357.2 mg/g DW for new roots associated with harvested spears during light harvest. Simultaneously, radioactivity of both root categories decreased sharply from 765 DPM to 367 DPM and 413 DPM respectively. For the heavy harvest, total soluble carbohydrate decreased further to 158.2 mg/g DW and 176.4 mg/g DW for new roots and new roots associated with harvested spears respectively. However, the decreases in radioactivity slowed down especially from new roots associated with harvested spears where the radioactivity decreased from 416 DPM at light harvest to 311 DPM at heavy harvest (decrease 105 DPM) while the radioactivity of new roots decreased from 367 DPM at light harvest to 201 DPM at heavy harvest (decrease 166 DPM). Thus, during heavy harvest radioactivity of new roots decreased about 158 % compared with that of new roots associated with harvested spears.

The level of total soluble carbohydrate of old 'empty' roots was only 184.7 mg/g DW before spear growth (at no harvest) and then decreased slightly to 92.1 mg/g DW and 72.7 mg/g DW at light and heavy harvest respectively. Similarly, radioactivity of old empty roots decreased slightly from 177 DPM (at no harvest) to 101 DPM (decrease 76 DPM) and 53 DPM (decrease 48 DPM) at light and heavy harvest, respectively.

Old full roots showed parallel decreases of total soluble carbohydrate and radioactivity during spear harvest. Total soluble carbohydrate decreased from 353.6 mg/g DW to 285.9 mg/g DW at light harvest and to 143.8 mg/g DW at heavy harvest while radioactivity decreased from 295 DPM (at no harvest) to 205 DPM (decrease 90 DPM) at light harvest and to 101 DPM (decrease 104 DPM) at heavy harvest.

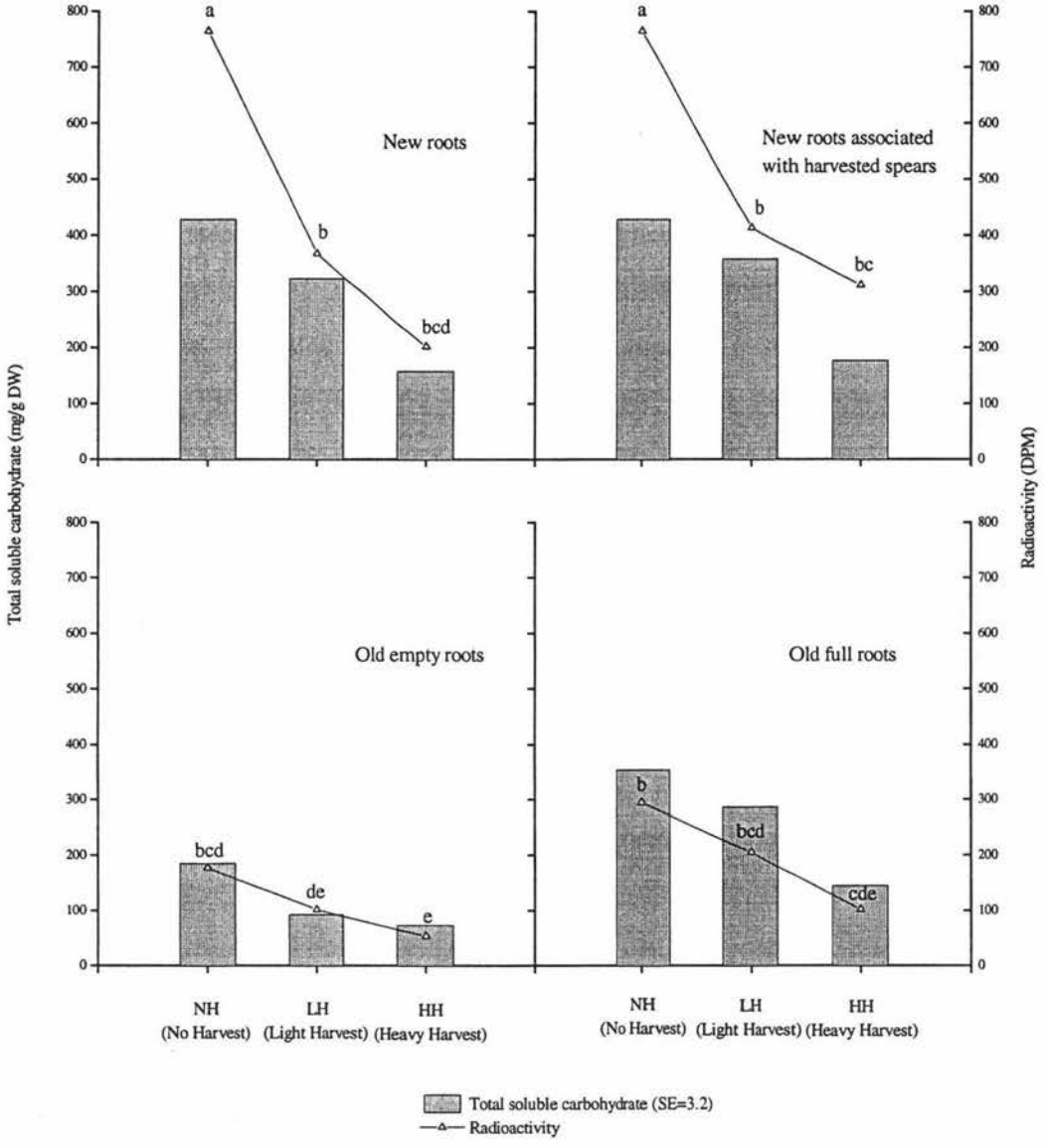


Figure 3.6. Total soluble carbohydrate content and radioactivity of different roots during spear harvest. Radioactivity means followed by the same letter are not significantly different at p=0.05, data log transformed.

3.3.3. Fructose, glucose, and sucrose contents of different roots during spear harvest

In all root categories, the concentration of sucrose was highest followed by fructose and then glucose (Figure 3.7).

Sucrose contents of new roots, new roots associated with spears, and old full roots showed similar trends, sucrose concentrations increased during light harvest and then decreased at heavy harvest. For instance, the level of sucrose of new roots increased sharply from about 42 mg/g DW up to about 61 mg/g DW at light harvest and then decreased sharply to about 38 mg/g DW. New roots associated with harvested spears showed similar result but the decrease of sucrose level at heavy harvest was not as sharp as that of new roots. In addition, old full roots showed a small increase at light harvest to about 48 mg/g DW from about 42 mg/g DW at no harvest. In contrast, the concentration of sucrose of old empty roots decreased slightly from about 29 mg/g DW to about 25 mg/g DW and then leveled off at heavy harvest.

Fructose content of new roots decreased gradually during spear harvest from about 30 mg/g DW to about 26 and 18 mg/g DW at light harvest and heavy harvest respectively. New roots associated with spears showed a slight increase in fructose level during light harvest, and then the level decreased at heavy harvest. The concentration of fructose in old full roots remained the same at about 34 mg/g DW during light harvest and then decreased up to about 22 mg/g DW at heavy harvest, while fructose concentration of old empty roots increased slightly at heavy harvest after a slight decrease occurred at light harvest.

In contrast to sucrose and fructose contents, the concentrations of glucose from all root categories decreased linearly during spear harvest. At the end of the heavy harvest the concentrations of glucose from all roots were about 5 mg/g DW.

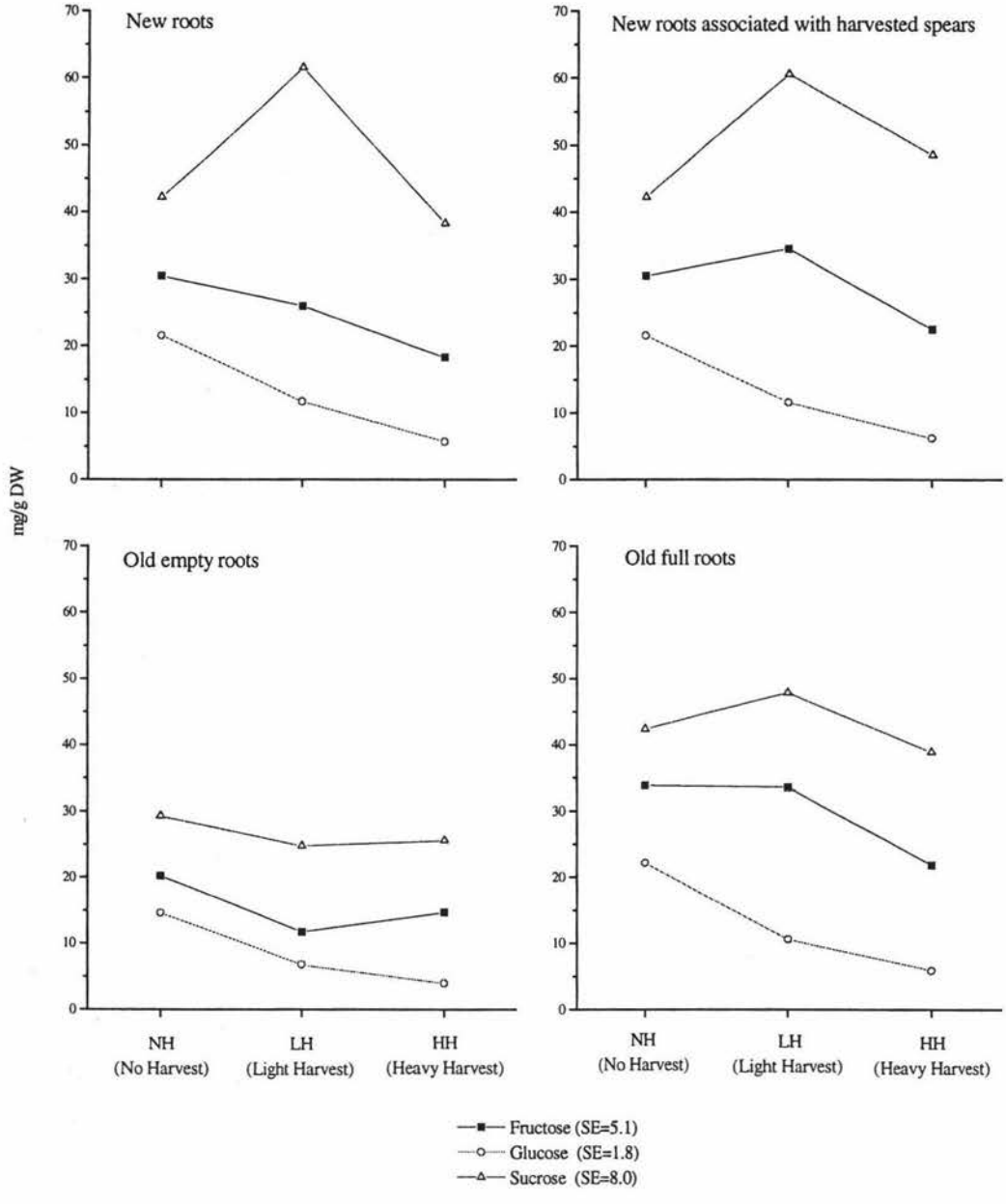


Figure 3.7. Fructose, glucose, and sucrose contents of different roots during spear harvest.

3.3.4. Changes in fructan composition of different roots during spear harvest

The changes in levels of fructans ranging from DP3 to DP10 from different roots during spear harvest are presented in Figure 3.8 and 3.9. Before spear growth (at no harvest), the concentrations of most fructans were around 9 to 12 mg/g DW in new roots and slightly lower in old roots, except fructan DP3 and DP10. The level of DP3 fructan before spear growth was only around 1 to 3 mg/g DW and around 4 to 6 mg/g DW for DP10 fructan.

Harvesting spears during light harvest depleted most fructan levels in roots except fructans DP9 and DP10 which increased in new roots and new roots associated with harvested spears. Increasing harvest pressure (heavy harvest) depleted fructans levels further except that DP3, which was barely discernible at light harvest, increased in new roots associated with harvested spears, old full roots, and old empty roots. The level of fructan DP4 during heavy harvest also increased in old empty roots and old full roots. From the graphs, it can be noticed that the level of fructan DP4 during heavy harvest was higher than the other fructans.

The depletion of short chain fructans (sum of fructans DP3 to DP10) compared with long chain fructans (sum of fructans with DP more than 10) during spear harvest can be seen in Figure 3.10. In all root categories, the depletion of short chain fructans was very slow. For example, before spear growth the concentration of short chain fructans in new roots was about 68 mg/g DW, then the concentration decreased to about 60 mg/g DW and 30 mg/g DW during light harvest and heavy harvest respectively. In contrast, the depletion of long chain fructans was very sharp during spear harvest. The long chain fructans from new roots, for example, decreased linearly from about 265 mg/g DW (at no harvest) to about 163 mg/g DW during light harvest and then decreased further to about 65 mg/g DW during heavy harvest. Similar fructan depletions occurred in other root categories.

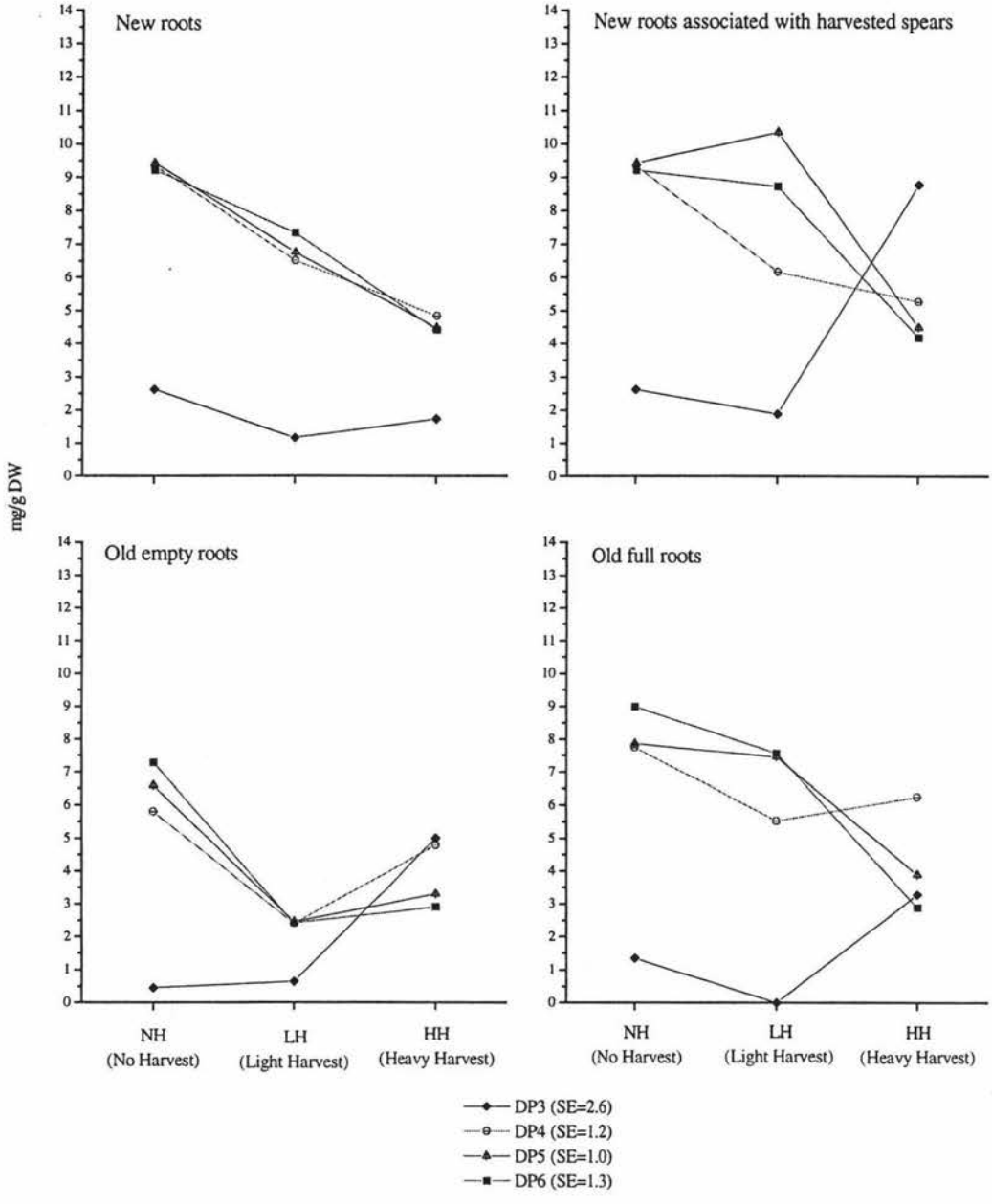


Figure 3.8. Changes in fructan composition (DP3 to DP6) of different roots during spear harvest.

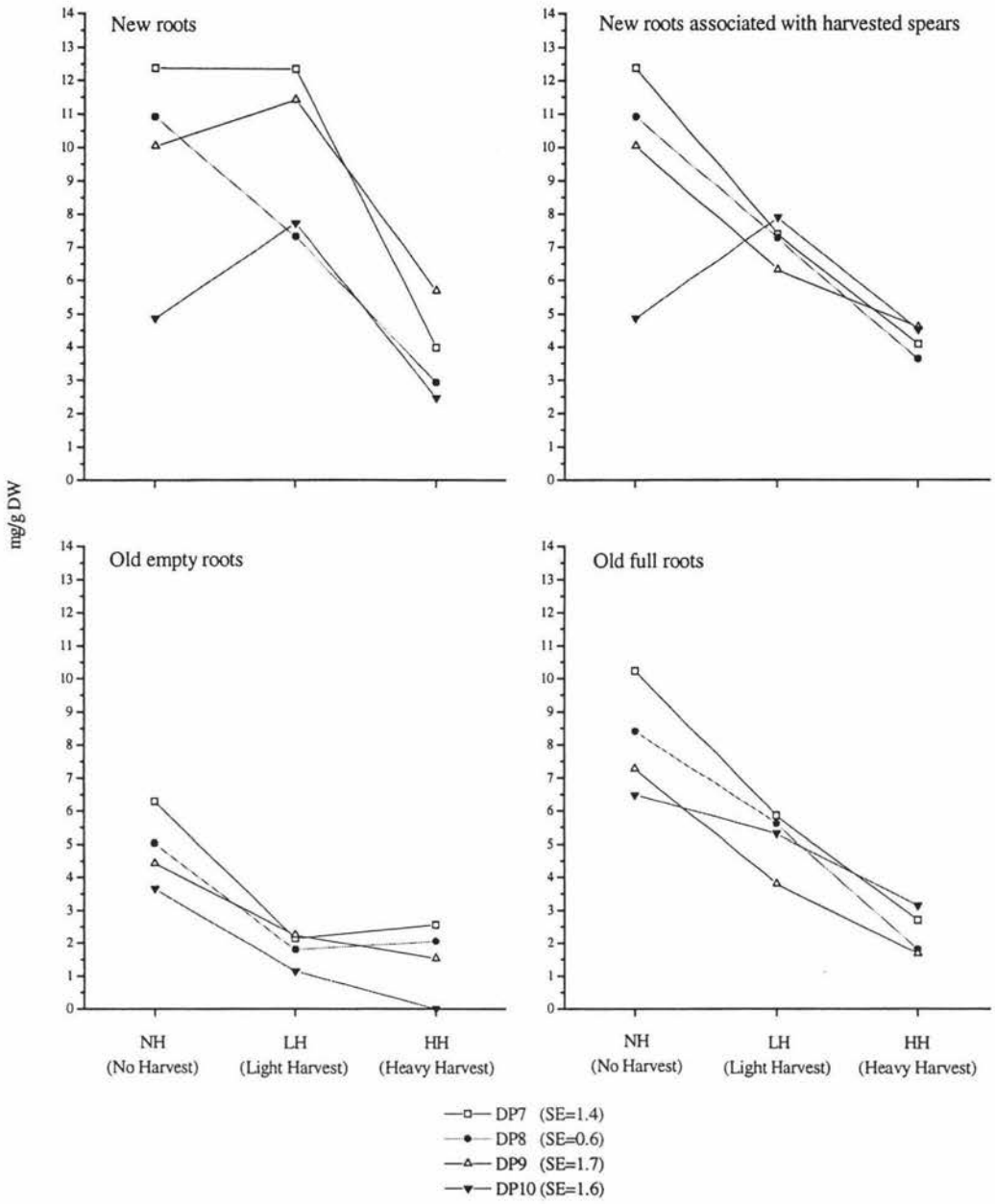


Figure 3.9. Changes in fructan composition (DP7 to DP10) of different roots during spear harvest.

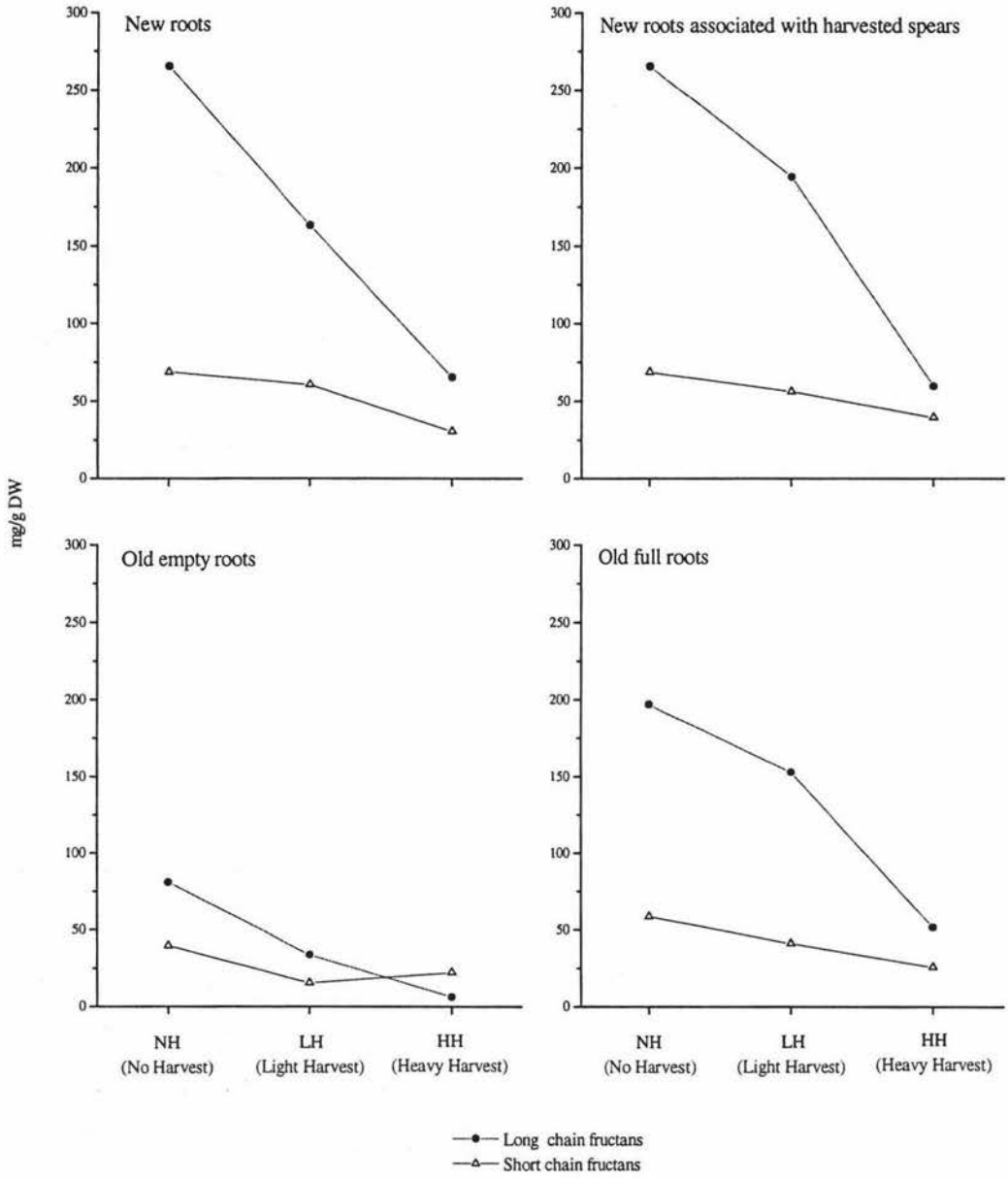


Figure 3.10. Changes in short chain (sum of DP3 to DP10) and long chain (sum of DP more than 10) fructans of different roots during spear harvest.

3.4. Discussion

3.4.1. Changes in total soluble carbohydrates and radioactivity

The carbohydrate accumulation stage, which starts when the fern is fully developed, is the most important stage in asparagus production as the storage carbohydrate will be used to support spear and fern growth during the harvest season. In this stage, the fern acts as strong source and starts to produce carbohydrate through photosynthetic activity while roots act as a strong sink. Unless the plants store adequate carbohydrate in the storage roots, spear production will be very poor in the following season (Nichols 1996). The concentration of total soluble carbohydrate in storage roots after ^{14}C feeding to actively photosynthesizing fern indicated that photosynthates were mainly accumulated in new roots (about 420 mg soluble carbohydrate/g dry weight) although some carbohydrates were stored in old roots (Figure 3.6 at no harvest treatment). Besides, radioactivity (^{14}C) recovered from the new roots was more than twice that in old full roots and about four times that in old empty roots, indicating that more carbohydrates were accumulated in new roots than in old roots.

During spear harvest, the concentration of carbohydrates of all root categories decreased as the harvest progressed. The radioactivity which, because of the time of 14C-labelling was also the most recently formed, also declined rapidly during spear harvest. Woolley et al. (1999) found that the decline in radioactivity in the crown was more rapid than that in dry weight during spear harvest and suggested that spear growth utilized a specific pool of highly labeled carbohydrate. In this experiment, a rapid decrease in radioactivity only occurred in new roots (both new roots and new roots associated with harvested spears) during light harvest but the decrease slowed down during heavy harvest. Radioactivity in old roots decreased linearly and parallel with decreasing carbohydrate concentration. These results suggested the priority for supply of carbohydrates for spear growth. Spear growth may use the most recently formed carbohydrates stored in new roots before using the other source of carbohydrate within the same or different roots.

Roots associated with younger parts of the rhizome where the buds grow would be expected to be the major carbohydrate source for initial spear growth (Woolley et al. 1999). In the present study, however, similar decreases of soluble carbohydrate and radioactivity between new roots and new roots associated with harvested spears indicated that spear growth initially used carbohydrate not from the nearest roots, but 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 (Figure 3.6).

3.4.2. Fructose, glucose and sucrose contents of roots during spear harvest

The concentration of sucrose was higher than that of fructose and glucose concentration was the lowest in all root categories, both before spear growth and during harvest. Before spear growth, the concentrations of sucrose of new roots, new roots associated with harvested spears and old full roots were around 40 mg/g DW (dry weight) and around 30 mg/g DW in old empty roots (Figure 3.7). Sucrose concentration increased during light harvest in all roots except old empty roots and then decreased during heavy harvest. The concentration increased about 50 % in new roots and about 25 % in old full roots and then decreased to about initial levels during heavy harvest. In old empty roots, sucrose decreased slightly during light harvest and leveled off during heavy harvest.

Some of the these results were similar to those of Dean and Skrzeczkowska (1996) and Pressman et al. (1993). Dean and Skrzeczkowska (1996) showed that sucrose levels of storage roots of "Jersey Giant' increased about 100 % from 25 mg/g DW at the first week harvest and then decreased and leveled off at about 40 mg/g DW in the following harvest. Pressman et al. (1993) using 10 years old asparagus plants found higher concentration of sucrose (80 to 100 mg/g DW) in the storage roots prior to harvest. They found different response of 'Junon' and 'UC157' to harvest. Sucrose levels of 'Junon' were similar to the results in this experiment, sucrose increased from 80 mg/g DW to about 90 mg/g DW and then decreased to the initial level. However, sucrose levels of 'UC157' was about 100 mg/g DW before harvest and the concentration decreased linearly up to about 50 mg/g DW during harvest period. Although the level of sucrose

may vary with cultivars and plant ages, the presence of a high concentration of sucrose in storage roots before and during spear harvest suggests an important role of sucrose in buds or spears growth in asparagus. Sucrose is the main form of translocated carbohydrate and Pressman et al. (1993) also suggested a physiological role of sucrose as a signal for bud break in asparagus.

The levels of fructose in new roots decreased gradually during harvest but in new roots associated with harvested spears, it increased slightly during light harvest and then decreased at a similar rate to new roots. In old empty roots fructose level remained the same during light harvest but decreased during heavy harvest while old empty roots showed a decrease during light harvest but the level of fructose increased slightly during heavy harvest (Figure 3.7). These results were different from those of Dean and Skrzeczkowska (1996) who found that fructose concentration in storage roots was less than 10 mg/g DW before spear growth, increased up to about 800 % during the first week of spear harvest and then decreased slightly in the following harvest. This difference in fructose level in storage roots may relate to the rate of fructan hydrolysis which results in fructose and the rate of conversion of fructose to sucrose for spear growth. Pressman et al (1989) showed that asparagus spears contained sucrose, fructose, and glucose while storage roots accumulated fructans and small amounts of sucrose, glucose, and fructose. Thus, spear growth utilizes the sugars hydrolyzed from fructans in storage roots. If the rate of fructan hydrolysis is higher than the rate of fructose conversion, the levels of fructose in storage roots will be high and vice versa.

Glucose concentration in new roots and old full roots was around 20 mg/g DW and slightly lower in old empty roots. In all roots, it decreased during light harvest and continued to decrease in the heavy harvest until the concentration was around 5 mg/g DW. These results were similar to those of Dean and Skrzeczkowska (1996); however, glucose levels in this experiment were slightly higher. Presumably glucose levels reflect the relative rates of conversion of fructose to glucose and glucose to sucrose, particularly if our HPLC column does not separate sugar phosphates from free sugar.

3.4.3. Changes in fructan concentrations in roots during spear harvest

In this study, fructan compositions in storage roots were dominated by long chain fructans (DP>10). Before spear growth (no harvest), the concentration of long chain fructans in new roots was around 270 mg/g DW compared with about 70 mg/g DW for short chain fructans (DP3-10). The concentrations were slightly lower in old full roots and very low in old empty roots (Figure 3.10). The compositions of fructans in the storage roots of asparagus are at variance with some previous reports. Martin and Hartmann (1990) showed that most fructans in the storage roots were low molecular weight fructans; however, they did not separated the fructans into different DPs but into low molecular weight and high molecular weight. Cairns (1992) separated fructo-polysaccharides from asparagus roots on an HPX-42C calcium-form ion exchange column and found that the majority of soluble carbohydrates was fructans with DP greater than 5 and only a small amount of oligosaccharides of DP3 and DP4. The study by Cairns, fructans were only separated until DP5 and all fructans with DP greater than 5 appeared in one a big peak so that the information about long chain fructans was not clear.

Shelton and Lacy (1980) reported that the highest fructo-oligosaccharides from asparagus roots were composed of 90 % fructose and 10 % glucose and had molecular weight less than 4000 (equivalent to DP9) as estimated by Gel Permeation Chromatography (Bio-Gel P-4 and P-6 400 mesh exclusion beads). However, using Anion Exchange chromatography (AEC), Shiomi (1993) found fructans with a high degree of polymerization in asparagus roots ranging from DP12 to 22 with the predominant size being DP13-16. Thus, the results from this experiment agree with those of Shiomi (1993) that fructan compositions in storage roots were mostly long chain fructans. Woolley et al. (1999) also found similar results.

Long chain fructans declined sharply during spear harvest. In new roots long chain fructans decreased from about 270 mg/g DW to about 150 and 70 mg/g DW during light and heavy harvest, respectively. Other root groups indicated similar decreases. In

contrast, short chain fructans decreased slightly during spear harvest (Figure 3.10). These results are different from those of Woolley et al. (1999) showing that long chain fructans decreased at a slower rate than short chain fructans. However, they included sucrose (DP2) in the short chain fructans so that the decrease in short chain fructans was higher than that of long chain fructans. In fact, the decrease in short chain fructans was mainly from the decrease of sucrose concentration while the decrease from short chain fructans (DP3-7) was quite low.

Some individual short chain fructans such as DP4, DP5, DP6 and DP7 were hydrolyzed in similar ways, the concentration decreased during light and heavy harvest. In contrast DP4 in old roots increased during heavy harvest. The concentration of DP4 at heavy harvest was relatively higher than the others. Fructans with DP9 and DP10 showed an increase during light harvest and then decreased during heavy harvest while the concentration of DP3 was very low during light harvest but increased markedly during heavy harvest (Figure 3.8 and 3.9) suggesting that the rate of conversion of DP3 to sucrose may be a rate limiting step in fructan hydrolysis during heavy harvest. Sucrose uptake for spear growth was very high during heavy harvest, indicated by a decrease in sucrose concentration. These results show some differences from those of Dean and Skrzeczkowska (1996) who found that all fructans were hydrolyzed at the similar rate during spear harvest except DP4. In the present work DP4 decreased in new roots but increased in old roots for the heavy harvest treatments.

The sharp decline in long chain fructans suggests an important role for them during spear harvest. These long chain fructans were hydrolyzed to produce simple sugars such as fructose. The fructose released is phosphorylated by hexokinase and is then converted by sucrose phosphatase and sucrose phosphate synthase to sucrose and transported to buds to support spear growth (Smith 1993). The hydrolysis of highly polymerized fructans in storage roots is catalyzed by fructan exohydrolase (FEH) by removing fructose molecules so that eventually only sucrose is left which can be hydrolyzed by invertase (Nelson and Spollen 1987). It is of interest to determine if the rate of hydrolysis is limited by any particular step. From results described here and those of Dean and Skrzeczkowska (1996), hydrolysis rates of DP5, DP4 and DP3 seem the most likely candidates.

CHAPTER FOUR

EFFECT OF CUTTING HEIGHT ON SPEAR YIELD AND QUALITY OF ASPARAGUS, AND STIMULATION OF ADDITIONAL SPEARS USING HORMONE TREATMENT

4.1. Introduction

Storage carbohydrate accumulated after fern establishment is a very important stage in asparagus production. During this stage, when photosynthesis is very active, further storage roots, buds, and bud clusters are produced (Nichols 1996). Then, the ability of plants to remobilize the carbohydrate reserves to available buds, and possibly number of buds capable of growth, determines spear yield.

Previous results from a cultivar trial in New Zealand indicated that spear yield varied between cultivars on the same site. For instance, 'Jersey Giant' produced about 3 times total yield and about 7 times marketable yield than 'Brunetto' (Nichols 1992). Bussell et al. (1996) suggested that relative differences between cultivars varied with cutting height. Asparagus spears are usually harvested at 18 cm cutting height for processing (normally canning), while 23 cm spear length is preferable for fresh export. However, different cultivars showed huge differences in spear quality (tip tightness) 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 are still questionable and should be considered in asparagus cultivar trials.

The experiments described in this chapter investigate the relationship between cutting height and total and marketable yield. The experiments also analyze the relative importance of bud number and carbohydrate resources on productivity by determining

changes in soluble carbohydrate, the number of buds remaining when spear production had almost ceased, and the ability of a hormone treatment to stimulate further spear production (bud break).

4.2. Materials and methods

4.2.1. Introduction

Two experiments are presented in this chapter. The first experiment was run from 5 March to 3 May 1999 to determine the effects of cutting height on spear yield and quality. The second experiment was run from 8 May to 2 June 1999 to analyze the effects of hormone solution on additional spear production using the same plants.

4.2.2. Experiment 1

4.2.2.1. Plant material

Four cultivars of asparagus ('Grande', 'Apollo', 'Atlas', and 'UC157') were used. The plants were grown in 20 liter containers for 9 months in a glass house vented at 25°C and heated at 18°C and then made dormant by stopping water on 1 February 1998 until fern dry down. During growth the plants were infected by stemphyllium so that considerable fern replacement was necessary. The dry fern was removed on 25 to 28 February 1998 so that the plans were ready for treatment. Initial levels of total soluble carbohydrate in the storage roots were around 30 % based on root dry weight.

4.2.2.2. Experimental design

The experiment design used was randomized block with two factors and three blocks according to plant size.

The first factor was cultivar which consisted of four cultivars : 'Grande', 'Apollo', 'Atlas', and 'UC157'. The second factor was height at which spears were cut: 5 cm, 15 cm, 20 cm, 30 cm, and 50 cm. Thus, there were 20 treatment combinations and each combination consisted of four individual plants per plot.

The experiment started on 5 March 1998 by rewatering the plants using overhead irrigation in a greenhouse at Plant Growth Unit, Massey University. The greenhouse temperature was semi-controlled using heating and ventilating systems with heating set point at 18°C and ventilating set point at 25°C. The irrigation was set for 30 minutes duration at 5 pm every day during the experiment.

4.2.2.3. Data collection

The asparagus spears were harvested every day for 8 weeks at the different cutting heights. All spears were weighed, and graded for head tightness into 3 classes : Tight (Quality 1), Medium (Quality 2), and Seedy (Quality 3) (See Figure 4.1. for detail). Spear diameter was also recorded at spear base using digital calipers. Spear dry weight was recorded weekly and total spear number was counted at the end of harvest. Total soluble carbohydrate of storage roots was determined by the Anthrone method as described in Chapter Two.

4.2.2.4. Data analysis

Cumulative data (all harvests) of fresh and dry weight of spears, spear quality and spear number were analyzed by analysis of variance (Anova) using general linear model procedure (proc glm) of SAS Package version 6.12 Differences among means were determined using Duncan's Multiple Range Test.

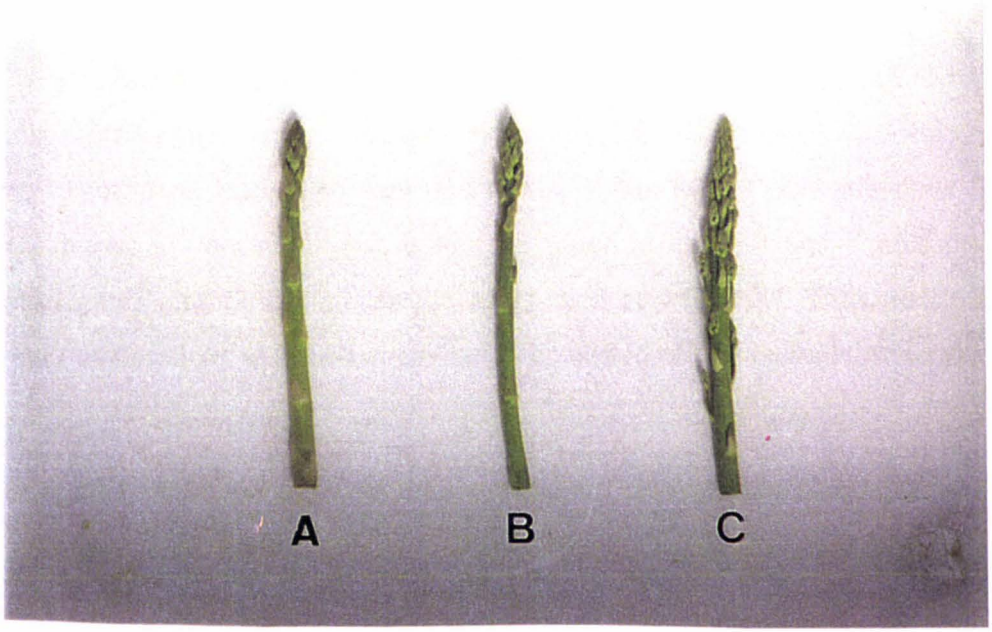


Figure 4.1. Three classes grading system for spear head tightness : (A) Tight (Quality 1), (B) Medium (Quality 2), and (C) Seedy (Quality 3).

4.2.3. Experiment 2

4.2.3.1. Plant material

Plant materials from the above experiment 1 (at the end of the experiment) were used in this experiment. When the spear production had nearly ceased, hormone solution was applied to the crown surface as the treatment and tap water was applied as a control.

4.2.3.2. Experimental design

Experimental design applied in this experiment was a split plot design with 3 replicates. The main plots were asparagus cultivars and cutting heights arranged randomly as in the previous experiment. Each four plant plot was split into two, 2 plant sub-plots. The sub plot was hormone concentrations: 0 M (tap water as control) and a mixture of BA (Benzyladenine) plus GA3 (Gibberellic acid) each at $4x10^{-4}$ M. Thus, there were 40 treatment combinations and each combination consisted of two individual plants per sub-plot.

The hormone mixture was made by diluting 90.01 mg BA (molecule weight=225.3) and 145.6 mg GA3 (molecule weight=364) into a small amount of ethanol and then water was added up to one liter to produce a hormone concentration of $4x10^{-4}$ M. Two drops Tween 20 per liter were added to the mixture as a wetting agent. The hormone mixture was sprayed onto crown surface on 8 May 1999 and repeated after two days.

4.2.3.3. Data collection

After treatment, the number of additional spears produced (compared with Experiment 1) was observed until 2 June 1999. Spears were cut at the same height (20 cm). At the end of harvest bud number remaining was counted and the storage roots were freeze dried and analyzed for total soluble carbohydrate using Anthrone method as described in the Chapter Two.

4.2.3.4. Data analysis

Data obtained from this experiment were analyzed by analysis of variance (Anova) using general linear model procedure (proc glm) of SAS Package version 6.12. Differences among means were determined using Duncan's Multiple Range Test.

4.3. Results

4.3.1. Experiment 1

4.3.1.1. Spear yield

4.3.1.1.1. Fresh weight of spears

Statistical analysis indicated that total fresh weight of spears was not affected by cultivar but highly significantly affected by cutting height ($p<0.001$). There was no interaction effect between cultivar and cutting height (Table 4.1).

Table 4.1. Total fresh weight (gram/plant) of spears of four asparagus cultivars harvested at different cutting heights.

Cutting height (CH)	Cultivar (CV)				Cutting height mean
	'Grande'	**'Apollo'**	**'Atlas'**	**'UC157'**	
5 cm	16.3	13.2	14.1	9.7	13.4 c
15 cm	32.1	34.3	27.1	23.8	29.3 b
20 cm	23.9	30.8	34.6	47.6	34.2 b
30 cm	40.4	44.6	46.0	28.5	39.9 b
50 cm	50.3	50.3	44.1	65.5	52.6 a
Cultivar mean	32.6 a	34.7 a	33.2 a	35.0 a	
CV	ns				
CH	***				
CV x CH	ns				

- Within the column and row, means followed by the same letter are not significantly different at 5 % level by Duncan's Multiple Range Test.
- ns, *, **, *** : non significant or significant at p=0.05, 0.01 or 0.001 respectively.

Total fresh weight of spears increased significantly with increasing cutting height (Table 4.1). At 5 cm cutting height the average fresh weight of spears was 13 g/plant. Increasing cutting height to 15, 20, and 30 cm increased fresh weight of spears to 29, 34, and 39 g/plant respectively. These increases are significantly different from that of 5 cm cutting height. At 50 cm cutting height the fresh weight of spears increased markedly up to about 52 g/plant which is significantly different from the other cutting height.

The spears grew about 6 days after watering and this was the starting point for harvest 1 and harvest continued until week 8 (Figure 4.2). Spear harvest started in the first week for most cutting heights except 50 cm where the spears did not reach 50 cm in the first week. However, in the following weeks, spear harvest of 50 cm cutting height exceeded the other cutting heights. In general spear yield increased with increasing cutting height; however, for 'Atlas', spear yield of 50 cm cutting height resulted in a somewhat lower yield than that of 30 cm cutting height. 'UC157' was the lowest yielding cultivar at 5 cm cutting height but spear yield was the highest at 50 cm cutting height compared with the other cultivars.

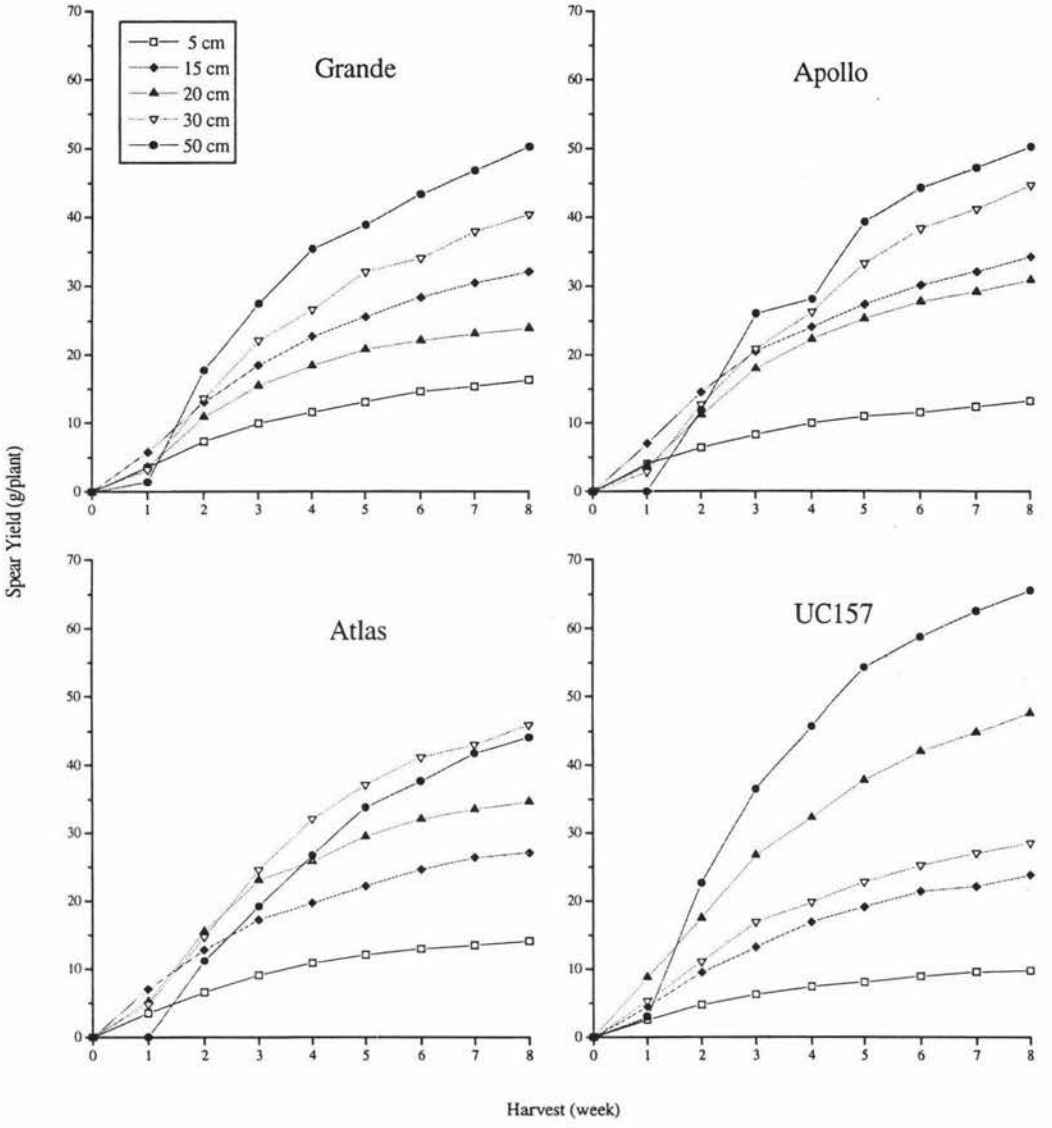


Figure 4.2. Cumulative spear fresh yield of four asparagus cultivars harvested at different cutting heights during 8 weeks harvest period.

4.3.1.1.2. Dry weight of spears

Similar to the results of fresh weight, total dry weight of spears was not affected by cultivar but highly significantly affected by cutting height ($p<0.001$). Besides that, there was no interaction effect between cultivar and cutting height on dry weight of spears (Table 4.2).

Table 4.2. Total dry weight (gram/plant) of spears of four asparagus cultivars harvested at different cutting heights.

Cutting height (CH)	Cultivar (CV)				Cutting height mean
	'Grande'	**'Apollo'**	**'Atlas'**	**'UC157'**	
5 cm	1.4	1.1	1.2	0.8	1.1 d
15 cm	2.7	2.9	2.4	1.9	2.5 c
20 cm	2.1	2.7	3.1	4.0	3.0 bc
30 cm	3.7	4.1	4.3	2.9	3.8 b
50 cm	3.6	6.0	5.0	7.3	6.0 a
Cultivar mean	3.1 a	3.4 a	3.2 a	3.4 a	
CV	ns				
CH	***				
CV x CH	ns				

- Within the column and row, means followed by the same letter are not significantly different at 5 % level by Duncan's Multiple Range Test.
- ns, *, **, *** : non significant or significant at p=0.05, 0.01 or 0.001 respectively.

Increasing cutting height increased total dry weight of spears significantly Table 4.2). The average dry weight of spears was around 1.1 g/plant at 5 cm cutting height. At 15 cm cutting height the dry weight increased significantly to about 2.5 g/plant while at 20 and

30 cm spear dry weight increased to about 3.0 and 3.8 g/plant respectively. Increasing cutting height to 50 cm increased the dry weight of spears further to 6.0 g/plant.

Similar to the results for fresh weight, there was no significant difference among cultivars in term of spear dry weight (3.0 to 3.5 g/plant).

4.3.1.2. Spear quality

In this work, spear quality is divided into 3 groups : Quality 1, Quality 2, and Quality 3 (see Material and Method for details).

Analysis of variance showed that the yield of quality 1 spear was significantly affected by cutting height ($p<0.001$) and there was a significant interaction effect between cultivar and cutting height ($p<0.05$) (Table 4.3). In addition, the yield of quality 2 and 3 spears were also significantly affected by cutting height ($p<0.001$) but not affected by cultivar and there was no interaction (Table 4.4 and Table 4.5).

All spears were regarded as quality 1 at 5 cm cutting height. Increasing cutting height to 15 cm increased the yield of quality 1 spear although a small amount of spear was quality 2. At 20 cm cutting height, the yield of quality 1 spears decreased for 'Grande', 'Apollo', and 'Atlas' but quality 1 spears still increased for 'UC157'. At this cutting height, quality 2 increased markedly for all cultivars. As a result, the proportion of quality 1 and quality 2 was about the same at 20 cm cutting height except 'UC157' where quality 1 spears were much higher than quality 2 spears. Quality 3 spears started to appear at this point. At 30 cm cutting height, spear yield was dominated by quality 2 spears while quality 3 spears also increased but only a small amount of spear yield was quality 1. Finally, most spear yield was quality 3 at 50 cm cutting height (Figure 4.3).

Table 4.3. Quality 1 spears (gram/plant) of four asparagus cultivars harvested at different cutting heights.

Cutting height (CH)	Cultivar (CV)				Cutting height mean
	'Grande'	**'Apollo'**	**'Atlas'**	**'UC157'**	
5 cm	16.3	13.2	14.1	9.7	13.4 b
15 cm	30.1	31.5	25.4	21.6	27.2 a
20 cm	11.3	11.2	18.7	32.7	18.5 b
30 cm	0.4	2.4	0.5	1.1	1.1 c
50 cm	0.0	0.0	0.0	0.0	0.0 c
Cultivar mean	11.6 a	11.7 a	11.7 a	13.1 a	
CV	ns				
CH	***				
CV x CH	*				

- Within the column and row, means followed by the same letter are not significantly different at 5 % level by Duncan's Multiple Range Test.
- ns, *, **, *** : non significant or significant at p=0.05, 0.01 or 0.001 respectively.

Table 4.4. Quality 2 spears (gram/plant) of four asparagus cultivars harvested at different cutting heights.

Cutting height (CH)	Cultivar (CV)				Cutting height mean
	'Grande'	**'Apollo'**	**'Atlas'**	**'UC157'**	
5 cm	0.0	0.0	0.0	0.0	0.0 c
15 cm	1.8	2.4	1.50	1.8	1.9 c
20 cm	12.4	16.7	15.4	15.2	14.9 b
30 cm	30.8	29.7	32.8	18.3	27.9 a
50 cm	1.1	0.0	1.6	0.0	0.7 c
Cultivar mean	9.2 a	9.7 a	10.3 a	7.1 a	
CV	ns				
CH	***				
CV x CH	ns				

- Within the column and row, means followed by the same letter are not significantly different at 5 % level by Duncan's Multiple Range Test.
- ns, *, **, *** : non significant or significant at p=0.05, 0.01 or 0.001 respectively.

Table 4.5. Quality 3 spears (gram/plant) of four asparagus cultivars harvested at different cutting heights.

Cutting height (CH)	Cultivar (CV)				Cutting height mean
	'Grande'	**'Apollo'**	**'Atlas'**	**'UC157'**	
5 cm	0.0	0.0	0.0	0.0	0.0 c
15 cm	0.3	0.0	0.0	0.4	0.2 c
20 cm	0.2	2.2	0.5	0.0	0.7 c
30 cm	9.2	10.6	14.1	9.1	10.7 b
50 cm	49.2	50.3	42.5	62.1	51.0 a
Cultivar mean	11.8 a	12.6 a	11.4 a	14.3 a	
CV	ns				
CH	***				
CV x CH	ns				

- Within the column and row, means followed by the same letter are not significantly different at 5 % level by Duncan's Multiple Range Test.
- ns, *, **, *** : non significant or significant at p=0.05, 0.01 or 0.001 respectively.

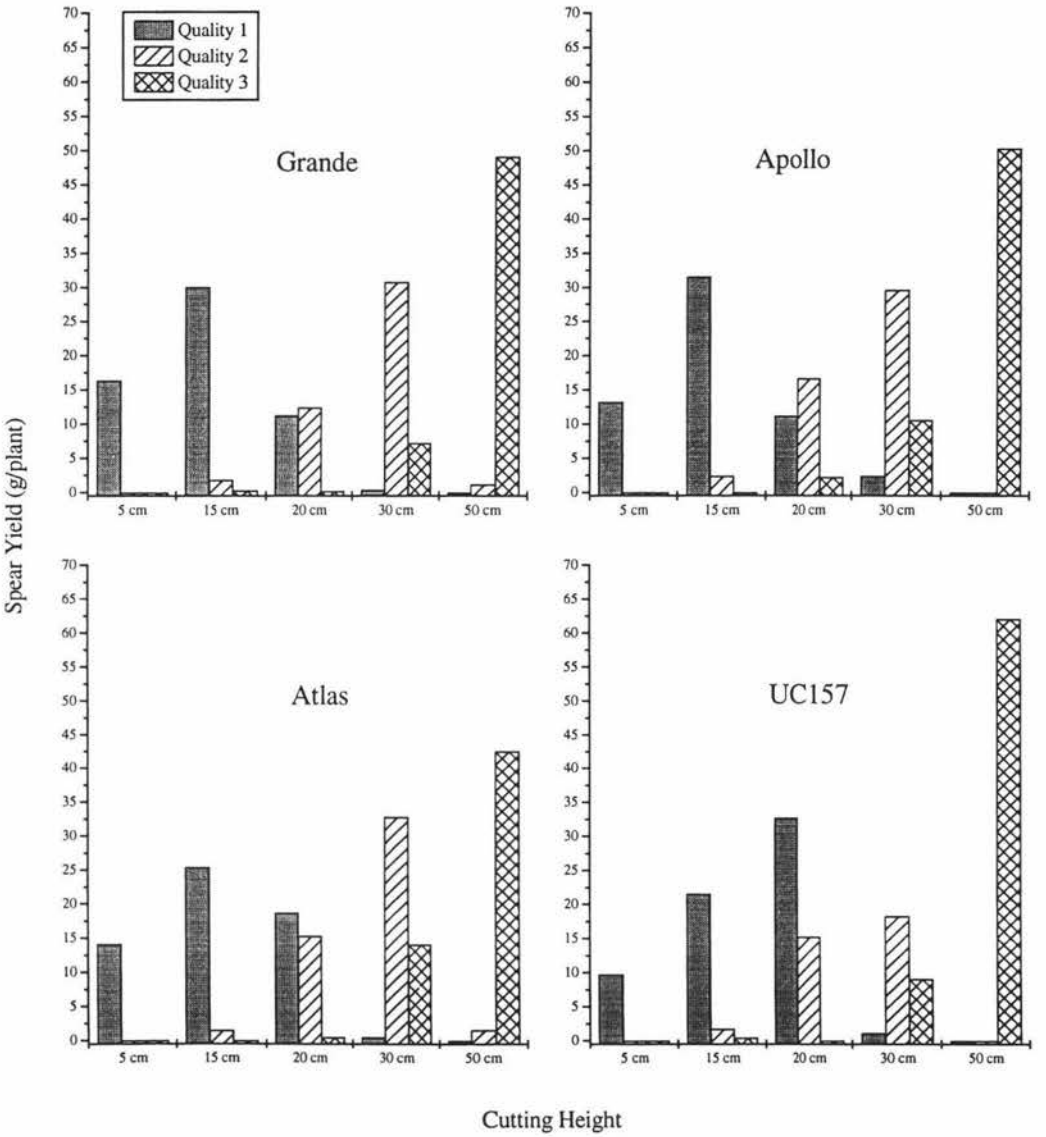


Figure 4.3. Spear quality of four asparagus cultivars harvested at different cutting heights.

4.3.1.3. Spear number and diameter

Total spear number of spears was not affected by cultivar but significantly affected by cutting height ($p<0.001$). The interaction effect between cultivar and cutting height was not significant (Table 4.5).

Table 4.5. Total spear number (per plant) of four asparagus cultivars harvested at different cutting heights.

Cutting height (CH)	Cultivar (CV)				Cutting height mean
	'Grande'	**'Apollo'**	**'Atlas'**	**'UC157'**	
5 cm	16.4	11.4	15.3	13.3	14.1 a
15 cm	10.3	11.5	10.5	9.8	10.5 bc
20 cm	7.3	9.8	11.6	15.4	11.0 b
30 cm	7.2	8.3	9.8	9.6	8.7 cd
50 cm	6.0	6.7	5.7	8.5	6.7 d
Cultivar mean	9.4 a	9.6 a	10.6 a	11.3 a	
CV	ns				
CH	***				
CV x CH	ns				

- Within the column and row, means followed by the same letter are not significantly different at 5 % level by Duncan's Multiple Range Test.
- ns, *, **, *** : non significant or significant at p=0.05, 0.01 or 0.001 respectively.

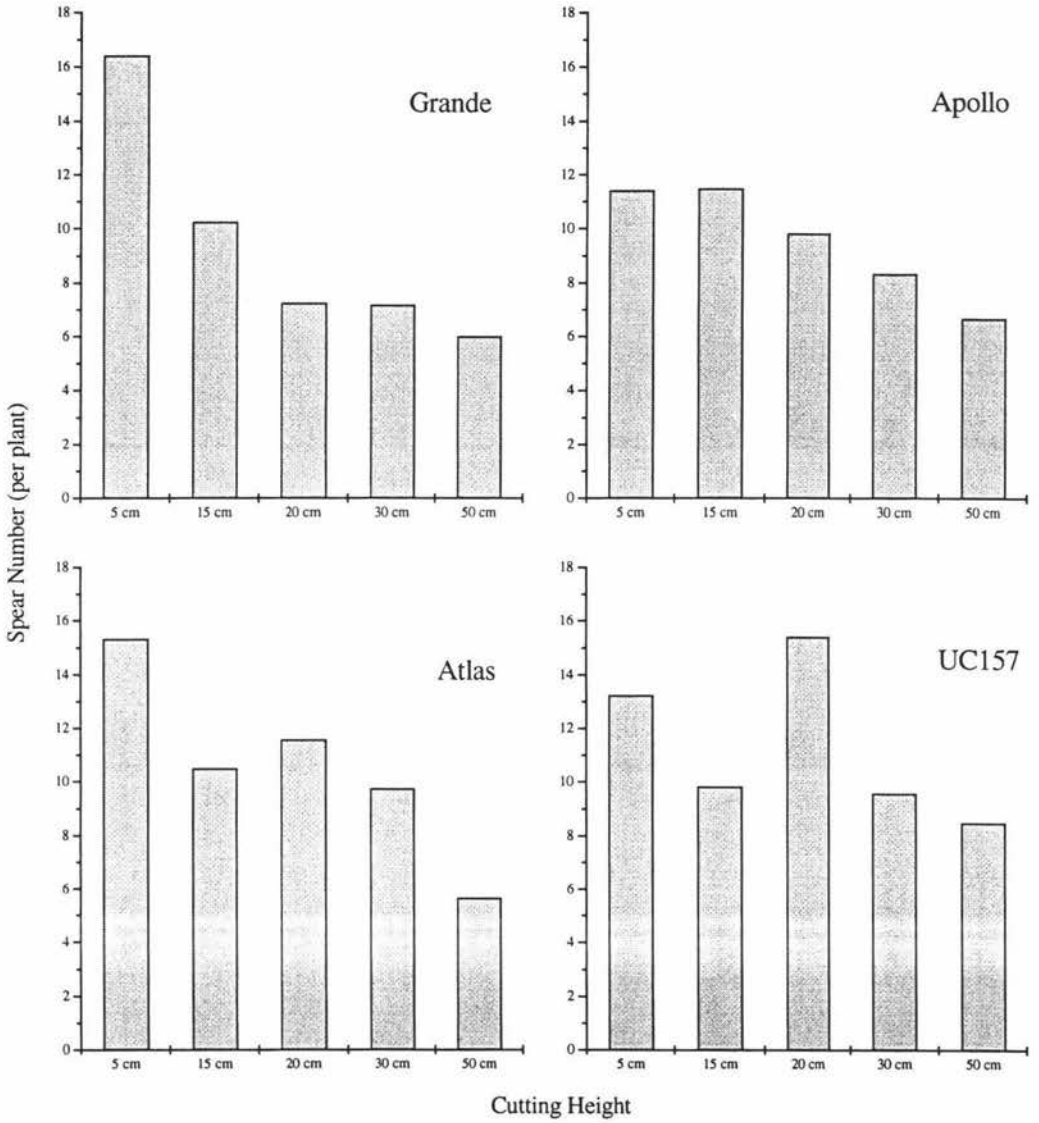


Figure 4.4. Total spear number (per plant) of four asparagus cultivars harvested at different cutting heights.

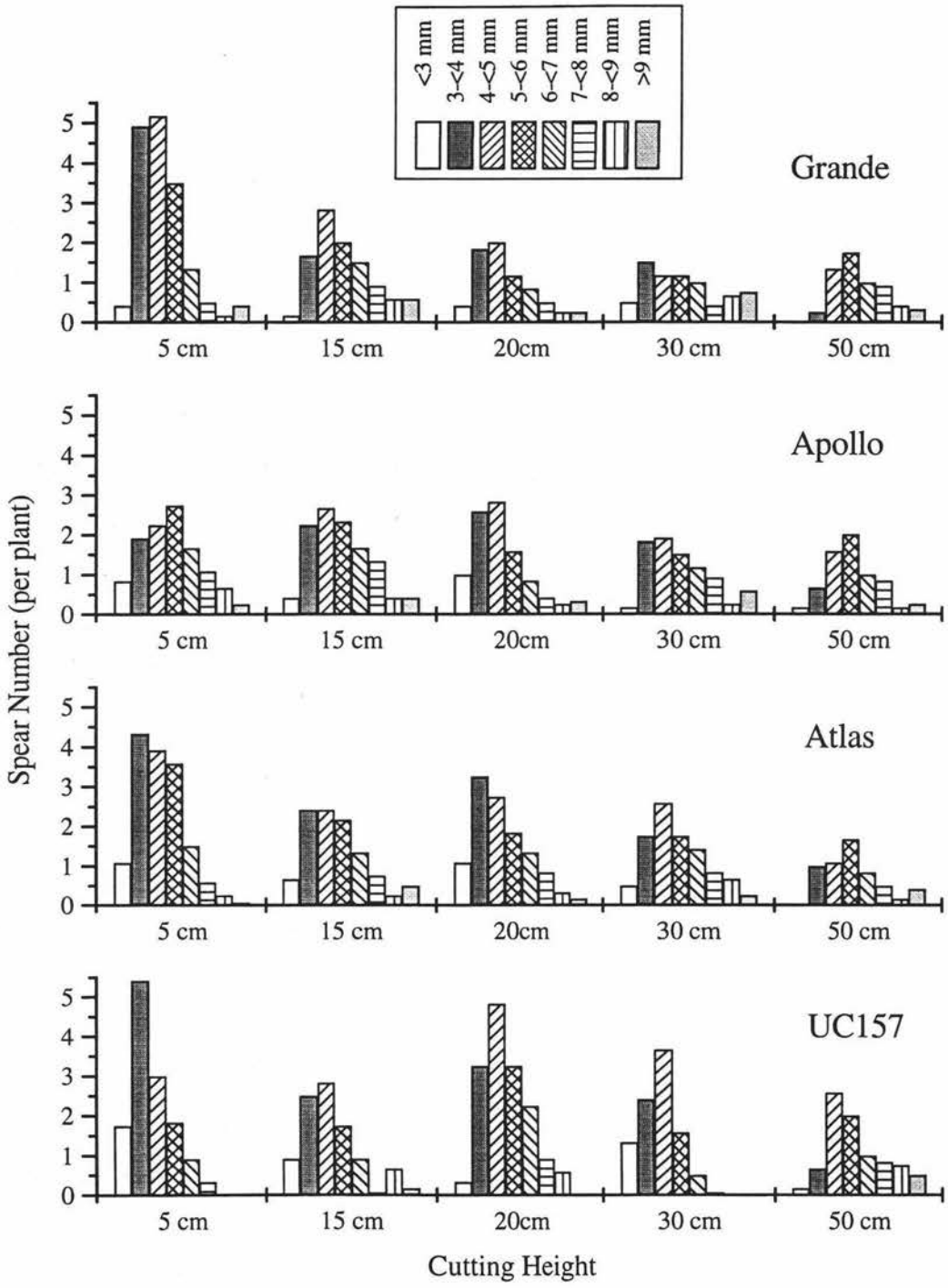


Figure 4.5. The distribution of spear diameter of four asparagus cultivars harvested at different cutting heights.

Total spear number decreased significantly with increasing cutting heights (Table 4.5 and Figure 4.4). For example, spear number of 'Grande' harvested at 5 cm cutting height was about 16 then decreased to about 10 at 15 cm cutting height. The spear number decreased further to about 6 as cutting height increased to 50 cm. Other cultivars such as 'Apollo' and 'Atlas' showed similar trend although spear number of cv 'Atlas' at cutting height of 15 cm was slightly lower than at the 20 cm cutting height. 'UC157' showed different results from the other cultivars; although spear number tended to decrease with increasing cutting height, cutting height at 20 cm resulted in the highest spear number ($p=0.05$).

Total spears consisted of many different diameters (Figure 4.5.). Most spears had diameter around 3 to 7 mm and only a few spears had diameter less than 3 mm or more than 7 mm. In general, at 5 cm cutting height, the proportion of spears were dominated by spears with diameter 3-<4 mm and then followed by 4-<5 mm diameter. However, at higher cutting height such as 15 cm, 20 cm, and 30 cm, the proportion of spears with diameter 4-<5 mm was higher than the other diameters. Then at 50 cm cutting height, spears with diameter of 5-<6 mm had higher proportion than the others. All these results were based on 9 month-old asparagus plants.

4.3.1.4. The relationship between cutting height and total yield and marketable yield

In this work, it is assumed that quality 1 spears were included in the first class marketable yield while the sum of quality 1 and quality 2 spears were regarded as total marketable yield. The response of cutting height to total yield was quadratic, the total yield increased linearly until 30 cm cutting height and then stayed nearly constant or slightly decreased after that. In contrast, the response of cutting height to both first class and total marketable yield 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 were lower than those for total marketable yield (Figure 4.6 and 4.7).

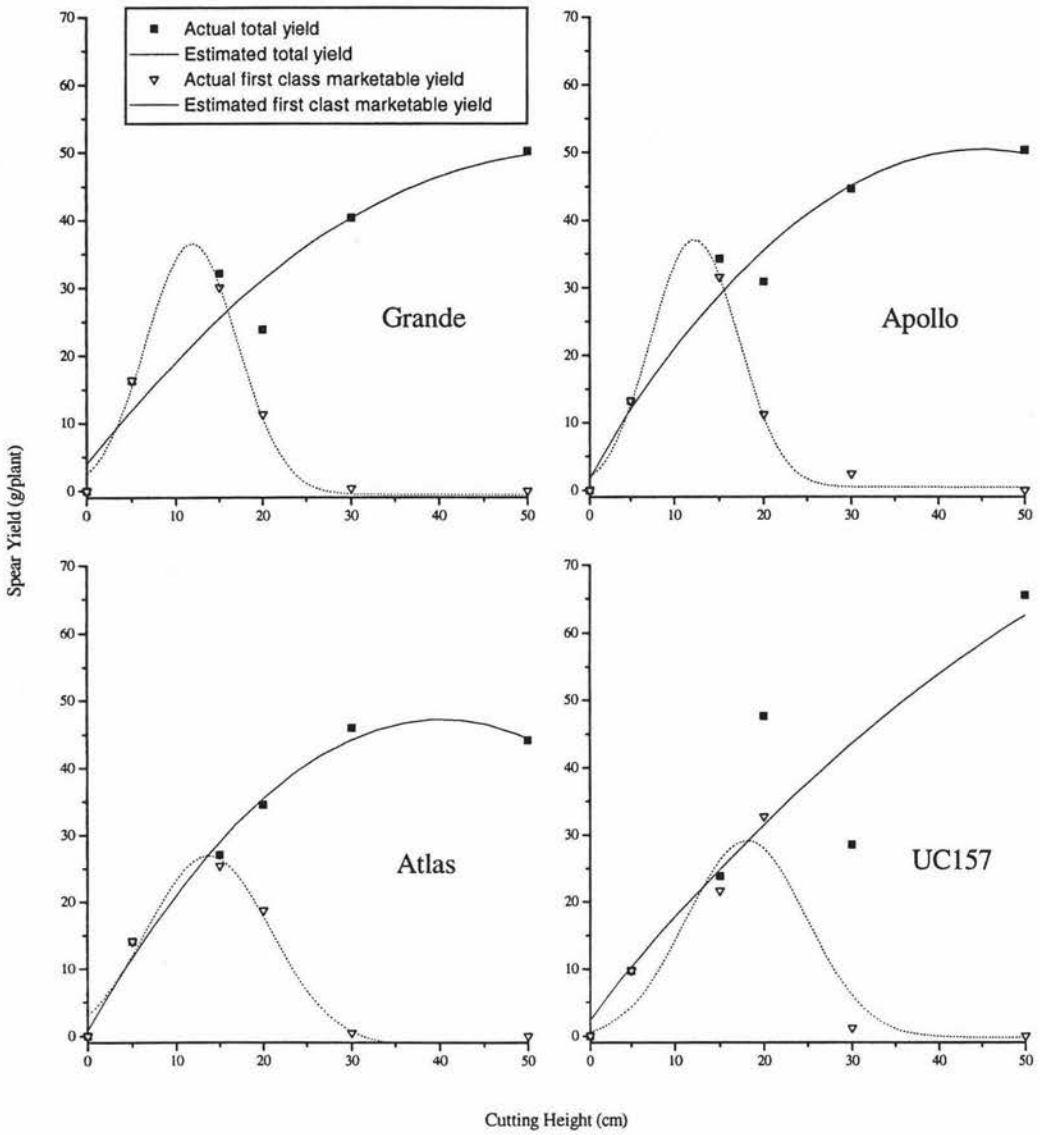


Figure 4.6. The relationship between cutting height and total yield and first class marketable yield of four asparagus cultivars.

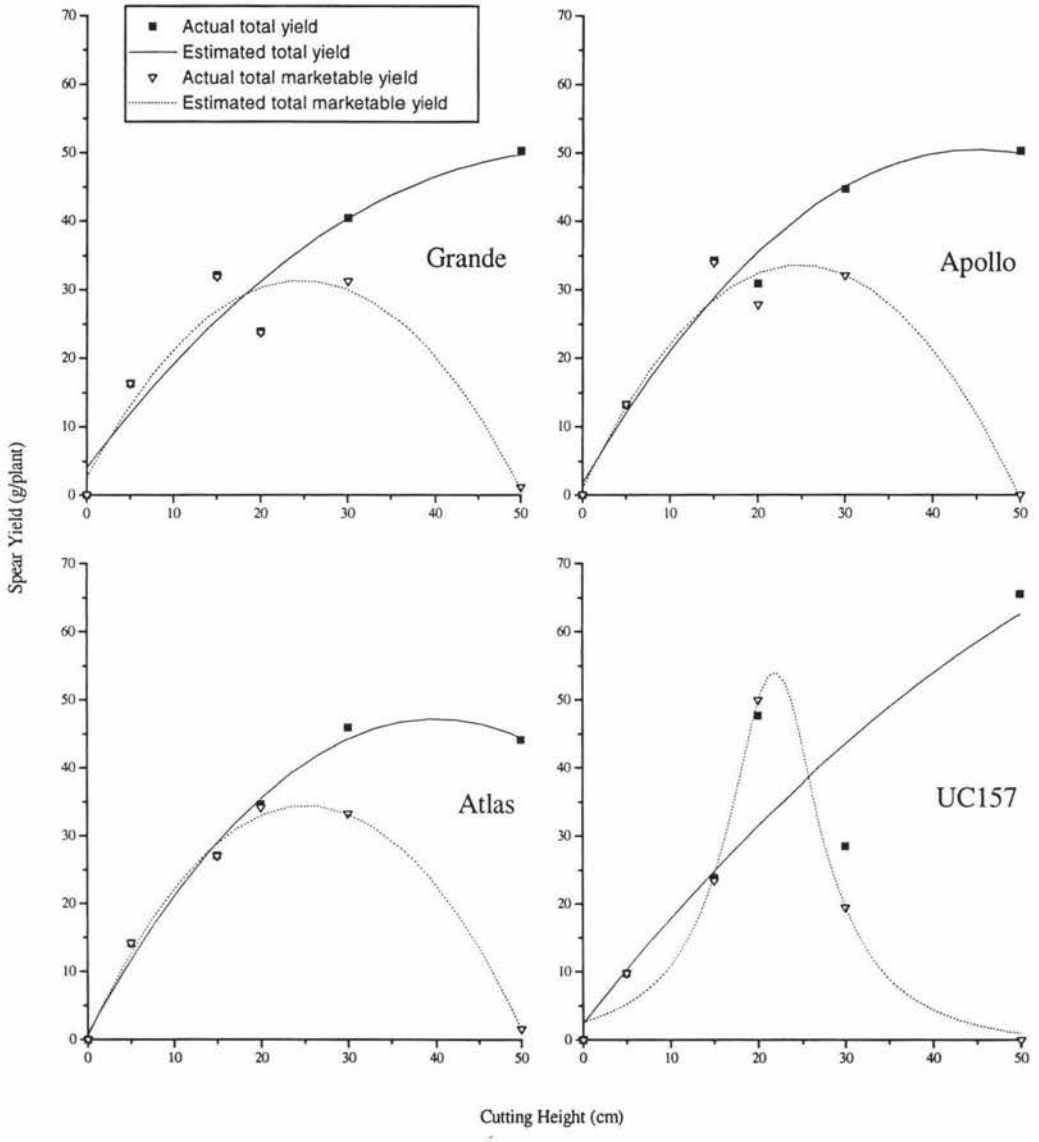


Figure 4.7. The relationship between cutting height and total yield and total marketable yield of four asparagus cultivars.

4.3.2. Experiment 2

4.3.2.1. Analysis of variance

Analysis of variance revealed that additional spear number and spear fresh weight were significantly affected by hormone treatment (p=0.001) but not affected by cultivar and cutting height. Bud number remaining was also significantly affected by hormone (p=0.001) treatment. Total soluble carbohydrate was significantly affected by cultivar and cutting height (p=0.05) but not affected by hormone treatment. No interaction effect between main factors was observed in this experiment (Table 4.6). Therefore, the main effects of cultivar, cutting height, and hormone treatment are presented and discussed.

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.

Source of variation	Additional spear number	Spear fresh weight (g/plant)	Bud number remaining	Total soluble carbohydrate of roots (%)
Cultivar (CV)	ns	ns	ns	*
Cutting height (CH)	ns	ns	*	*
CV x CH	ns	ns	ns	ns
Hormone (H)	***	***	***	ns
CV x H	ns	ns	ns	ns
CH x H	ns	ns	ns	ns
CV x CH x H	ns	ns	ns	ns

- ns, *, **, *** : non significant or significant at p=0.05, 0.01 or 0.001 respectively.

4.3.2.2. Effects of cultivar

Additional spear number and bud number left were not significantly different between cultivars. However, the results showed that 'UC157' produced higher additional spear number than the other cultivars and had lower bud number left than the other cultivars (Table 4.7).

In contrast, spear fresh weight was significantly different between cultivars. 'Grande' had 0.9927 g/plant spear fresh weight which was significantly higher than that of 'Atlas' (0.4433 g/plant) but not significantly different from 'Apollo' (0.7277 g/plant) and 'UC157' (0.6040 g/plant). In addition, 'UC157' showed significantly lower total soluble carbohydrate (4.55 %) than 'Apollo' and 'Atlas' which had total soluble carbohydrate of 6.58 % and 6.33 %, respectively. 'Grande' had 5.11 % total soluble carbohydrate which was not significantly different from the other cultivars (Table 4.7).

Table 4.7. Effects of cultivar on additional spear number, spear fresh weight, bud number remaining, and total soluble carbohydrate of roots.

Cultivar	Additional spear number (per plant)	Spear fresh weight (g/plant)	Bud number remaining (per plant)	Total soluble carbohydrate of roots (%)
'Grande'	0.83 a	0.99 a	2.43 a	5.11 ab
'Apollo'	0.75 a	0.73 ab	2.05 a	6.58 a
'Atlas'	0.67 a	0.44 b	2.37 a	6.33 a
'UC157'	0.98 a	0.60 ab	1.80 a	4.55 b

- Within columns, means followed by the same letter are not significantly different at 5 % level by Duncan's Multiple Range Test.

4.3.2.3. Effects of cutting height

Additional spear number and bud number remaining were significantly affected by cutting height. Plants harvested at 15 cm and 30 cm showed lower additional spear number than the other cutting heights with the highest number at 5 cm cutting height. However, although spear fresh weight showed a similar trend, it was not significantly different between cutting heights (Table 4.8).

Bud number remaining was also significantly affected by cutting height. The lowest bud number was at 20 cm cutting height (1.52) then followed by 50 cm cutting height (1.67). These values were significantly lower than those of other cutting heights with the highest bud number at 15 cm cutting height (2.65). In addition, the effect of cutting height on total soluble carbohydrate was also significant. Plants harvested at 20 cm and 50 cm showed significantly lower total soluble carbohydrate than those harvested at 5 cm and 15 cm (Table 4.8).

Table 4.8. Effects of cutting height on additional spear number, spear fresh weight, bud number remaining, and total soluble carbohydrate of roots.

Cutting height	Additional spear number (per plant)	Spear fresh weight (g/plant)	Bud number remaining (per plant)	Total soluble carbohydrate of roots (%)
5 cm	1.10 a	0.87 a	2.46 a	6.43 ab
15 cm	0.65 b	0.50 a	2.65 a	7.05 a
20 cm	0.79 ab	0.75 a	1.52 b	4.22 c
30 cm	0.63 b	0.64 a	2.52 a	5.67 abc
50 cm	0.88 ab	0.71 a	1.67 b	4.84 bc

- Within columns, means followed by the same letter are not significantly different at 5 % level by Duncan's Multiple Range Test.

4.3.2.4. Effects of hormone treatment

The application of hormone mixture ($4x10^{-4}$ M BA and GA_3) significantly stimulated additional spear number. The treatment resulted in an average of 1.22 spears compared with 0.4 spears for control. Spear fresh weight averaged 1.1 g/plant for hormone treatment which was significantly higher than 0.27 g/plant for control (Table 4.9).

Bud number remaining on the crown after harvest was significantly different between hormone treatment and control. For control, the average of bud number remaining was 2.93 compared with 1.40 for hormone treatment. However, total soluble carbohydrate of roots was not significantly different between hormone treatment and control (Table 4.9).

Bud number remaining varied from zero to 7 buds for control plants. Hormone treatment induced additional spear growth. As a result, bud number remaining on the crown decreased to around 3 or less (Figure 4.8).

Table 4.9. Effects of hormone treatment on additional spear number, spear fresh weight, bud number remaining, and total soluble carbohydrate of roots.

Hormone	Additional spear number (per plant)	Spear fresh weight (g/plant)	Bud number remaining (per plant)	Total soluble carbohydrate of roots (%)
Treatment	1.22 a	1.1100 a	1.40 b	5.84 a
Control	0.40 b	0.2738 b	2.93 a	5.45 a

- Within columns, means followed by the same letter are not significantly different at 5 % level by Duncan's Multiple Range Test.

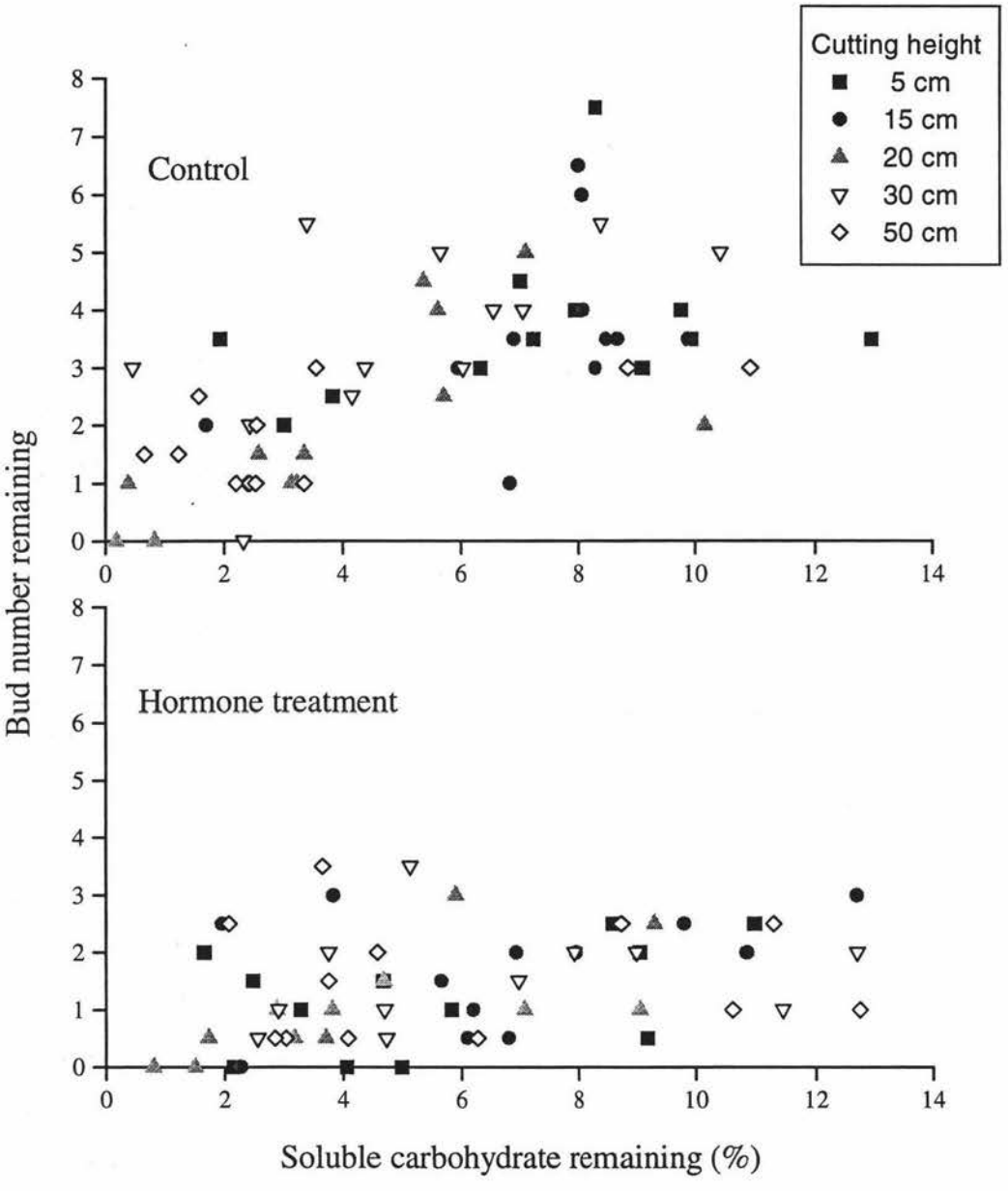


Figure 4.8. Effects of hormone treatment on bud number remaining in relation to soluble carbohydrate remaining in storage roots.

4.4. Discussion

4.4.1. Effects of cutting height on spear yield and spear number

Total spear yield was significantly affected by cutting height ($p<0.001$) but not significantly affected by cultivar. The response of spear yield to cutting height was quadratic, spear yield increased linearly up to 30 cm cutting height and then slightly increased ('Grande' and 'Apollo') or slightly decreased ('Atlas') at the higher cutting height. Spear yield of 'UC157' increased nearly linear up to 50 cm cutting height. Nichols and Fisher (1999) suggested similar trends. They hypothesized that the relationship between total spear yield and cutting height up to 30 cm is essentially linear. These current results also agree with those of Dean (1993) that total spear yield increased significantly from 1553 kg/ha at 13 cm cutting height to 2250 and 2881 kg/ha at 18 and 23 cm cutting heights, respectively over 3 yearly harvests. He suggested that harvesting taller spears up to 23 cm would increase grower returns as long as carbohydrate supply from storage roots were not depleted too greatly by cutting height.

The present results showed that the higher the cutting height, the higher the spear yield. This means that increasing cutting height increased the utilization of carbohydrate from storage roots for spear growth so that carbohydrate storage was depleted more with increasing cutting height. These effects of cutting height are similar to those of harvest pressure but fewer buds are utilized. Too high pressure of harvest time leads to reduced future yield while too short a harvest period results in reduced current yield. Takakori et al. (1970) showed the effect of harvest pressure from 30 to 120 days on spear yield and found that spear yield was optimum at 60 days of harvest. Sanders (1985) also found that 60 and 70 days of harvest tended to produce higher yield than 50 days, but 60 days of harvest was optimum for spear yield as 70 days of harvest produced more small spears. In addition, 60 days harvest period tended to have a greater carbohydrate reserve in roots as the season progressed while longer harvest duration reduced root dry weight. Shelton and Lacy (1980) also 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. Similarly, spear yield would be optimum at certain cutting heights without severely affecting carbohydrate reserve for supporting the following harvest season.

While spear yield increased with increasing cutting height, spear number decreased significantly ($p<0.001$) as cutting height increased. However, spear number was not significantly different between cultivars. The average spear number at 5 cm cutting height was about 14, the highest spear number among cutting height treatments. Spear number decreased significantly to 10.5 and 11 at 15 cm and 20 cm cutting heights, respectively. Spear number continued to decrease significantly to around 8.7 and 6.7 as cutting heights increased to 30 cm and 50 cm, respectively. These results suggest that at high cutting heights, spear yield was not limited by bud number but by other factors such as carbohydrate concentration in storage roots or apical dominance as explained below.

As the taller cutting height would be expected to deplete carbohydrate reserves faster than the shorter one, after certain harvest there may not be enough carbohydrate within storage roots to support the next spear growth so that bud break and spear growth were limited by carbohydrate supply. However the results only indicated that the two shortest cutting heights may have more carbohydrate than the longer cutting heights even though more spears were produced (Table 4.8).

Another possibility is the effect of apical dominance on bud break and spear growth. Nichols and Woolley (1985) suggested that within bud cluster, usually only one spear grows at a time. The buds grow starting from the oldest bud followed by the next buds; however, the next buds on the cluster will not grow ,or grows only slowly, until the previous spear loses its apical dominance due to harvest or growth into fern. Nichols and Woolley (1985) showed that the relative growth rate of spear decreased from spear number 1 to at least spear number 3 within a bud cluster. Any subsequent spears in the same flush showed an inhibited growth rate. When the earlier spears were removed the growth rate of the inhibited spears increased abruptly indicating the release of apical dominance. Thus, decreasing spear number due to cutting height could be influenced by

apical dominance. The taller the cutting height, the longer the effect of apical dominance on the subsequent bud. As a result, the spear number decreased (Table 4.5).

4.4.2. Effects of cutting height on spear quality

Spear quality generally 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, many researchers use different grade. Some researchers such as Dean (1993) and Jayamangkala (1992) used spear diameter for determining spear quality. Although other parameters are important, most above classification was based on spear diameter.

In the present study, however, spear quality was only assessed from spear tip tightness as most harvested spears had diameter less then 7 mm. This was probably because of plant age and low carbohydrate levels (around 30 %) due to stemphylium. Normally, for commercial purposes, asparagus spears are harvested three years after planting.

The results showed that the yield of quality 1 spear increased as cutting height increased, reached peak at a certain cutting height and decreased at higher cutting heights. An interaction effect occurred as the yield of 'UC157' increased up to 20 cm cutting height while the other cultivars ('Grande', 'Apollo', and 'Atlas') showed a decrease of spear yield at 20 cm cutting height. These results suggested that the response of cultivars to cutting height were different (Figure 4.3 and Table 4.3).

As the yield of quality 1 spears decreased at 20 cm cutting height, the yield of spear quality 2 started to increase, suggesting that the spears starting to lose their tip tightness at 20 cm. The yield of quality 2 spears increased up to 30 cm cutting height when spears' tip started to open to become quality 3 spears and at 50 cm cutting height most spears' tip opened. Thus, 20 cm was critical cutting height as increasing cutting height reduced spear quality markedly; however, the response varied dependent on cultivars. Krarup et al. (1997) found that degree of opening of the tip of asparagus was significantly different

between cultivars and showed that 'UC157' and 'UC72' opened their tips at a higher cutting height than the other cultivars as suggested in this present study. They suggested that spear elongation and tip opening of spear were affected by temperature. An increase in temperature resulted in greater daily elongation of spears and higher total yield but spears' tips tended to open at a lower height. Therefore, cutting height is a very important factor in spear harvest in order to produce optimum yield of high quality spears.

4.4.3. Marketable yield versus total yield

As discussed above, total yield increased linearly until 30 cm cutting height and then slightly increased or decreased after that. This suggested that increasing cutting height increased carbohydrate uptake from storage roots so that carbohydrate reserve decreased with increasing cutting height (Table 4.8). In contrast, 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 (Figure 4.6 and 4.7).

Based on the condition of the plants in this study (9 months old asparagus plants with carbohydrate concentration in storage roots of about 30 % dry weight), optimum marketable yield was different dependent on cultivars. The optimum of estimated first class marketable yield of 'Grande' and 'Apollo' was around 12 cm cutting height; 'Atlas' was around 15 cm cutting height; and 'UC157' was around 18 cm cutting height. However, for total marketable yield 'Grande', 'Apollo' and 'Atlas' showed similar optimum cutting height at 25 cm while 'UC157' was around 22 cm (Figure 4.6). This different response of asparagus cultivars to cutting height suggested that variation in cutting height during spear harvest should be used for cultivar evaluation.

Spear yield of asparagus is mainly dependent on carbohydrate reserves in storage roots while the goal of growing asparagus is to make profit; thus carbohydrate reserves have economical implication. These reserves should be used as much as possible to produce marketable spears. At this point, cutting height is a critical factor in asparagus

production. As indicated by this study that marketable yield reached a maximum value at certain cutting height, harvesting spears at lower than optimum cutting height will result in lower yield because storage carbohydrates in the roots are not used optimally. Similarly, harvesting spears at higher than optimum cutting height will reduce marketable spears as some spears start to lose their quality. In this case, some carbohydrate reserves are used to produce unmarketable spears, meaning economic loss. Therefore, to get an optimum profit, asparagus spears should be harvested at optimum cutting height which varies with asparagus cultivars as indicated by this present study.

4.4.4. Effects of hormone treatment on additional spear production

The asparagus plants used in this experiment were harvested for 8 weeks until bud growth stopped before hormone application. Data from control plants indicated that several buds were still available but the buds did not grow (Table 4.9). Lack of growth could be caused by factors such as hormone balance or nutrient supply. As the concentration of soluble carbohydrate in the storage roots was very low (less than 6 % dry weight), bud break may have been limited by low carbohydrate reserves in storage roots because the buds might have difficulties mobilizing it. However, the application of an hormone mixture of 4x10-4 M benzyladenine (BA) and gibberellic acid (GA_3) on the crown surface significantly stimulated additional spear production, both spear number and spear fresh weight, compared with control plants. This indicates that bud break and spear growth could be induced by hormone application even though carbohydrate was very low (less than 7 %).

Gibberellic acid (GA) has been reported to induce bud break in sweet potato (Tompkins and Bowers 1970). They reported that GA treated plants showed significantly higher number of sprouts and produced 39 to 92 % more plants than control. However, Mahotiere (1976) found that GA was not effective in stimulating shoot growth in asparagus. In this present study, the effects of GA alone cannot be ascertained as it was applied together with BA.

Uesugi (1991) found that foliar application of BA in fall on asparagus promoted spear sprouting and increased spear thickness. The effect of BA on spear production was rapid and continued for three weeks after treatment. Mahotiere et al. (1993) found similar results, application of BA promoted shoot emergence in three asparagus cultivars compared with control plants.

Although the conditions of plants used in this study (low carbohydrate reserves in storage roots) were different from those used by Uesugi (1991) and Mahotiere et al. (1993), the results were similar. In both plant conditions, the application of hormone increased shoot production in asparagus. The results suggested that induction of spear growth was effective for crown which have more than 4 buds and the induction may stop when 3 or less buds remained on the crown (Figure 4.8). The application of hormone mixture (BA and GA_3) may increase sink strength of the buds so that they could remobilize carbohydrate to induce bud growth. However, further experiments are needed to determine the effects of BA and GA_3 separately and the interaction effect between them.

At least, the present study implies that hormone solution can be used to boost fern renewal after a certain period of harvest so that fern can be produced for renewal of carbohydrate reserves.

CHAPTER FIVE

GENERAL DISCUSSION

5.1. Carbohydrate partitioning in relation to daylength, remobilization and utilization of carbohydrate for spear production

Carbohydrate reserves in storage roots play a major role in asparagus production. The ability of plants to partition carbohydrate to storage roots tends to determine the spear yield as spear production depends mainly on these carbohydrate levels in storage roots. In addition, the ability of plants to remobilize carbohydrate from storage roots to available buds, and possibly the number of buds capable of growth, determines total spear yield. Finally, the management of carbohydrate utilization by cutting height can determine marketable yield of asparagus spears.

This thesis evaluated the effect of daylength on dry matter partitioning between fern and storage roots (Chapter Two), remobilization of soluble carbohydrates (fructans) from different roots during spear harvest (Chapter Three), and the effect of cutting height at harvest on spear yield and quality and stimulation of additional spears using hormone treatment (Chapter Four).

The effect of daylength on dry matter partitioning was evaluated in controlled climate rooms set at constant daylength (15.5 h) and reducing daylength (Chapter Two). The results demonstrated that partitioning carbohydrates between fern and crown was influenced by daylength. Plants exposed under reducing daylength showed reductions in plant height, shoot number, number of lateral per shoot, length of lateral, and fern dry weight. Although reducing daylength was associated with decreasing NAR (net assimilation rate), relatively more carbohydrate partitioned to crown than to fern. This was indicated by root:shoot dry weight ratio and allometric ratio between crown and fern. The results also suggested that daylength around 13.5 h have 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.

These results raise other questions such as do cultivars differ in their daylength requirement?; is there a critical daylength at which the change in partitioning occurs?; and do cultivars differs in yield partly because of differences in daylength at different latitudes? If there is a critical daylength range (windows of opportunity) at which fern production slows, but increased partitioning carbohydrate to the crown occurs, then crop management could be modified to take advantage of the phenomenon. For example : (a) ensure establishment of adequate fern area before the critical daylength, lack of adequate fern may explain failure of increased crown growth rates, compared with the long day controls, at daylengths below 13 hours in the growth room experiments; (b) the use of low intensity lingt ningth-breaks, or daylength extension, to obtain favorable daylengths when natural daylengths sre below 13 hours in tunnel house or white asparagus out-of-season production; (c) supplementary low intensity in the field if daylengths decrease below the optimum is possible, but may be uneconomic.

After carbohydrates (fructans) partitioned to storage roots, remobilization of these fructans from storage roots during spear growth is another important issue in asparagus production (Chapter Three). The results revealed 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. Probably spear growth utilized the most recently formed carbohydrates stored in new roots before using the other source of carbohydrate within the same or different roots.

The HPLC system used in this work could separate fructans up to DP10, plus a 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 frutans decreased sharply during spear growth while short chain fructans (DP3 to DP10) decreased slightly.

Prior to remobilization the fructans are hydrolyzed by fructan exohydrolase (FEH) by cutting fructose molecules once at a time (Nelson and Spollen 1987). Fructose is then converted to sucrose by sucrose phosphate synthase and sucrose phosphatase and transported to buds to support spear growth (Smith 1993). Our results indicate that sucrose may accumulate slightly during light harvests, but is quickly exported to the spears during a heavy harvest (Figure 3.7).

Changes in individual fructans suggested that hydrolysis rates of DP4 and DP3 seem to be a limiting process during fructan hydrolysis as also suggested by Dean and Skrzeczkowska (1996). Thus, it is of interest to determine if the rate of hydrolysis is limited by any particular step and if this step can be manipulated by environmental, hormonal or genetic modification.

Finally, remobilization (utilization) of storage carbohydrates to produce marketable yield from available buds is the most practical issue in asparagus production (Chapter Four). This can be managed by managing 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. Cutting height also involves the use of carbohydrate reserves and buds. Increasing cutting height from the optimum height will increase carbohydrate uptake although less buds will be used. In this case, spear productions is likely to be limited by carbohydrate reserves. Marketable yield will also be reduced as some spears start to lose their tip tightness. At low cutting heights there will be reduced requirement for carbohydrate but increased requirements for the use of buds. Thus bud number may limit spear yield.

The results from hormone treatments showed that the application of hormone mixture (BA and GA_3) induced additional spear production indicating that spear production was not limited by bud number but by carbohydrate level in storage roots or some unknown factor, at least for cutting heights above 30 cm. However, if spear size is crucial,

spear production may be limited by bud size even if total bud number is not limiting. In addition, even if the carbohydrate level is very low hormone treatment may increase sink strength of the buds so that they could remobilize carbohydrate to induce bud growth for fern establishment. Another possibility is that hormone treatment may increase fructan hydrolysis of DP4 and DP3, as this appears to be a limiting factor during heavy harvest, so that additional fructose could be released to support additional spear growth.

5.2. Further work

- Further work on daylength effects is needed to accurately define daylength periods that are particularly favorable for storage carbohydrate in the roots, in order to increase yield; to investigate further cultivar differences in response to daylength; and to investigate how crop management can be modified, and cultivars selected, to take advantage of differences in response to daylength.

- As the HPLC system used in this study of fructans only separated fructans up to DP10 while most fructans in storage roots are long chain fructans (DP more than 10) further studies using a different column system are needed.

- Plant material used in cutting height experiments was too young (nine months) and carbohydrate levels were low due to stemphyllium infection. Thus, further investigations using better plant material are needed to develop a greater understanding of the relationship between bud numbers and carbohydrate sources in asparagus. In addition, field experiments are needed to evaluate the response of different cultivars on cutting height in order to develop a more efficient technology for cultivar evaluation.

- The application of a hormone mixture (BA and GA_3) induced additional spear production but further experiments are needed to determine the effects of BA and GA3 separately and the interaction effect between them. In addition, it is of interest to investigate the effect of hormone treatments on fructan hydrolysis, particularly the hydrolysis of fructans DP3 and DP4 which, based on this study, are probably limiting factors in fructan hydrolysis.

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Appendix 1. Statistical analysis of the effect of daylength on crown dry weight.

(a) Table of means (g/plant)

Main effect of daylength

Daylength	harvest 0	harvest 1	harvest 2	harvest 3	harvest 4	harvest 5	harvest 6	harvest 7	harvest 8
Reducing	0.27	0.32	0.45	1.20	1.71	2.09	3.08	4.25	6.72
Constant	0.28	0.29	0.45	0.68	1.25	1.73	2.99	5.33	7.17
SE	0.01	0.02	0.05	0.07	0.08	0.04	0.08	0.20	0.18
p-value	0.68	0.28	0.92	0.01	0.01	0.003	0.43	0.02	0.14

Effect of cultivar (within daylength)

Daylength	Cultivar	harvest 0	harvest 1	harvest 2	harvest 3	harvest 4	harvest 5	harvest 6	harvest 7	harvest 8
Reducing	UC	0.27	0.32	0.56	1.34	1.88	2.11	3.09	4.13	6.86
	JG	0.25	0.33	0.42	1.19	1.73	2.12	3.10	4.73	6.27
	IH	0.28	0.31	0.38	1.09	1.51	2.05	3.06	3.88	7.02
Constant	UC	0.26	0.26	0.45	0.71	1.27	1.82	3.01	5.27	7.15
	JG	0.31	0.34	0.52	0.78	1.29	1.64	3.17	5.17	7.34
	IH	0.26	0.26	0.37	0.54	1.19	1.74	2.78	5.55	7.01
	SE	0.03	0.03	0.05	0.07	0.08	0.11	0.16	0.37	0.38
	p-value	0.59	0.45	0.02	0.01	0.04	0.81	0.55	0.51	0.64

(b) Sas program

```
option ls=78 ps=63 nodate nocenter nonumber;

title'root:shoot ratio - nested design';
data nested;
        infile 'A:\crown.dt';
  input block dlength cultivar reps harvest0 harvest1 harvest2
        harvest3 harvest4 harvest5 harvest6 harvest7 harvest8;
run;

proc print data=nested;
        var block dlength cultivar reps harvest0 harvest1 harvest2
        harvest3 harvest4 harvest5 harvest6 harvest7 harvest8;
run;

proc glm data=nested;
        class block dlength cultivar;
        model harvest0 harvest1 harvest2 harvest3 harvest4 harvest5
        harvest6 harvest7 harvest8=block dlength cultivar(dlength)/ss3;
        random block dlength cultivar(dlength) / test;
        lsmeans dlength/e=cultivar(dlength) pdiff stderr;
        lsmeans cultivar(dlength)/pdiff stderr;
run;
```

(c) Sas output for harvest 4

General Linear Models Procedure
Class Level Information

Class	Levels	Values
BLOCK	2	1 2
DLENGTH	2	1 2
CULTIVAR	3	1 2 3

Number of observations in data set = 60

crown dry weight - nested design

General Linear Models Procedure

Dependent Variable: HARVEST4

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	6	3.96622403	0.66103734	9.85	0.0001
Error	53	3.55764194	0.06712532		
Corrected Total	59	7.52386597			

R-Square	C.V.	Root MSE	HARVEST4 Mean
0.527152	17.50470	0.259086	1.480092

Source	DF	Type III SS	Mean Square	F Value	Pr > F
BLOCK	1	0.14467806	0.14467806	2.16	0.1480
DLENGTH	1	3.09133141	3.09133141	46.05	0.0001
CULTIVAR(DLENGTH)	4	0.73021455	0.18255364	2.72	0.0392

General Linear Models Procedure

Source	Type III Expected Mean Square
BLOCK	Var(Error) + 30 Var(BLOCK)
DLENGTH	Var(Error) + 10 Var(CULTIVAR(DLENGTH)) + 30 Var(DLENGTH)
CULTIVAR(DLENGTH)	Var(Error) + 10 Var(CULTIVAR(DLENGTH))

Dependent Variable: HARVEST4

Source: BLOCK
Error: MS(Error)

DF	Type III MS	Denominator DF	Denominator MS	F Value	Pr > F
1	0.1446780615	53	0.0671253196	2.1553	0.1480

Source: DLENGTH
Error: MS(CULTIVAR(DLENGTH))

DF	Type III MS	Denominator DF	Denominator MS	F Value	Pr > F
1	3.0913314135	4	0.1825536383	16.9338	0.0147

Source: CULTIVAR(DLENGTH)
Error: MS(Error)

DF	Type III MS	Denominator DF	Denominator MS	F Value	Pr > F
4	0.1825536383	53	0.0671253196	2.7196	0.0392

Least Squares Means

Standard Errors and Probabilities calculated using the Type III MS for CULTIVAR(DLENGTH) as an Error term

DLENGTH	HARVEST4 LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	Pr > |T| H0: LSMEAN1=LSMEAN2
1	1.70707667	0.07800719	0.0001	0.0147
2	1.25310667	0.07800719	0.0001	

CULTIVAR	DLENGTH	HARVEST4 LSMEAN	Std Err LSMEAN	Pr > |T| H0:LSMEAN=0	LSMEAN Number
1	1	1.87720000	0.08193004	0.0001	1
2	1	1.73193000	0.08193004	0.0001	2
3	1	1.51210000	0.08193004	0.0001	3
1	2	1.27267000	0.08193004	0.0001	4
2	2	1.29268000	0.08193004	0.0001	5
3	2	1.19397000	0.08193004	0.0001	6

Pr > |T| H0: LSMEAN(i)=LSMEAN(j)

i/j	1	2	3	4	5	6
1	.	0.2154	0.0027	0.0001	0.0001	0.0001
2	0.2154	.	0.0632	0.0002	0.0004	0.0001
3	0.0027	0.0632	.	0.0437	0.0637	0.0082
4	0.0001	0.0002	0.0437	.	0.8635	0.4999
5	0.0001	0.0004	0.0637	0.8635	.	0.3981
6	0.0001	0.0001	0.0082	0.4999	0.3981	.